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Original Article

Seropositivity of Leptospira in rodents, shrews, and domestic animals in Unguja, Tanzania

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Abstract

Background: Leptospirosis is one of the most commonly neglected zoonoses in developing nations including Tanzania. This study aims to find out the seroprevalence of leptospirosis in rodents, shrews, and domestic animals in different regions in Unguja Island, Tanzania.

Methods: A cross-sectional study was carried out from January to April 2022. The blood samples were collected from rodents and shrews (n=248), cattle (n=247), goats (n=130), sheep (n=32), and dogs (n=80). The blood samples were allowed to clot in a slanted position and serum samples were harvested. A microscopic agglutination test (MAT) was performed on the sera to check for leptospiral antibodies using five Leptospira serovars as antigens (Sokoine, Lora, Pomona, Grippotyphosa and Hebdomadis).

Results: The overall seropositivity of leptospiral antibodies was 9.68% in rodents and shrews, 14.57% in cattle, 10.01% in goats, 31.25% in sheep, and 26.25% in dogs. The seropositivity of Leptospira varied significantly with animal species (OR=1.9, 95 % CI:1.1-3.3, p=0.03). The most frequently detected serovar was Sokoine (27.89%), followed by Pomona (19.47%), Lora (18.26%), Grippotyphosa (17.98%), and Hebdomadis (8.16%), respectively.

Conclusion: Our study suggests that further research should be conducted to find out factors of high seropositivity of leptospiral in Unguja. Vaccination of domestic animals with vaccines against local Leptospira strains should be encouraged, and rodent control and public awareness should be emphasized.

Keywords: Leptospirosis, Animals, Microscopic Agglutination Test (MAT), Unguja, Tanzania

Background

Leptospirosis is a zoonotic infectious disease caused by a spirochete of the genus Leptospira [1]. Rodents are the major reservoir host of Leptospira worldwide [1]. So, humans and other animals can become ill upon contact with contaminated water or soil with urine or other materials from infected animals Leptospirosis is a public health concern, especially in tropical and subtropical countries where the environment is ideal for the survival of pathogenic Leptospira [2]. It is a life-threatening disease that causes 1.03 million severe cases report and 60, 000 death each year globally [3]. In Africa, leptospirosis poses a huge disease burden to society as it impacts livestock productivity and human health [4]. In Tanzania, the annual incidence of human leptospirosis is estimated to be 75 to 102 cases per 100,000 population [5]. Tanzania is the second-largest livestock producer in Africa, after Ethiopia, with 87.7 million chickens, 3.2 million pigs, 3.2 million goats, 8.5 million sheep, and 33.9 million cattle. Zanzibar has 270 998 cattle, 111 623 goats, 934 sheep, 2209 pigs, and 3.8 million chickens [6]. Furthermore, it is estimated that Zanzibar has 8095 dogs, found in Kaskazini 'A' (1810), Kaskazini 'B' (476), Kusini (1080), Kati (1865), Magharibi 'A' (229), Magharibi 'B' (341), Micheweni (346), Wete (736), Chakechake (260) and Mkoani (952) [7]. Findings from previous studies from Tanzania have shown that leptospiral infection is very common in domesticated and wild animals, rats, shrews, and people in several regions of the country [2,8–11]. Moreover, serovars circulating in Tanzania reported in rodents, shrews, and domestic animals were identified as Sokoine, Lora, Kenya, Grippotyphosa, Hebdomadis, Pomona, and Canicola [2]. In Zanzibar, studies on the seroprevalence of leptospirosis have been reported to be 7.7% in humans [12] and less than 1.0% in patients at Mnazi Mmoja Hospital [13]. Thus, the disease is underreported or goes unnoticed. So, this is the first study conducted on the island, to find out the prevalence of leptospiral

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infection in rodents, shrews, ruminant animals, and dogs. It is necessary to understand the prevalence of circulating Leptospira species in these hosts to obtain baseline information on the disease for effective zoonotic disease management for public health welfare in Zanzibar, specifically on Unguja Island.

