EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 4 (4): September 2023

Leptospira infections among rodents and shrews trapped in public markets in Unguja Island, Zanzibar: Untold silent public health threat

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Abstract

Leptospirosis is a zoonosis caused by spirochete bacteria belonging to the genus Leptospira. The disease is recognized as an occupational hazard where rodents and shrews are primary reservoirs of infection for animals and humans. A cross-sectional study was conducted from January to March 2022 to assess the seroprevalence of leptospira infection among rodents and shrews trapped in public markets namely Darajani, Mombasa, Jumbi, Mkokotoni, and Kwerekwe C. The study involved the capture of 210 live rodents and shrews for serum sample collection. The sera were then tested for antibodies against five leptospira serovars using the microscopic agglutination test (MAT). The findings of this study indicated that 16 out of 210 samples were seropositive for leptospira serovars. The overall seroprevalence of leptospira infection was 7.6% (95% CI =4.4-12.1), with a prevalence of 8.0 % (14/174) in rodents and 5.6 % (2/36) in shrews. The range of titers was between 1:20 and 1:160. Rattus rattus were shown to have the highest seroprevalence (5.2%), followed by Rattus norvegicus (1.7%) and Mus spp (1.1%). Samples of rodents and shrews captured from Darajani markets recorded a highest seroprevalence (4.2%). The most prevalent serovars were Sokoine 11 (5.2%), Lora 4 (1.9%), Pomona 2 (1.0%) and Grippotyphosa 1 (0.5%). These findings suggest that market workers, buyers, and sellers are at risk of being infected with leptospira pathogens when they come into contact with urine or contaminated water and soil. Hence, the findings of this study call for awareness creation about leptospiral infection and its association with rodents and shrews in market environments, and the need to control rodents and shrews in marketplaces by relevant government authorities.

Keywords: *Seroprevalence, leptospira infection, rodents and shrews, public markets, Unguja Island*

Cite as: Ally *et al.*, (2023). Leptospira infections among rodents and shrews trapped in public markets in Unguja Island, Zanzibar: Untold silent public health threat. *East African Journal of Science, Technology and Innovation 4(4).*

Received: 21/04/23
Accepted: 21/08/23
Published: 29/09/23

Introduction

Leptospirosis is one of the most significant neglected zoonotic diseases worldwide, caused by *Leptospira* spp. The disease is a public health challenge and economic threat (Ricardo *et al.,* 2018; Motto *et al.,* 2021). The pathogenic *Leptospira* bacteria are capable of causing disease in animals and humans. Around 20 serogroups and more than 250 pathogenic Leptospira serovars are known to affect animals and humans worldwide (Tilahun *etal.,* 2013; Motto *et al.,* 2021). However, Leptospira serovars vary from country to country depending on the ecological niche in a given area. For example, in Tanzania, the common Leptospira serovars that have been reported among wild animals, domestic animals, and humans are *L. borgpetersenii* serogroup Ballum serovar Kenya, *L. kirschneri* serogroup Ictero-haemorrhagiae serovar Sokoine, *L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, *L. interrogans* serogroup Australis serovar Lora, and *L. interrogans* serogroup Pomona and Hebdomadis (Mgode *et al.,* 2019; Mgode *etal.,*2021). Similarly, *L. interrogans* serovars Mwogolo and Canicola were reported in wild and domestic animals (Assenga *et al.,* 2015). Likewise, L. *interrogans* serogroup Sejroe serovar Hardjo was found in humans, wildlife, and domestic animals (Assenga *et al.,* 2015). The disease causes morbidity and mortality globally, with an estimated 1.03 million cases and killing nearly 60,000 people yearly (Costa *et al.,* 2015). Tropical and subtropical countries are more vulnerable to this disease, which is attributed to favourable climatic conditions and environmental conditions which contributes to the survival and replication of the bacteria (Machang'u *et al.,* 2004; Mgode *et al.,* 2014; Mwachui *et al.,* 2015; Sato *et al.,* 2022). Rodents and shrews are considered primary reservoirs of infection in animals and humans due to their survival in different environments. However, domestic animals such as cattle, goats, dogs, and pigs can be carriers of infection, in which the bacteria may harbor in the renal tubules of the kidneys for several months before being discharged into environment through urine (Mgode *et al.,* 2006; Tilahun *et al.,* 2013; Mwachui *et al.,* 2015; Said *et al.,* 2018; Ngugi *et al.,* 2019).

Humans are considered accidental hosts of the disease and usually get infected by *Leptospira* spp either through direct contact with the urine or tissues of infected animals or indirectly through contact with contaminated environmental components such as water and soil. *Leptospira* spp penetrate into humans through cuts or abrasions on the skin or through mucous membranes of the mouth, nose, and eyes (WHO, 2003; Tilahun *et al.,* 2013; Mgode *et al.,* 2017; Chin *et al.,* 2020). The disease is an occupational hazard and affects vulnerable groups such as public market workers, abattoir workers, sewage workers, agricultural workers, vegetable farmers, gardeners, fishermen and animal handlers upon contact with the urine of infected animals or urine-contaminated environments (Tilahun et *al.,* 2013; Assenga *et al.,* 2015; Rahman *et al.,* 2018; Mgode *et al.,* 2019). The market workers are among the most vulnerable groups for leptospira infection because of the food and other products they sell attract rodents and shrews to reside and reproduce within the market areas. Poor market hygienic conditions further contribute to the survival of the *Leptospira* spp for an extended time (Azali *et al.,* 2016; Rahman *et al.,* 2018). In Zanzibar, the availability of heterogeneous foods in public markets attracts rodents and shrews, thus subjecting humans, including market workers, buyers, and sellers, to close contact with rodents, shrews and their excreta. These animals act as reservoirs for various pathogenic agents, including *Leptospira* spp, thus putting humans at risk of multiple infections, including leptospirosis. Therefore, this study aimed at assessing the seroprevalence of leptospira infection among rodents and shrews in public markets in Unguja Island. The findings of this study call for education and awareness creation of leptospira infection and its association with rodents and shrews in the open markets. In addition, it will support strategies to be used for safe control of rodents and shrews in marketplaces.

