Seroprevalence of Leptospira antibodies in rodents and shrews of Kibondo and Kakonko Districts, Kigoma region, Tanzania

Clara A. Majawa[1,2,3], Athumani M. Lupindu[1], Ginethon G. Mhamphi[2], Robert S. Machang'u[4], Abdul A.S. Katakweba[2]

[1] Department of Veterinary Medicine and Public Health,
Sokoine University of Agriculture,
P. O. Box 3015,
Chuo Kikuu, Morogoro, Tanzania

[2]Institute of Pest Management,
Sokoine University of Agriculture,
P.O. Box 3110,
Chuo Kikuu, Morogoro, Tanzania

[3]Balaka District Hospital,
P.O. Box 138,
Balaka, Malawi.

[4]St. Francis University College of Health and Allied Sciences,
P.O. Box 175,
Ifakara-Morogoro, Tanzania.

Abstract

Leptospirosis is a worldwide neglected bacterial zoonotic disease caused by pathogenic species of the genus *Leptospira*. Humans get leptospirosis through contact with an environment contaminated with bacteria from reservoir hosts, which are mainly rodents. A cross-sectional epidemiological study was carried out in Kibondo and Kakonko districts of Kigoma region, Tanzania, to determine the seroprevalence of Leptospira species in rodents and shrews. Blood sera were collected from rodents and shrews (n = 582) and tested for leptospiral antibodies using the Microscopic Agglutination Test (MAT) against five live serovars with titers ranging from 1:20 to 1:160 and a cut-off point of 1:160. The overall prevalence of leptospira antibodies was 11.9% with rodents showing 11.6% (95% CI 9.1% to 14.3%) and shrews having 0.3% (95% CI 0.1% to 1.1%). Number and prevalence per species (in brackets) were as follows; *Aethomys kaiseri* 16 (2.7%), *Arvicanthis niloticus* 1 (0.2%), *Lemniscomys rosalia* 2 (0.3%), *Lemniscomys striatus* 10 (1.7%),

Lophuromys flavopunctatus 2 (0.3%)*, Mastomys natalensis* 30 (5.2%)*, Rattus rattus* 6 (1.0%) and C*rocidura tansaniana* 2 (0.3%). No antibodies were revealed in *Grammomys dolichurus, Mus musculus, Praomys delectorum,* and *Tatera indica.* In terms of prevalence, there was no significant variation with regard to sex or between rodents and shrews, but it was found across species (P<0.05). The most prevalent Leptospira serovar and titer were Lora (4%) and titer 1:40 respectively. Kakonko had a prevalence of 18 (3.1%) compared to Kibondo, 51 (8.8%). Fallow land was leading in the prevalence of leptospira antibodies in its captured rodents and shrews with a prevalence of 36 (6.2%), followed by farmland 16 (2.7%), indoor 11 (1.9%), grassland 4 (0.7%), forest 1 (0.2%) and wetland 1 (0.2%). The findings of this study denote a potential public health risk among the people of Kigoma region, Tanzania, and hence the need to raise awareness of the disease among the study population and the country as a whole.

Keywords: *Leptospirosis, rodents, shrews, Kibondo, Kakonko, Kigoma, Tanzania*

1.0 INTRODUCTION

The bacteria of the genus Leptospira cause leptospirosis, which is recognized as a re-emerging zoonosis in tropical and sub-tropical regions of the world (Goarant, 2016). In humans, leptospirosis manifests clinically in phases, starting with the acute stage, progressing to the sub-acute stage, and chronic stages. The disease leads to multisystem damage, including injury to skeletal muscles, renal, and hepatic systems (Plank et al., 2000). It generally displays signs and symptoms similar to those of other febrile diseases, such as malaria, dengue, and influenza (De Brito et al., 2018). Due to the similarity, it is commonly misdiagnosed and listed as one of Tanzania's most underreported and neglected condition. Leptospirosis is thought to be responsible for up to 20% of ill-defined febrile illnesses worldwide (World Health Organisation, 2011). The leptospires are typically found in the renal tubules and voided with the urine of accidental and maintenance hosts like cattle, pigs, dogs, sheep, goats, and rodents (Allan et al., 2018). However, rodents and other small mammals are major reservoir vectors of leptospires (Boey et al., 2019). Leptospirosis is also associated with certain occupations like growing rice, fishing, handling animal products, handling cattle, and engaging in aquatic sports. (Cutler et al., 2010).

According to recent estimates, leptospirosis causes 1.03 million cases and 58,900 deaths annually, making it the top zoonotic cause of mortality and morbidity worldwide. WHO (2011), reports that Africa has the highest average yearly incidence, with 96 cases per 100,000 people. Leptospirosis has been studied in some parts of Tanzania, where it is found to affect humans, domestic animals, and small

wild mammals at significant rates (Mgode et al., 2014). Even with these reports, there is still a paucity of information on this disease as studies have only been carried out in 10 of the country's 26 regions. The extent to which leptospirosis in rodents contributes to the spread of the disease to people and domestic animals in Kigoma region has not been sufficiently studied. Commensal rodents, in particular, increase the risk of transmitting leptospirosis to humans (Holt et al., 2006). It is, therefore, important to understand the prevalence of leptospirosis in rodents and shrews by researchers, clinicians, farmers, and policymakers for appropriate disease surveillance and control.