Methods

Study locations

The research was done in Unguja, Zanzibar (Figure 1), an island in the Indian Ocean. It is located between latitudes 04°50' and 06°30'S and longitudes 39°10' and 39°50'E. Unguja and Pemba are Zanzibar's two largest Islands. Unguja has a total area of 1 554 km2, while Pemba has a total area of 990 km^2. The total population of Zanzibar is about 1.9 million [14]. According to [15], Zanzibar's economy is based significantly on agriculture, which generates 31% of the country's GDP. The sampling sites (farms, domestic, peridomestic, forest, and grazing habitats) were selected randomly with assistance from the Department of Livestock Development. Sites were located across the entire island including Kaskazini B, Kati, Kaskazini A, Kusini, Magharibi A, Mjini, and Magharibi B districts to ensure a representative sample population. Moreover, a total of 31 Shehia (ward) and 155 households were purposely selected based on the animal population while domestic animals were selected randomly. The following shehia were selected in each district: Kaskazini A (Kinyasini, Donge Muwanda, Kibokwa, Kikobweni, Kisongoni, and Pwanimchangani), Kaskazini B (Kilombero, Mahonda, Mangapwani, Mkadini, Zingwezingwe, Kiwengwa, and Kitope), Mjini (Maruhubi, Darajani, and Mwemberadu), Magharibi A (Kizimbani, Dole, Kianga, Mwera, Bubwisudi and Mkwajuni), Kati (Dunga, Bambi, Kiboje and Mpapa), and Kusini (Unguja-Ukuu, Pungume, and Kizimkazi) and Magharibi B (Kisakasaka).

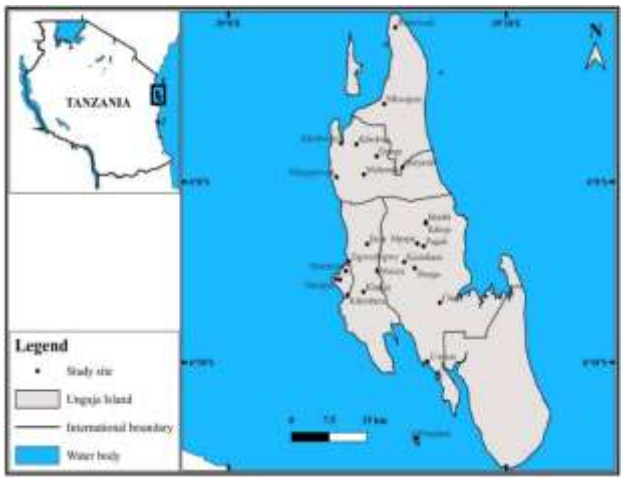

Figure 1: The distribution of seven different districts, shehia, and habitat types in Unguja Island; Sources: QGIS: v.3.24'Tisler'. Coordinate Reference System (CRS): WGS 84 EPSG:4326" retrieved on September 16, 2022

Inclusion and exclusion criteria

The study included live rodents and shrews ranging from adult, sub-adult, and juvenile, and all dead rodents and shrews were not included. While on the other hand, the research included adult and Juvenile livestock older than one year (>1 year). Young animals (<1 year) and animals in 3rd trimester of pregnancy were excluded. Likewise, household owners who were not ready to be involved were excluded. In dogs, the finding included only individuals one or more years of age and excluded aggressive animals and those with poor health conditions.

Data collection and blood sample

This study utilized a cross-sectional design to collect samples and related information from January to April 2022. Sherman live traps (7.5 x 9.0 x 23.0 cm) were used to catch rodents and shrews (n = 248) in domestic, peridomestic, and farm (cultivated, fallow lands), woodland, and grazing areas habitats. A total of one hundred Sherman live traps were set per site in ten lines each with ten trapping stations, positioned ten meters apart in each station and line for four consecutive nights. Traps were daily baited using a mixture of peanut butter and maize brans [16]. The traps were inspected early in the morning (06:00 and 07:00h) and late in the evening (18:00h). The traps were then washed with water to remove any old feces, food, and smell that may discourage other species from entering such as shrews, the bait was replaced to new one after each trap's inspection for four consecutive night per month, because of inactive of rodents at day and active at night time. All captured individuals were shipped in ventilated plastic buckets to the Department of Livestock and Development Laboratory and anesthetized using diethyl ether. Basic descriptive characteristics (gender, age, and species) and morphometric data were recorded [17]. Blood (1 to 2 ml) was aseptically collected from the retro-orbital sinus and through heart puncture for both rodents and shows using sterile syringes and needles. Blood samples from livestock (cattle, sheep, goats, and dogs) were collected by manually restraining the animal and retrieving 4 to 10 ml from the jugular vein while in dogs blood sampling was performed from the cephalic vein. Collected samples were immediately transferred into plain vacutainer tubes and allowed to clot for separation of serum, before completing the serum separation by centrifugation at 4000rpm for 5 minutes. The sera obtained were subsequently transferred into appropriately labeled Eppendorf tubes and stored at-20^{oC} until subjected to serology analysis [18].