2.0 MATERIALS AND METHODS

2.1 Study area

The cross-sectional study was carried out in Kibondo and Kakonko districts, in Kigoma region, Tanzania from mid-February to mid-March 2022. Kakonko district is situated between 3.0 and 5.0 degrees south of the equator and 30.2 and 31.5 degrees east of GMT. Kibondo is situated between latitudes 3.9 and 5.0 S and longitudes 30.2 and 31.5 E (National Bureau of Statistics, 2013). The districts have a tropical climate with two distinct rainy seasons that occur between October and December, and between March and May. The highest amounts of rainfall occur in April, and the yearly rainfall ranges from 800 to 1600 mm. Between June and September, it is a dry season with an average humidity of 10% (Tanzania Meteorological Authority, 2021). The study sites (Figure 1), were selected randomly and were recorded and mapped using a global positioning system (GPS).

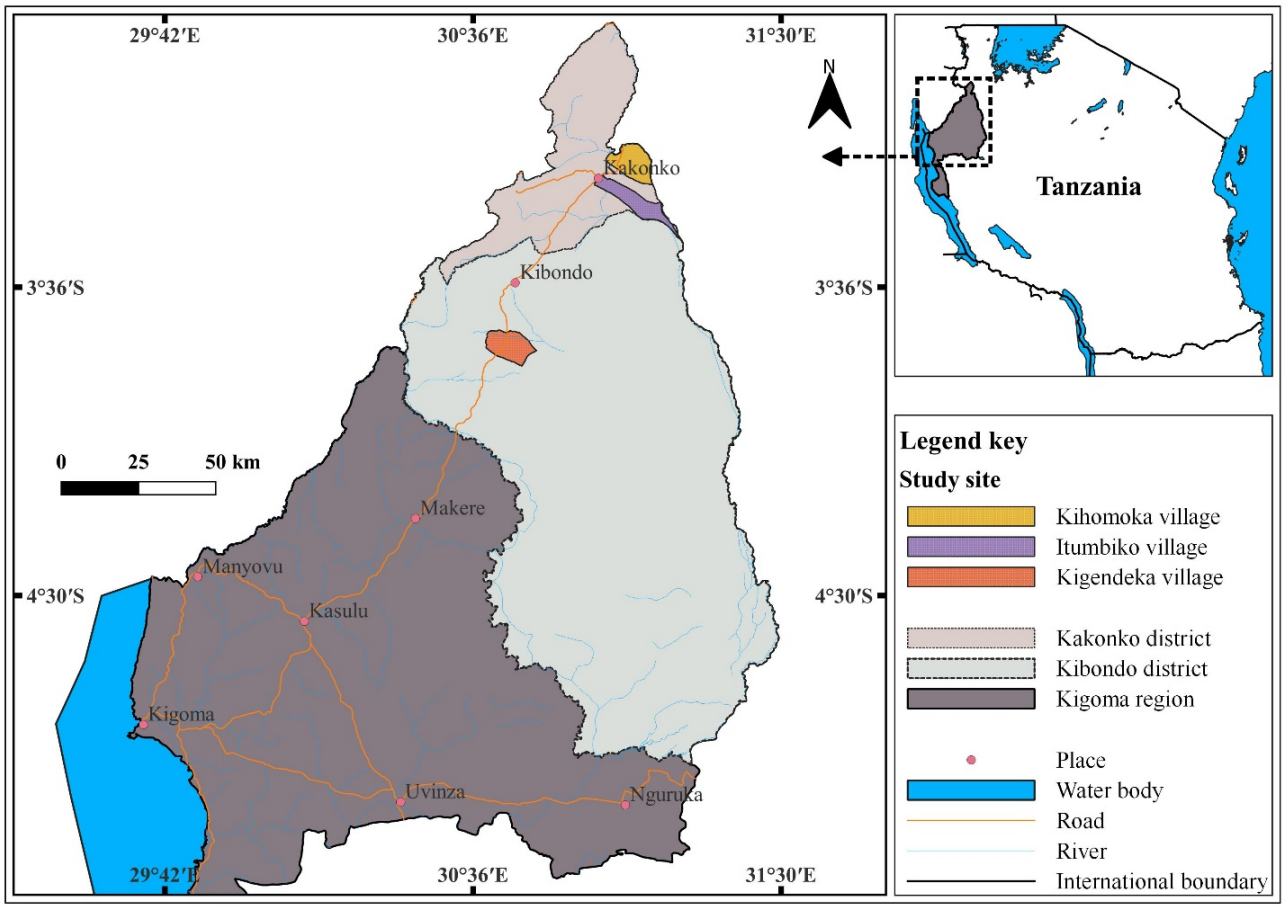

Figure 1: *Map of Kigoma region showing Kibondo and Kakonko districts. Source: QGIS Version 3.24 Tisler' retrieved on 31 August 2022*

2.2 Animal trapping

A total of 100 Sherman® traps measuring 8x9x23 cm (H.B. Sherman Traps Inc., Tallahassee, USA) were used to capture live rodents in grasslands, fallow lands, farmlands, forests, and wetlands. Twelve improvised wire traps measuring 12×15×20, were used in indoors. The Sherman traps were baited with peanut butter mixed with maize bran, while the improvised wire traps were baited with the same, plus tomatoes and dried sardines. The traps were set in the evening, and the bait was changed after each trap check, that is every morning and late in the afternoon for three nights per habitat. This is because most rodents are nocturnal and few are diurnal, as well as to alleviate the heat stress that the caught small mammals would be subjected to (Mulungu *et al*., 2008; Magige, 2016).