Serological detection of leptospiral antibodies

Microscopic agglutination test (MAT) which is the gold standard for serological analysis was used to detect antibodies against Leptospira in rodents and shrews [19]. Five Leptospiral serovars belonging to two pathogenic Leptospira species commonly circulating in our locality namely L. Interrogans (serovar Lora, Pomona, and Hebdomadis) and L. kirschineri (serovars Sokoine and Grippotyphosa) were used in the test. Thereafter, they were divided into five serogroups, including Hebdomadis (serovar Hebdomadis), Pomona (serovar Pomona), Australis (serovar Lora), Grippotyphosa (L. kirschineri serovar Grippotyphosa) and Icterohaemorrhagiae (serovar Sokoine) [20]. Leptospira stock cultures of serovars "Pomona, Sokoine, Hebdomadis, Lora and Grippotyphosa" were purified by subculturing into Ellinghausen–McCullough–Johnson–Harris (EMJH) medium. Pure leptospira cultures were subcultured and incubated for five to seven days at 30^{o}C. The purity of the leptospira serovars was observed by a dark field microscope. The recommended maximum leptospira density for MAT is 3 X 108 cells/ml on the MacFarland scale (Goris et al., 2013). MAT was conducted on a microtitre plate. All wells of a microtiter plate were filled with 50µl of phosphate-buffered saline (PBS), pH 7.2, except the wells of row 2 which contained 90µl of PBS. Ten microlitres of

serum were added to the wells of row 2 (dilution was 1:10). then serially double diluted with PBS to obtain dilution of 1:10, 1:20, 1:40, and 1:80 by pipetting 50 µl from the wells of row 2 to the next rows. Finally, the remaining 50 µl were discarded. Then after, volumes of 50 µl of Leptospira antigen were added to all wells of the microtitre plate for initial screening. The antigen–serum mixtures were examined under a dark field microscope, by taking a drop of antigen PBS mixture to a microscopic slide. Positive samples titer was noted by detecting 50% Leptospira agglutination [19].

Statistical analysis

Data entry, storage, descriptive statistics, and graph creation were all done using Microsoft Office Excel 2007. Epi-Info version 7.2.5.0 Epi-Info version 7.2.5.0 (CDC Atlanta, USA) was used to calculate the seroprevalence of leptospiral antibodies. Categorical data were presented as frequencies and percentages, and numerical variables were reported as means and standard deviations (SD).

Logistic regression analyses (LR) were conducted to compute the correlation been explanatory variable (age, breed, gender, sites, and species) and the presence of the seroprevalence (Leptospiral seropositive), Odds ratios, and a confidence interval of 95% were calculated and Chi-square test ($\chi 2$) was used calculate the statistical significance of the difference between proportions of seroprevalence of antibodies against Leptospira and associations were considered statistically significant at P-values ≤ 0.05.

Results

Rodents and shrews captured

A total of 248 rodents and shrews were sampled from farm, forest, domestic, grazing, and peridomestic settings as shown in Table 1. Out of the rodents and shrews sampled, 133 were males, and 115 females. In urban and peri-urban regions, Rattus rattus was the most frequent species in domestic habitats, R. norvegicus the most frequent in peridomestic habitats, and M. natalensis in farms.

Table 1: Prevalence of leptospiral antibodies in different species of rodents and shrews in Unguja Island, Tanzania.

Type	Species	Number	Male	Female	Habitat	Proportion	Tested	Positive (%)	P-value	Chi-square (χ^2)
Rodents	*Cricetomys gambianus*	10	4	6	Peridomestic/ Domestic	4.03	10	1 (0.40)	0.04	0.54
	Rattus rattus	69	37	32	Domestic/ Grazing	27.82	69	7 (2.82)		
	Mus spp.	39	24	15	Domestic	15.73	39	4 (1.61)		
	Rattus norvegicus	62	32	30	Peridomestic	25	62	6 (2.42)		
	Mastomys natalensis	56	29	27	Farm	22.58	56	5 (2.02)		
Shrews	Crocidura spp	12	7	5	Forest	4.84	12	1 (0.40)		
Total		**248**	**133**	**115**	**5**	**100**	**248**	**24 (9.67)**		

Prevalence of Leptospiral antibodies

The overall prevalence of leptospiral antibodies in cattle, goats, sheep, dogs, rodents, and shrews were 14.57%, 10.01%, 31.25%, 26.25%, and 9.68% respectively. Five leptospiral serovars used to test leptospiral antibodies of the different hosts in this study were; - serovar Sokoine, serovar Lora, serovar Pomona, serovar Grippotyphosa and serovar Hebdomadis. All hosts had positive leptospiral serovar tests. Except for sheep, which were not tested for serovar Hebdomadis. Serovar Sokoine showed the highest seropositivity of Leptospiral antibodies, followed by Pomona, Lora, Grippotyphosa, and Hebdomadis (Table 2).

Titres were highest in cattle followed by rodents and shrews and dogs as indicated in Table 3. However, the highest titre of 1:80 was common. On the other hand, 1:160 titer was not tested in sheep.

It may be considered that the different antibody titre observed may be caused by different immune response among species. Serovar sokoine showed high titers and high frequencies for all the titres shown in Table 4. While 1:80 titer was more abundant compared to other titers, 1:20 titer was not observed in serovar "Pomona, Grippotyphosa and Hebdomadis." (Table 4). A high prevalence of Leptospiral antibodies (4.03%) of rodents and shrews (n=10/248) was observed in Kaskazini A district, followed by Magharibi A district (2.02% or 5/248) and Kaskazini B district (1.61%) as shown in Figure 2. In cattle, sheep, and dogs, the high prevalence was observed in the Kaskazini A district (5.67%), 18.75%, and 10% respectively); while goats' high prevalence was observed in the Kati district (3.85%).

Table 2: Several Leptospira serovars' seroprevalence in tested animal species

Leptospiral serovars	Rodents and shrews	Cattle	Goats	Sheep	Dogs	Total	χ^2	p-value
Sokoine	9(3.63%)	11(4.45%)	3(2.31%)	4(12.5%)	4(5.00%)	31(27.89%)	22.83	0.0004
Lora	7(2.82%)	5(2.02%)	2(1.54%)	3(9.38%)	2(2.50%)	19(18.26%)		
Pomona	5(2.02%)	7(2.83%)	6(4.62%)	2(6.25%)	3(3.75%)	23(19.47%)		
Grippotyphosa	1(0.40%)	6(2.43%)	1(0.77%)	1(3.13%)	9(11.25%)	18(17.98%)		
Hebdomadis	2(0.81%)	7(2.83%)	1(0.77%)	0(0.00%)	3(3.75%)	13(8.16%)		
Total	24(9.68%)	36(14.57%)	13(10.01%)	10(31.25%)	21(26.25%)	104(91.76%)		

Table 3: Different host species' composition and proportion of Leptospiral antibody titers

Host	1:20	1:40	1:80	1:160	Total positive
Rodents and shrew	2(0.80%)	10 (4.04%)	11(4.43%)	1(0.40%)	24(9.68%)
Cattle	3(1.21%)	11(4.45%)	17(6.88%)	10(4.05%)	41(16.60%)
Sheep	1 (3.13%)	6(18.75%)	4(12.5%)	0(0.00%)	11(34.38%)
Goats	3(2.30%)	5(3.85%)	7(5.38%)	2(1.54%)	17(13.08%)
Dogs	2(2.50%)	5(6.25%)	6(7.5%)	11(13.75%)	24(30.00%)
Total	11(9.94%)	37(37.34%)	45(36.69%)	24(19.74%)	117(103.71%)

Table 4: Titres of the tested serovars

Titers	Sokoine	Lora	Pomona	Grippotyphosa	Hebdomadis	Total
1:20	9	2	0	0	0	11
1:40	12	8	9	4	4	37
1:80	13	7	13	7	5	45
1:160	7	3	3	7	4	24
Total	41	20	25	18	13	117