2.3 Handling and processing of captured rodents and shrews

The animals were first removed from the traps with a cotton bag, then euthanized in a container with cotton wool soaked with diethyl ether. Weight, tail, ear, head and body, and hind foot lengths were measured in millimeters (mm) and recorded for species identification, as stated by Happold *et al*., (2013). Soon after, one to two milliliters of whole blood were drawn by cardiac puncture and placed in micro vials. For serum separation, the blood was allowed to coagulate and then centrifuged for 10 min at 4000 RCF to obtain sera. The sera were collected, placed in micro vials, and then kept in freezers at the Kakonko Veterinary Office and Kibondo District Hospital at -20°C until the completion of the field session. The frozen sera were then sent to Sokoine University of Agriculture, Institute of Pest Management (IPM) Laboratory under cold conditions and stored at -20°C before serology tests.

2.4 Detection of leptospiral antibodies

For this purpose, the microscopic agglutination test (MAT) was used to demonstrate antibodies in rodent sera to five individual antigens (serovars) consisting of the *L. interrogans* serovar Lora, *L. interrogans* serovar Grippotyphosa, and *L. kirschneri* serovar Sokoine, as well as the reference serovars *L. interrogans* serovar Hebdomadis and *L. interrogans* serovar Pomona. The antigens were grown for 5 to 8 days in Ellinghausen - McCullough, modified by Johnson and Harris (EMJH) culture medium, with growth density and purity being checked regularly using a dark field microscope. The antigens utilized for screening had a density of $3x10^{\wedge 8}$ leptospires/ml. Using U-bottom microtiter plates, volumes of 10 µl of the sera were combined with 90 µl of phosphate-buffered saline (PBS) to create 100 µl (1:10 dilutions). Then 50 µl of the sera-PBS combination was pipetted into succeeding wells with 50 µl of PBS to create serial serum dilutions with a final 50µl being discarded. The diluted sera were mixed with 50 µl of the live leptospira antigens, gently mixed, and then incubated at 30°C for two hours. The serum-antigen mixture was then examined on a dark-field microscope for the presence of agglutination and the titers were determined. The highest dilution at which approximately 50% of the leptospires remained agglutinated in comparison to the control was the MAT end-point titer (Hartskeerl et al., 2001; Mgode et al., 2014). A serum sample was deemed positive if it had 50% agglutination and an end point titer equal to or higher than 160 (MAT titer 1:160) for a serovar.

2.5 DATA ANALYSIS

Data were entered into Microsoft Excel 2016 for coding and cleaning and then transferred to SPSS version 25 (IBM Corporation, Armonk, NY, USA) for analysis. Proportions were used to present categorical data such as sex, district, habitat, and species. A Chi-square test was used to show associations between leptospira seropositivity and independent variables. A p-value of <0.05 was considered statistically significant.

3.0 RESULTS

The study involved a total of 582 rodents and shrews from Kibondo (n=273) and Kakonko (n=309) districts, in six different habitats, namely fallow land, farmland, wetland, forest, grassland, and indoors. Of the captured rodents and shrews, 46.7% and 53.3% were males and females respectively. Eleven different species of rodents and one shrew species (C*rocidura tansaniana*) were captured. The most common rodent species, *Mastomys natalensis*, comprised 49.1% of all captures, while *Tatera indica* and *Praomys delectorum* contributed the least (0.7%) of all captures. The abundance and composition varied among habitats, with fallow land accounting for the majority of captures 305(52.4%) and forest for the minority 12(2.1%). Using a cut-off titer of 1:160, a total of 69 of the 582 sera were positive giving a prevalence of 11.9% (95% CI 9.4%–14.7%) whilst 88.1% of the total captures tested negative. Of the seropositive, rodents contributed 11.6% (95% CI 9.1%-14.3%), while shrews contributed 0.3% (95% CI 0.1%-1.1%). In contrast, rodents made up 87.4% of the negatives, while shrews made up 0.7%. Although there was a difference in the seroprevalence rates between the rodents and the shrews, the difference was not statistically significant (P = 0.102). Seven of the eleven rodent species, (*Aethomys kaiseri, Arvicanthis niloticus, Lemniscomys rosalia, Lemniscomys striatus, Lophuromys flavopunctatus, Mastomys natalensis,* and *Rattus rattus*) tested positive with *Mastomys natalensis* having the highest prevalence (43.5%) and *Arvicanthis niloticus* having the lowest prevalence (1.4%). There were statistically significant variations in the seropositivity among the species (P=0.004). The apparent seroprevalence in each district was 8.8% in Kibondo and 3.1% in Kakonko and the difference was statistically significant (P= 0.001). There was a slight variation in the prevalence of leptospira antibodies between males and females as they had prevalence rates of 5.5% and 6.4% respectively, however, the difference was not statistically significant. The level of seropositivity by habitat varied from 6.2% in fallow land to 0.2% in the wetlands (Table 1).

Table 1: *Rodents and shrew species composition, the proportion of MAT results, and seroprevalence of Leptospira, Kigoma region (n=582)*

Variable	Category	N=582(%)	MAT Positive within n=69 (%)	MAT Positive overall n=582(%)	MAT Negative within N=513(%)	MAT Negative overall n=582(%)	Overall Prevalence (95% CI)
District	Kibondo	273(46.9)	51(73.9)	51(8.8)	222(43.3)	222(38.1)	8.8(6.7-11.3)
	Kakonko	309(53.1)	18(26.1)	18(3.1)	291(56.7)	291(50.0)	3.1(1.9-4.7)
Sex	Male	272(46.7)	32(46.4)	32(5.5)	240(46.8)	240(41.2)	5.5(3.9-7.6)
	Female	310(53.3)	37(53.6)	37(6.4)	273(53.2)	273(46.9)	6.4(4.6-8.6)
Species							
Rodents	*Aethomys kaiseri*	56(9.6)	16(23.2)	16(2.7)	40(7.8)	40(6.9)	2.7(1.6-4.3)
	Arvicanthis niloticus	15(2.6)	1(1.4)	1(0.2)	14(2.7)	14(2.4)	0.2(0.0-0.8)

Variable	Category	N=582(%)	MAT Positive within n=69 (%)	MAT Positive overall n=582(%)	MAT Negative within N=513(%)	MAT Negative overall n=582(%)	Overall Prevalence (95% CI)
	Grammomys dolichurus	5(0.9)	0(0.00)	0(0.00)	5(1.0)	5(0.9)	0.00
	Lemniscomys rosalia	13(2.2)	2(2.9)	2(0.3)	11(2.1)	11(1.9)	0.3(0.1-1.1)
	Lemniscomys striatus	69(11.9)	10(14.5)	10(1.7)	59(11.5)	59(10.1)	1.7(0.9-3.0)
	Lophuromys flavopunctatus	9(1.5)	2(2.9)	2(0.3)	7(1.4)	7(1.2)	0.3(0.1-1.1)
	Mastomys natalensis	286(49.1)	30(43.5)	30(5.2)	256(49.9)	256(44.0)	5.2(3.6-7.2)
	Mus musculus	23(4.0)	0(0.00)	0(0.00)	23(4.5)	23(4.0)	0.00
	Praomys delectorum	4(0.7)	0(0.00)	0(0.00)	4(0.8)	4(0.7)	0.00
	Rattus rattus	92(15.8)	6(8.7)	6(1.0)	86(16.8)	86(14.8)	1.0(0.4-2.1)
	Tatera indica	4(0.7)	0(0.00)	0(0.00)	4(0.8)	4(0.7)	0.00

Variable	Category	N=582(%)	MAT Positive within n=69 (%)	MAT Positive overall n=582(%)	MAT Negative within N=513(%)	MAT Negative overall n=582(%)	Overall Prevalence (95% CI)
Shrews	*Crocidura tansaniana*	6(1.0)	2(2.9)	2(0.3)	4(0.8)	4(0.7)	0.3(0.1-1.1)
Habitat	Grassland	20(3.4)	4(5.8)	4(0.7)	16(3.1)	16(2.7)	0.7(0.2-1.6)
	Fallow land	305(52.4)	36(52.2)	36(6.2)	269(52.4)	269(46.2)	6.2(4.4-8.4)
	Farmland	124(21.3)	16(23.2)	16(2.7)	108(21.1)	108(18.6)	2.7(1.6-4.3)
	Forest	12(2.1)	1(1.4)	1(0.2)	11(2.1)	11(1.9)	0.2(0.0-0.8)
	Indoor	105(18.0)	11(15.9)	11(1.9)	94(18.3)	94(16.2)	1.9(1.0-3.2)
	Wetland	16(2.8)	1(1.4)	1(0.2)	15(2.9)	15(2.6)	0.2(0.0-0.8)

Table 2 shows the proportions of Leptospira serovars among 69 seropositive sera that were determined by MAT. The most common serovar among the five was Lora (3.4%), followed by Sokoine (3.1%) and Hebdomadis (0.3%). *Lemniscomys rosalia* and *Crocidura tansaniana* only reacted to serovars Lora and Sokoine. Only the *Mastomys natalensis* reacted to all serovars, while *Lemniscomys striatus* and *Aethomys kaizeri* reacted to all serovars, except Grippotyphosa and Hebdomadis. *Rattus rattus* was positive to all except serovars Hebdomadis and Pomona while *Arvicanthis niloticus* reacted to serovar Lora only (Fig. 2). The findings also revealed that four rodent sera had serological cross-reactions with two serogroups, specifically Lora and Pomona and Sokoine and Hebdomadis. Table 3 shows the frequency of MAT antibody titer of rodents and shrew sera by serovar in Kibondo and Kakonko districts. A total of nine sera in all had titers of 1:160, indicating active Leptospira infection, whereas the other positive sera had lower antibody levels (1:20 to 1:80), which are below the cut-off threshold of 1:160 as stipulated by Hartskeerl et al., (2001).

Table 2: *Seroprevalence of Leptospira serovars by MAT (titer 1:20-1:160) among positive rodents and shrew's sera in Kibondo and Kakonko districts*

Serovar tested	**Total animals tested (N)**	**Leptospirosis positive (n)**	**Serovar prevalence (%)**
Lora (local)	582	20(3)	3.4
Sokoine (local)	582	18(1)	3.1
Hebdomadis (reference)	582	2	0.3
Pomona (reference)	582	17	2.9
Grippotyphosa (local)	582	12	2.1

* Number of specimens in brackets reacted to more than one *Leptospira* serovar (cross-reactions) hence are not included in overall prevalence (69 positive animals out of 582)