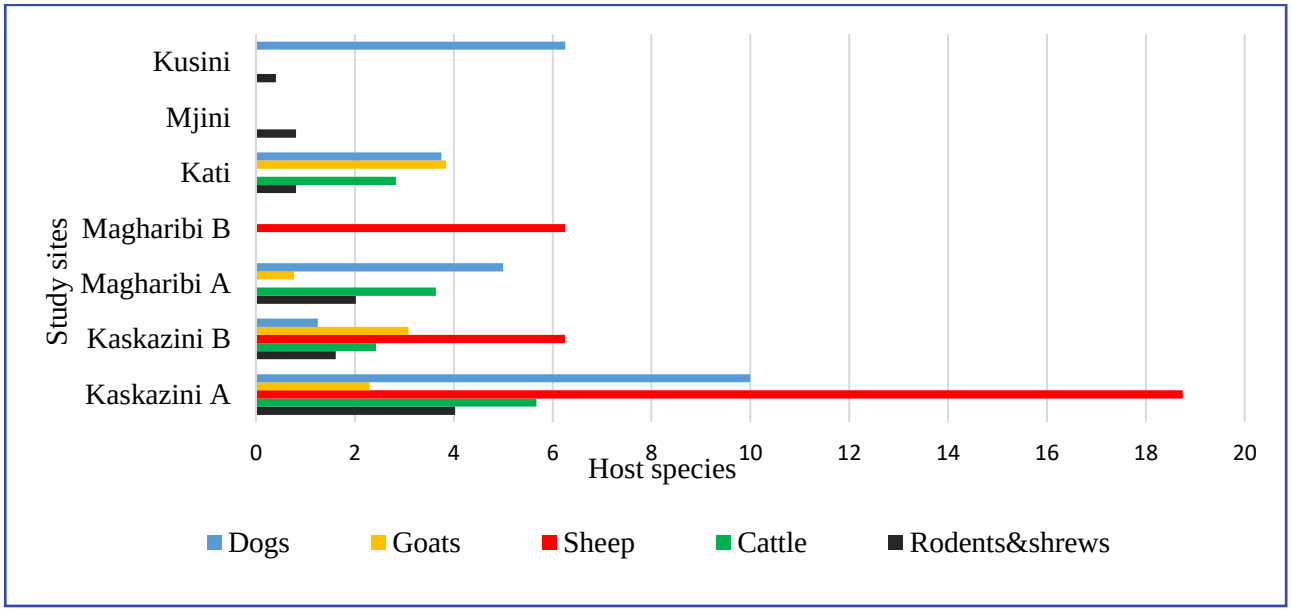

Figure 2: Distribution of Leptospiral antibodies in different Host species in different sites

Comparison of seroprevalence of leptospiral antibodies in different variables

Seroprevalence of leptospiral antibodies (dependent variable) among dogs, cattle, sheep, goats, rodents, and shrews in different predictor variables such as age, sex, breed, species, and serovars was compared to determine whether certain groups were at great risk of contracting disease than others by using logistic regression. The study demonstrated that adult age was 0.7 times more likely to be infected with the disease than juvenile (OR=0.7,95% CI:0.5-1.2, 0.27). The seroprevalence of leptospiral antibody in male animals was 0.9 times higher than in females, which was not statistically significant (OR=0.9,95% CI:0.6-1.3, p=0.45). In domestic animal breeds, local breeds were 0.8 times more likely to be infected with leptospirosis compared to improved breeds (OR=0.8,95% CI:0.5-1.4, p=0.54). All animals were 0.6 times more likely to be infected by serovar Sokoine compared to other serovars, which was not statistically significant (OR=0.6,95% CI:0.3-1.4, p=0.25). In contrast, animal species revealed a significantly higher likelihood to be infected with the disease (OR=1.9,95% CI:1.1-1.3, p=0.03), although, sheep was 1.9 times significantly more likely to be infected with leptospirosis compared to other animals (Table 5). In addition, the results showed that samples from three sheep, five cattle's and one dog reacted to more than one leptospiral serotype (co-infection).

Discussion

To the best of our knowledge, this is the first study to document the seropositivity of Leptospira spp. among rodents, shrews, cattle, goats, sheep, and dogs in Unguja, Tanzania. The previous reports in the same settings documented Leptospira seropositivity among hospitalized febrile patients [12,13]. The current study aimed to address that gap of animal leptospirosis by examining the seroprevalence of leptospirosis among rodents, shrews, cattle, goats, sheep, and dogs. The overall prevalence in rodents and shrews was 9.68%. This may be explained by the fact that for this study the high-risk factors and climate conditions include high temperature and tropical climate that favors infection and allow Leptospira to multiply in the environment resemble with the study by Mgode et al. [20] in Morogoro, Tanzania, which seropositivity was 10.8% are also almost similar with this study.

Table 5: Logistic regression analyses (LR) associated with *Leptospira* seropositivity in Unguja.

Variable	Categories	OR	95% C. I	P-Value
Age	Adult	0.7	0.5-1.2	0.27
	Juvenile			
Sex	Male	0.9	0.6-1.3	0.45
	Female			
Breed	Local Improved	0.8	0.5-1.4	0.54
Species	Dogs			
	Cattle			
	Rodents and shrews			
	Sheep	1.9	1.1-3.3	0.03
Serovars	Hebdomadis			
	Grippotyphosa			
	Lora			
	Pomona			
	Sokoine	0.6	0.3-1.4	0.25