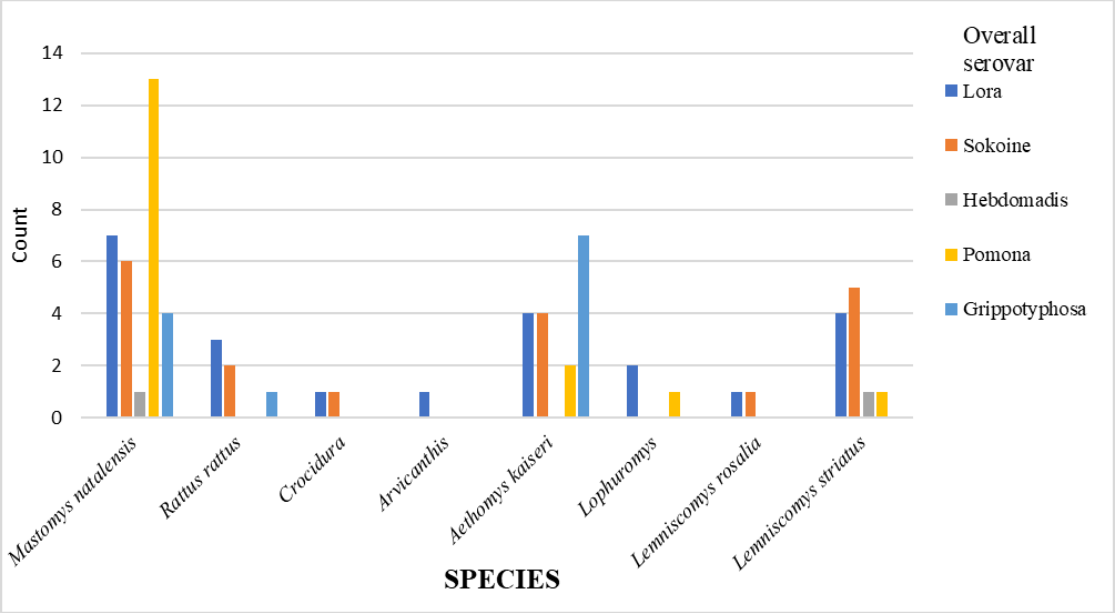

Figure 2: *Distribution of* ***Leptospira*** *serovars in rodents and shrews in Kibondo and Kakonko districts*

Table 3: *Frequency of MAT antibody titers of rodents and shrew sera by serovar in Kibondo and Kakonko districts*

	Overall titer				
Leptospira serovar	**1:20**	**1:40**	**1:80**	**1:160**	**Total (N=73)**
Lora	1	11	9	2	23(31.5)
Sokoine	1	11	5	2	19(26.0)
Hebdomadis	1	0	1	0	2(2.7)
Pomona	2	7	5	3	17(23.3)
Grippotyphosa	0	3	7	2	12(16.4)
Total % (N=73)	5(6.8%)	32(43.8%)	27(37.0%)	9(12.3%)	

4.0 DISCUSSION

This study documents the seroprevalence of leptospira antibodies in rodents and shrews in Kibondo and Kakonko districts in Kigoma region, Tanzania. To our knowledge, this is the first research that details *Leptospira* spp. seropositivity in rodents and shrews in these study areas.

The results of the study are significant for public health because leptospirosis is a neglected zoonotic disease and is more likely to spread among people who live close

to small mammals. In Tanzania, few leptospirosis studies have been conducted in some districts where human disease outbreak has occurred (Chopra et al., 2022).

The current study identified 11 different species of rodents and one shrew species of which *M. natalensis* was the most frequently captured; a finding consistent with that of Habtamu et al. (2008), who also noted high capture rates of these species. *M. natalensis* is the most prevalent and dominant rodent pest species in most of Sub-Saharan Africa, (Mulungu et al., 2013), due to its high prolificacy, adaptability to different environments, generalist feeding habits, and ability to coexist with different kinds of rodent species (Habtamu et al., 2008; Mulungu et al., 2013).

The results also showed that the total catches varied depending on habitat. According to Datiko et al., (2013), the complexity of the environment to food supply and cover affects the overall distribution of rodents in an area. Makundi et al., (2005), found a correlation between the composition of small mammals and the resources present in their environment. The study also identified a gender gap, which is attributed to the fact that males are more mobile than females because of their mating behavior (Borremans et al., 2014).

This study has reported a seroprevalence of 11.9% which is lower than the 25.8% reported by Mgode et al., (2014), in rodents and shrews in cultivated and fallow lands. The lower prevalence rates could be attributed to the smaller sample size and the species captured as *Leptospira* spp. are host-specific (Cordonin et al., 2020) and these findings collectively suggest that there is a significant risk of human exposure to pathogenic Leptospira spp. in Kibondo and Kakonko districts. As compared to Kakonko, Kibondo showed a higher prevalence in our study. This variation in seroprevalence might be explained by variations in the meteorological conditions like temperature and humidity of the studied locations which influence Leptospira carriage in rodents (Perez et al., 2011). Contrary to a study by Cosson et al., (2014), this study showed that female rodents were more susceptible to infection than males. The results of a research by Mosallanejad et al., (2013) in Ahvaz, Iran, found that leptospira was more prevalent in males than females, different from what was observed in this study.

Additionally, susceptibility to leptospiral infection varied across species. *Leptospira* spp. were found to affect most species and our statistical analysis showed that species was a determinant of infection. The observed variance in rodent species prevalence is likely a collateral effect of their unique habitat preferences (Cosson et al., 2014). For instance, the presence of infection in the *Crocidura* species may be because of their frequent visits to moist and damp areas, which are conducive to the survival of the bacterium outside the host (Desvars et al., 2011). Rarely studied species including *Lemniscomys* spp., *Aethomys kaiseri*, and *Lophuromys* spp. showed prevalence of leptospira infection as well. This finding shows that the

diversity of rodent species serving as leptospirosis reservoirs may be greater than previously assumed (Thaipadungpanit et al., 2007).

In most captured species, the local serovars Sokoine and Lora were the most common, indicating that they are the prominent circulating serovars in the research area. This study demonstrates that several leptospiral serovars are found in Tanzania in different rodent species and *Crocidura tansaniana*. Some rodent species likely harbor more leptospires than others, for instance, the *M. natalensis,* yielded more leptospiral serovars. This finding, however, conflicts with results from a study conducted by Mgode et al., (2015). The disparities in the prevalence of *Leptospira* spp. between the small mammals could be explained in part by the differences in population densities of the species. Another potential contributing factor could be differences in their ecosystems (Boey et al., 2019). For example, the *M. natalensis* is a peri-domestic rodent that is found in both wild and domestic environments (Halliday et al., 2013). These environments might increase the likelihood to contract leptospires and thus account for the high rates of leptospira antibodies in these species. Contrarily, despite the widely held notion that moist habitats are the best for leptospira survival, the *Crocidura tansaniana*, which commonly inhabits damp surroundings, showed a lower infection rate. (Trueba et al., 2004).

Although they were not captured in large numbers in the current investigation, the *Arvicanthis niloticus* and the *Lophuromys flavopunctatus* were found to be carriers of leptospires. In a study conducted in Niamey, Niger by Dobigny et al. (2015), similar results were reported. This strongly shows that *Leptospira* spp. also circulates in species other than the widespread species like *M. natalensis*, necessitating further research on these species.

The current findings showed that four of the positive *R. rattus* were captured indoors which served as a key host reservoir for leptospires, thus posing a risk of Leptospira transmission to humans. This is supported by findings from a prospective study conducted in Salvador, Brazil, by Felzemburgh et al., (2014), which revealed that residing in areas with high rodent infestation was associated with a high risk of Leptospira transmission. Several studies have documented infections in *R. rattus* and *M. musculus* (Foronda et al., 2011); however, in the current study, *M. musculus* was not confirmed to be a carrier of the leptospires. This finding is different from that reported in Kenya by Halliday et al., (2013), but is comparable to the one in Tanzania by Mgode et al., 2015. The absence of Leptospira in *M. musculus* could be explained by the lower proportion of captures. Furthermore, no Leptospira was found in *Tatera* species which is similar to the findings reported in a study by Mgode et al., (2015).

Although it was not statistically significant, the study also showed a difference in serovar prevalence, with local serovar prevalence being greater than reference

serovar prevalence (Table 2). This is similar to findings from a study carried out in western Kenya by Ngugi et al., (2019). Moreover, it was discovered that serovars Pomona, Hebdomadis, and Grippotyphosa were not detected in shrews (*Crocidura* spp.). The absence of these serovars in the *Crocidura* spp. was also reported in a study conducted in Morogoro municipal, Tanzania, by Mgode et al. (2015).

Four of the tested sera showed multiple serovar reactions, which was another finding of the current investigation. Similar findings were drawn in research by Mgode et al., (2014) on rodents and shrews from Morogoro, Tanzania. This could be explained by the interactions among various serovars (Adler et al., 2010), which means that an animal infected with one serovar is likely to have antibodies against the serovar that caused the infection that cross-react with other serovars, usually at a lesser level (Olivera et al.,2018). In most cases, the serovar that reacts with sera is assumed to be the infecting serovar, however, when serovars cross-react, the serovar that yields the highest antibody titer is assumed to be the infecting serovar (Chirathaworn et al., 2014). The likelihood that the rodents had multiple serovar infections may potentially be the source of the various serovar reactions (Adler et al., 2010). In this instance, the newly acquired serovar may have interacted with the previously infective serovar (Evangelista et al., 2010). As a result, the memory reaction against past serovars is triggered. If so, the antibody titer specific to the previous serovar may be higher than the antibody titer against the newly infecting serovar (Adler et al., 2010; Chirathaworn et al., 2014).

In our study, Leptospira serovar positivity was associated with low antibody titers; the majority of sera had titers between 1:20 and 1:80, which is below the threshold of 1:160 used in Tanzania (Mgode et al., 2015). In a study conducted in Tanzania by Mgode et al. (2015), the majority of seropositive rodents also showed lower titers against the tested serovars, which is comparable to the findings in this study. The lower antibody titers (1:20 -1:80) in our present study indicated persistent leptospira infections (Mgode et al., 2014) and on the other hand, titers (1:160) indicated recent infections (Chirathaworn et al., 2014). The cut-off titer employed in our investigation was comparable to that used in a study by Villanueva et al., (2010), in the Philippines. Although a lower cut-off yields higher prevalence, it enables both recent and chronic infections, which are often characterized by lower antibody titers, to be recorded as positive, a strategy used frequently in prevalence studies (Kessy et al., 2010).

5.0 CONCLUSION

This study reports an overall prevalence (11.9%) of *Leptospira* spp. in rodents and shrews captured in two distinct districts. Both a link between prevalence and rodent species and a correlation between prevalence and district were found. Since there is

an existing rodent and human interaction in the studied districts, the presence of *Leptospira* spp. in the rodents and shrews raises concerns for public health. Leptospirosis, for example, is typically underdiagnosed, thus there is a need to raise public awareness of these rodent-borne illnesses. To promote public health, more efforts should be made to guarantee that leptospirosis is routinely screened.

Ethical statement: Ethical approval was obtained from the Directorate of Research, Technology Transfer, and Consultancy Review Board of the Sokoine University of Agriculture with reference and publication committee reference number (SUA/DPRTC/R/186/16) on 06/01/2022. The study was also approved by the Regional Administrative Authorities of the Kigoma region reference number (DA.73/274/02K/326) on 04/02/2022.

Acknowledgment: This work was sponsored by the African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE IRPM and BTD) at the Institute of Pest Management of the Sokoine University of Agriculture.

REFERENCES

Adler, B. & de la Peña Moctezuma, A. 2010. Leptospira and leptospirosis. *Veterinary Microbiology* 140(3-4):287-296.