However, the findings are in agreement with the report by Mirambo et al. [21] in Mwanza, Tanzania, in which seropositivity was 10.0% is also almost similar to this study. This may be attributed to the fact that for this study the associated-risk factors and climate conditions resemble those of the study conducted by Mgode et al. [20] in Morogoro, Tanzania. In domestic animals, the overall prevalence was 16.36%, with the highest seroprevalence being observed in sheep (31.25%) followed by dogs (26.25%), cattle (14.57%), and goats (10.01%). A high number of sheep originated from Tanzania's mainland. The present study revealed that farmers rarely vaccinate their animals against Leptospira. Thus, resulted in a high prevalence of Leptospiral antibodies positive. These findings resemble the report by Yupiana et al. [22], from New Zealand. In cattle and goats which mainly originated from Unguja. The observed prevalence may probably be due to the grazing system (zero grazing and tethering) which are commonly practiced. As in this grazing system animals are kept within a fenced homestead and feeds and water are brought to them. Rodents easily share feed with domesticated animals, and there is a high human–animal interaction, thus increasing the risk of this zoonotic disease to humans [12,18]. In comparison with the observation made in a previous study which reported a prevalence of 38.0% in sheep in Morogoro, Tanzania [20], probably due to a small number of sheep in the Morogoro study areas, most of these animals are imported from the outside the island and are used as a source of meat consumption. In dogs, the results showed that 26.25% were seropositive Leptospira antibodies, the results are in agreement with the study by Msemwa et al. [11] in Mwanza, Tanzania in which the seropositivity was 16.1%. This could be justified by the fact that this study included a cluster (farmers and livestock keepers) of a higher risk for Leptospira than those enrolled in the previous study. Moreover, the study previously conducted by Assenga et al. [9], in Katavi-Rukwa Ecosystem, Tanzania. reported seropositivity of 29.9%. This difference could be explained by the fact that only a small number of serovars were explored in the present study compared to the previous one. Cattle may act as maintenance hosts of Leptospira [23] and the overall prevalence for cattle in this study was 14.57%. A study done in Tanga by Karimuribo et al. [24], in East Usambara Mountains, Tanzania, reported, a prevalence of 21.3% in cattle, which is slightly higher than what is reported in this study, which implies that animals may serve as a host for Leptospira maintenance and a potential source of leptospirosis in humans [25]. Rodents are likely the carriers of several leptospiral serovars, as evidenced by the discovery of antibodies to various leptospiral serovars in six different species of rodents captured in various Unguja environments, thus playing a pivotal role in humans and domestic animals' leptospirosis transmission. In this study, serovar Leptospira Sokoine had the highest seroprevalence (3.63%), followed by serovar Leptospira Lora (2.82%), Leptospira Pomona (2.02%), Leptospira Hebdomadis (0.81%) and Leptospira Grippotyphosa (0.41%), in the tested rodent and shrew samples, this indicated that serovar Sokoine is the common serovar circulating among rodents and shrews in Unguja Island. The present finding was similar to three studies conducted in Morogoro, which revealed the existence of leptospiral antibodies in domestic animals, wildlife, rodents, and pet animals in Tanzania [20,26,27]. although in this study, serovar Kenya was not investigated. The interactions between rodents, shrews, domestic animals, and humans occurred regularly, as rats share the same habitats with people and domesticated animals, hence providing a suitable environment for Leptospira transmission across species. Furthermore, Other studies in Tanzania reported that serovar Sokoine was most prevalent and widespread in different regions, including Kagera [2] and Mwanza [21]. Among the five serovars identified in rodents, shrews, cattle, and sheep in the present study, L. kirschneri, serovar Sokoine of the serogroup Icterohaemorrhagiae, was the most frequent. With the serogroup Icterohaemorrhagiae, rodents are recognized to be the natural reservoir, and the high prevalence of serovar Sokoine in these hosts would be evidence of frequent contacts between the cattle, rodents, shrews, and sheep in the study site. This finding agrees with the study reported by Mgode et al. [20] and Assenga et al. [9]. For rodents and shrews, these results are in agreement with the research findings by Mgode et al. [18] in Bahi District, Dodoma, Tanzania. Also, Serovar Sokoine has been mostly reported in rodents [28], in contrast with the study by Allan, and Bvm [29], in Kilimanjaro, Northern Tanzania. It was notable that Rattus norvegicus was absent from this site. Furthermore, the seropositivity of serogroup Icterohaemorrhagie (serovar Sokoine) can be influenced by the abundance of commensal rats in urban and peri-urban locations. These small mammals could