Allan, K. J., Halliday, J. E., Moseley, M., Carter, R. W., Ahmed, A., Goris, M. G. ... & Cleaveland, S. 2018. Assessment of animal hosts of pathogenic Leptospira in northern Tanzania. *PLoS Neglected Tropical Diseases* 12(6): e0006444.

Boey, K., Shiokawa, K. & Rajeev, S. 2019. Leptospira infection in rats: A literature review of global prevalence and distribution. *PLoS Neglected Tropical Diseases* 13(8):e0007499.

Borremans, B., Hughes, N. K., Reijniers, J., Sluydts, V., Katakweba, A. A., Mulungu, L. S., ... & Leirs, H. 2014. Happily together forever: temporal variation in spatial patterns and complete lack of territoriality in a promiscuous rodent. *Population Ecology* 56:109-118.

Chirathaworn, C., Inwattana, R., Poovorawan, Y. & Suwancharoen, D. 2014. Interpretation of microscopic agglutination test for leptospirosis diagnosis and seroprevalence. *Asian Pacific Journal of Tropical Biomedicine* 4: S162-S164.

Chopra, H., Bibi, S., Aggarwal, N., Singh, I., Jose, J. & Emran, T. B. 2022. Leptospirosis: Recent outbreak and control measures. *International Journal of Surgery* 106:106907. doi: 10.1016/j.ijsu.2022.106907

Cordonin, C., Turpin, M., Bringart, M., Bascands, J. L., Flores, O., Dellagi, K. ... & Tortosa, P. 202).0Pathogenic Leptospira and their animal reservoirs: testing host specificity through experimental infection. *Scientific Reports* 10(1):1-8.

Cosson, J. F., Picardeau, M., Mielcarek, M., Tatard, C., Chaval, Y., Suputtamongkol, Y.,... & Morand, S. 2014. Epidemiology of Leptospira transmitted by rodents in Southeast Asia. *PLoS Neglected Tropical* Diseases 8(6):e2902.

Costa, F., Zeppelini, C. G., Ribeiro, G. S., Santos, N., Reis, R. B., Martins, R. D., ... & Ko, A. I. 2021. Household rat infestation in urban slum populations: Development and validation of a predictive score for leptospirosis. *PLoS Neglected Tropical Diseases* 15(3): e0009154.

Cutler, S. J., Fooks, A. R., & Van Der Poel, W. H. (2010). Public health threat of new, reemerging, and neglected zoonoses in the industrialized world. *Emerging Infectious Diseases* 16(1):1.

Datiko, D. & Bekele, A. 2013. Species composition and abundance of small mammals in Chebera-Churchura National Park, Ethiopia. *Journal of Ecology and the Natural Environment* 5(6):95-102.

De Brito, T., Silva, A. M. G. D. & Abreu, P. A. E. 2018. Pathology and pathogenesis of human leptospirosis: a commented review. *Revista do Instituto de Medicina Tropical de São Paulo*, *60*.

Desvars, A., Jégo, S., Chiroleu, F., Bourhy, P., Cardinale, E. & Michault, A. 2011. Seasonality of human leptospirosis in Reunion Island (Indian Ocean) and its association with meteorological data. *PloS One* 6(5):e20377.

Dobigny, G., Garba, M., Tatard, C., Loiseau, A., Galan, M., Kadaoure, I., ... & Bertherat, E. 2015. Urban market gardening and rodent-borne pathogenic Leptospira in arid zones: a case study in Niamey, Niger. *PLoS Neglected Tropical Diseases 9*(10):e0004097.

Evangelista, K. V., & Coburn, J. 2010. Leptospira as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiology* 5(9):1413-1425.

Felzemburgh, R. D., Ribeiro, G. S., Costa, F., Reis, R. B., Hagan, J. E., Melendez, A. X., ... & Ko, A. I. 2014. Prospective study of leptospirosis transmission in an urban slum community: role of poor environment in repeated exposures to the Leptospira agent. *PLoS Neglected Tropical Diseases 8(5):e2927.*

Foronda, P., Martin-Alonso, A., del Castillo-Figueruelo, B., Feliu, C., Gil, H., & Valladares, B. 2011. *Pathogenic Leptospira spp. in wild rodents*, Canary Islands, Spain.

Goarant, C. 2016. Leptospirosis: risk factors and management challenges in developing countries. *Research and Reports in Tropical Medicine* 7: 49-62. doi: 10.2147/RRTM.S102543

Goris, M. G., Leeflang, M. M., Loden, M., Wagenaar, J. F., Klatser, P. R., Hartskeerl, R. A. & Boer, K. R. 2013. Prospective evaluation of three rapid diagnostic tests for diagnosis of human leptospirosis. *PLoS Neglected Tropical Diseases* 7(7):e2290. Doi.https://doi.org/10.1371/journal.pntd.0002290

Habtamu, T. & Bekele, A. 2008. Habitat association of insectivores and rodents of Alatish National Park, northwestern Ethiopia. *Tropical Ecology* 49(1):1.

Halliday, J. E., Knobel, D. L., Allan, K. J., Bronsvoort, B. M. D. C., Handel, I., Agwanda, B., ... & Breiman, R. F. (2013). Urban leptospirosis in Africa: a cross-sectional survey of Leptospira infection in rodents in the Kibera urban settlement, Nairobi, Kenya. *The American Journal of Tropical Medicine and Hygiene* 89(6): 1095–1102.doi: 10.4269/ajtmh.13-0415.

Happold, M. & Happold, D. C. D. (Eds.). 2013. *Mammals of Africa* (Vol. 3). London: Bloomsbury.

Hartskeerl, R. A., Smits, H. L., Korver, H., Goris, M. G. A., Terpstra, W. J. & Fernández, C. 2001. International course on laboratory methods for the diagnosis of leptospirosis. *Netherlands: Royal Tropical Institute Department of Biomedical Research*.

Holt, J., Davis, S. & Leirs, H. 2006. A model of leptospirosis infection in an African rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. *Acta Tropica* 99(2-3):218-225.

Kessy, M. J., Machang'u, R. S. & Swai, E. S. 2010. A microbiological and serological study of leptospirosis among pigs in the Morogoro municipality, Tanzania. *Tropical Animal Health and Production 42:*523-530.

Magige, F. 2016. Variation of small mammal populations across different habitat types in the Serengeti ecosystem. *Tanzania Journal of Science* 42(1):15-22.

Makundi, R. H., Massawe, A. W. & Mulungu, L. 2005. Rodent population fluctuations in three ecologically heterogeneous locations in northeast, central and south-west Tanzania.

Mgode, G. F., Katakweba, A. S., Mhamphi, G. G., Fwalo, F., Bahari, M., Mdangi, M., ... & Mulungu, L. S. 2014. Prevalence of leptospirosis and toxoplasmosis: A study of rodents and shrews in cultivated and fallow land,

Morogoro rural district, Tanzania. *Tanzania Journal of Health Research* 16(3).

Mgode, G. F., Machang'u, R. S., Mhamphi, G. G., Katakweba, A., Mulungu, L. S., Durnez, L., ... & Belmain, S. R. 2015. Leptospira serovars for diagnosis of leptospirosis in humans and animals in Africa: common Leptospira isolates and reservoir hosts. *PLoS neglected Tropical Diseases 9*(12):e0004251.

Mosallanejad, B., Najafabadi, M. G., Avizeh, R., & Abdollahpour, G. 2013. A serological survey on leptospiral infection among wild rats (Rattus rattus) of Ahvaz district, southwest of Iran: a preliminary study. *Jundishapur Journal of Microbiology* 6(10).

Mulungu, L. S., Makundi, R. H., Massawe, A. W., Machang'u, R. S. & Mbije, N. E. 2008. Diversity and distribution of rodent and shrew species associated with variations in altitude on Mount Kilimanjaro, Tanzania.

Mulungu, L. S., Ngowo, V., Mdangi, M., Katakweba, A. S., Tesha, P., Mrosso, F. P., ... & Kilonzo, B. S. (2013). Population dynamics and breeding patterns of multimammate mouse, Mastomys natalensis (Smith 1834), in irrigated rice fields in eastern Tanzania. *Pest Management Science* 69(3):371-377.

National Bureau of Statistics 2013. Population and housing census report. [https://www.nbs.go.tz] site visited on 31/03/23

Ngugi, J. N., Fèvre, E. M., Mgode, G. F., Obonyo, M., Mhamphi, G. G., Otieno, C. A., & Cook, E. A. J. 2019. Seroprevalence and associated risk factors of leptospirosis in slaughter pigs; a neglected public health risk, western Kenya. *BMC Veterinary Research* 15(1):1-11.

Olivera, M., Chaparro, J. J., Chaparro, Y., Piedrahita, D., Fernández-Silva, J., Londoño, J., ... & Villar, D. 2018. Cross-sectional study of 13 Leptospira serovars in cows in a Colombian dairy region. *Revista Colombiana de Ciencias Pecuarias 31*(1):10-16.

Perez, J., Brescia, F., Becam, J., Mauron, C. & Goarant, C. 2011. Rodent abundance dynamics and leptospirosis carriage in an area of hyper-endemicity in New Caledonia. *PLoS Neglected Tropical Diseases 5*(10):e1361.

Plank, R. & Dean, D. 2000. Overview of the epidemiology, microbiology, and pathogenesis of Leptospira spp. in humans. *Microbes and Infection* 2(10):1265-1276.

Tanzania Meteorological Authority. 2021. [https://www.meteo.go.tz/] site visited on 18/04/23

Thaipadungpanit, J., Wuthiekanun, V., Chierakul, W., Smythe, L. D., Petkanchanapong, W., Limpaiboon, R. ... & Peacock, S. J. 2007. A dominant clone of Leptospira interrogans associated with an outbreak of human leptospirosis in Thailand. *PLoS Neglected Tropical Diseases* 1(1): e56.

Torres-Castro, M. A., Gutiérrez-Ruiz, E., Hernández-Betancourt, S., Peláez-Sánchez, R., Agudelo-Flórez, P., Guillermo-Cordero, L. & Puerto, F. I. 2014. First molecular evidence of Leptospira spp. in synanthropic rodents captured in Yucatan, Mexico. *Revue Méd Vét* 165(7-8):213-218.

Trueba, G., Zapata, S., Madrid, K., Cullen, P., & Haake, D. 2004. Cell aggregation: a mechanism of pathogenic Leptospira to survive in fresh water. *International Microbiology* 7(1):35-40.

Vadell, M. V., Cavia, R. & Suarez, O. V. 2010. Abundance, age structure and reproductive patterns of Rattus norvegicus and Mus musculus in two areas of the city of Buenos Aires. *International Journal of Pest Management* 56(4):327-336.

Villanueva, S. Y., Ezoe, H., Baterna, R. A., Yanagihara, Y., Muto, M., Koizumi, N., ... & Yoshida, S. I. 2010. Serologic and molecular studies of Leptospira and leptospirosis among rats in the Philippines. *The American journal of Tropical Medicine and Hygiene* 82(5):889.

World Health Organization. 2011. Report of the second meeting of the leptospirosis burden epidemiology reference group.