be the natural carriers of these serogroups. Consequently, these species could potentially be the cause of leptospirosis in both humans and animals. Serovar Grippotyphosa was the most prevalent in dogs and cattle. This finding is consistent with the study by Okewole, and Ayoola [30] in Southwestern, Nigeria. However, these differ from those reported by Assenga et al. [9] in Katavi-Rukwa, Tanzania, which found Grippotyphosa as the most prevalent in goats. The high prevalence of these serovars would imply that there is likely close interaction between dogs and cattle kept in peri-urban settings in our study areas. Serovar Pomona showed a high prevalence in goats, as well as in rodents, shrews, cattle, dogs, and sheep. These animals could be important maintenance hosts of this serovar, probably due to the close contact of commensal rodents with domestic animals in the study sites. This is similar to the study by Haji Hajikolaei et al. [31] in Ahvaz, Iran, which found that serovar Pomona was predominant in sheep and goats, implying that small ruminants potentially play a role in the epidemiology of the disease in animals and humans due to close interactions. In this finding, a higher seroprevalence of leptospiral infection was observed in peri-urban areas than urban ones, probably due to associated occupational risk in peri-urban sites [2,18,20], including farming, sewage cleaning, and livestock keeping. A high prevalence of rodents and shrews was observed in Kaskazini A district at (4.03% (10/248), followed by Magharibi A district (2.02% (5/248)) and Kaskazini B district (1.61% (4/248)). This agrees with a previous study reported by Motto et al. [10] from Kilimanjaro, Tanzania, which observed a high prevalence of the disease in rural rice field rats. In the study area, most livestock feeds were not stored properly and served as rodent nesting sites because feed was plentiful for rodents and domestic animals frequently come into contact with the rodents. As a result, the feeds became contaminated with rodent urine and feces, thus posing the risk of animals and humans contracting leptospirosis. A similar finding was reported by National Report [6], Tanzania, which showed that the main reason is that in Zanzibar, 180,220 (51.8%) were involved in agricultural activities including crops production (64.2%), crops and livestock farming (34.6%) and 1.2% in livestock only. Moreover, on the Island during the rainy season, floods in trenches, ponds, and water streams, pose the chance of disease outbreaks. Floods have a significant role in the spread of leptospirosis in this area because runoff and soil polluted with rodents and shrew pee end up in water sources. This finding revealed that the seropositivity of leptospirosis in roof rats was (2.82%) and the brown rat was (2.42%). These rats were important reservoirs of Leptospira in domestic, peridomestic, and farms proximal to the human settlements. The comparison of the serovars found in rodents and shrews showed no statistically significant difference because they share habitats and also probably due to the relatively large sample size of commensal rodents collected compared to shrews in the study area. This finding is similar to those other researchers by Haake, and Levett [32] from Los Angeles, USA and Mgode et al. [2] from Kagera, Tanzania, who recorded that the Rattus norvegicus and Rattus rattus, were plentiful in urban environments and are potentially the major sources of Leptospira infection. In rodents and shrews, the seropositivity for the five Leptospira serovars was characterized by high antibody titers except for serovar Hebdomadis, but also two animals demonstrated a relatively lower titre (1:20). According to Goris et al. [19] from Amsterdam, Netherlands, cut-off point adopted should be below 1:160. In contrast to the study by Mgode et al. [25] from Morogoro, Tanzania. The majority of Leptospira serovars were characterized by low antibody titre. The difference is due to variations in hosts and environmental, serovars, and methodology used in this study. High titres were found in peri-urban districts and were associated with human occupational activities which require water to be achieved [2]. Also, this study has stated the possibility of contracting the disease without including the livestock, but pet animals such as dogs and cats [26]. The study has shown the urban prevalence of leptospirosis was slightly lower than in peri-urban, probably due to a lack of enough habitats such as grazing and agricultural activities as well as forest habitats. Also, risk factors such as sewage systems, and the presence of the rodent and shrews were found in domestic habitats as well as in peridomestic habitats providing a broader environment for the commensal animals to multiply. Additionally, on the Island, pet animals were allowed kept in urban homes which may act as a source of leptospirosis transmission to people through their urine and fluids, but also through contaminated feeds. High titres (double fold rise) were observed in dogs and cattle, suggesting that acute leptospirosis infection had high levels of IgG but also non-specific of the host. On the other hand, the lower titre may suggest chronic leptospirosis infection with lower levels of IgG, that are host specific which can be below the detection threshold of MAT Test, [25]. The low prevalence of the serovar Sokoine, Pomona, Lora, Hebdomadis, and Grippotyphosa in rodents, shrews, and goats and the absence of serovar Hebdomadis in sheep, may suggest host specificity. But also, the variation of antibody titre observed may be caused by different immune responses among species. This is in agreement with the study by Machang'u et al. [27] from Morogoro, Tanzania, in which the serovar Kenya was common in Cricetomys spp. in Morogoro. No significant difference ($p>0.05$) in seroprevalence by age, breed, and sex, suggesting that all groups may face an equal risk of being infected by Leptospira. Seropositivity varied significantly with animal species (OR=1.9,95% CI:1.1-1.3, p=0.03). This implies that animal species were more likely to be infected with the disease. Although the results showed that sheep had a 1.9 times significantly higher likelihood of contracting leptospirosis than other animals, this may be because sheep imported from Tanzania's mainland were not routinely immunized and were kept inside a fenced homestead, which increases human-animal interaction and increased the likelihood of this zoonotic disease spreading to the population in Unguja. Antibodies against pathogenic Leptospira spp. were detected in livestock, wild animals, and companion dogs in both settings, this imply that there is high close interaction between commensal rodents with companion dogs, livestock, and human, which poses the risk of disease transmission to human. The frequent rodents contact with reservoir hosts (dogs, cattle, sheep, and goats) was observed in the peri-urban and urban locations. This finding is similar to the studies by Ally et al. [12] and, Mlowe et al. [16] from Unguja. Zanzibar, Tanzania. The present study observed serum agglutination in more than one serovar in sheep, cattle, and dogs. This may imply two or more frequent serological cross-reactions in past infections. Therefore, a previously infected host by one serovar may, later on, become infected by another serovar. The current serovars may cross-react with the previous one by

activating the memory response [9]. Higher antibody titers were evident in the serological cross-reactivity antibody titer attributable to past infection. The current study had some methodological limitations including, the absence of previous research studies on the seroprevalence of animal leptospirosis in the study areas, the scarcity of sufficient grazing and agricultural activities, and the fact that only pet animals like dogs and cats were permitted in towns while livestock keeping was not. As a result, some data and the connection between livestock and dogs were missed. In addition, due to a small population and insufficient data on some animals such as sheep in the study sites resulted to opt for purposive sampling. A minimum of two serum samples were advised to be collected because the estimation of seroprevalence of leptospirosis was constrained by the use of a single serum sample per species.

Conclusion

In conclusion, the present study showed high Leptospira prevalence in domestic animals, rodents, and shrews, suggesting that leptospirosis could be a major animal and public health threat. The study areas, characterized by high interactions of commensal rodents with domestic animals, have a high risk of leptospirosis transmission. Therefore, preventive measures, including rodent 'control such as reducing rodents' contact with reservoir hosts (dogs and ruminant animals), environmental sources of Leptospira (water sources), and vaccination of domestic animals with vaccines against local Leptospira strains, should be emphasized in both urban and rural settings to reduce the spread of pathogenic Leptospira spp. to people. Additionally, this study's findings show that common Leptospira serovars are present in rodents, shrews, domestic ruminants, and dogs, which will help in the planning of interventions to reduce the effects of infection on both domestic animals and people. Thus, we recommend that further research should be conducted to find out factors of high seropositivity of leptospiral in Unguja Island.

Abbreviation

MAT: Microscopic Agglutination Test; RDTs: Rapid Diagnostic Tests: EMJH; Ellinghausen McCullough Medium-Johnson and Harris; OCGS: Office of the Chief Government Statistician; ZALIRI: Zanzibar Livestock Research Institute; PBS: Phosphate Buffered Saline; GDP: Growth Domestic Product; URT: United Republic of Tanzania; NBS: National Bureau of Statistics; ACE: African Centre of Excellence; BTD: Biosensor Technology Development; IRPM: Innovative Rodent Pest Management; PBS: Phosphate Buffered Saline; CDC: Centre for Disease Control and Prevention; PHC: Population and Housing Census.

Declaration

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Availability of data and materials

Data will be available by emailing geraldmlowe@gmail.com

Authors' contributions

Gerald Dickson Mlowe (GDM) is the principal investigator (PI) who contributed to the conceptualization, data curation, formal analysis, and writing of the original draft of the manuscript. Isaac Makundi (IM) and Robert Machang'u (RM) are the core supervisors and Abdul Selemani Katakweba (AASK) is the main supervisor. IM, RM, and AASK contributed to the methodology, supervision, review, editing, and re-writing of the manuscript. All authors have read and accepted to be published final version of the manuscript.

Ethics approval and consent to participate

The ethical clearance for conducting this study was granted by the Research Ethics Committee at Sokoine University of Agriculture (Ref No. SUA/ADM/R.1/8/779 on January 10, 2022). Research protocols were revised and approved by the Zanzibar Livestock Research Institute (ZALIRI) and permission to conduct research in Zanzibar was obtained from the Research Committee of the Office of the Second Vice President and the Office of the Chief Government Statistician (OCGS), Ref No. OMPR/M.95/ C.6/2/VOL.XVIII/187 on January 20, 2022).

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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