Materials and Methods

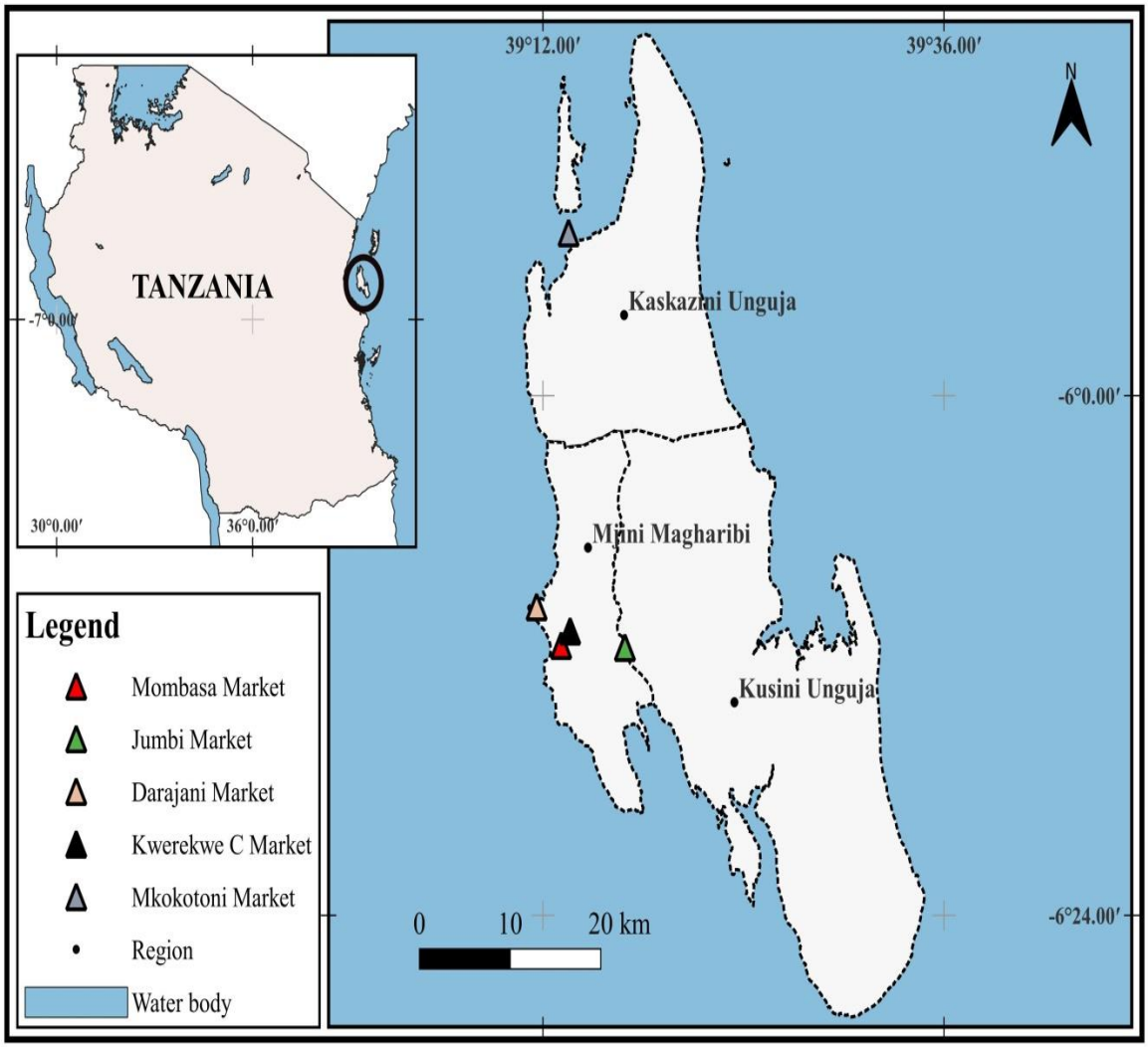
Study area

The study was conducted in Unguja Island from January to March 2022 at five public markets, namely Darajani, Mombasa, Jumbi, Mkokotoni,

and Kwerekwe C. These markets were selected because they are largest public markets in Unguja which provides services to many people from different locations. Unguja Island is the largest and most populated island in the Zanzibar archipelago (6° 08′ 26.00" S, 39° 20′ 11.57" E), which lies off the coast of Tanzania mainland (Figure 1). Unguja Island consisting of three regions: namely Kaskazini Unguja, Kusini Unguja and Mjini Magharibi. Mjini Magharibi has the largest population of about 893,169 people, which is 47.3% of the total population of Zanzibar (URT, 2022).

Figure 1

Map of Unguja Islands indicating the study location of public markets



Note. From QGIS Version 3.24 "Tisler" visited on August 10, 2022.

Study Design

In this study, a cross-sectional design was used, where personal observation and interview by using structured questionnaire were undertaken to assess the market features and practices as well as rodents and shrews trapping for serological assessment of *Leptospira* spp antibodies.

Determination of the sample size

A formula for the infinite population was used to determine the sample size for rodents and shrews as described by Kothari and Gaurav (2014); $\mathbf{n = Z^2*P\ (1\text{-}P)/d^2}$, where n = estimated sample size, Z = test statistic (1.96 for 95% confidence interval), P = an approximate prevalence rate from a previous study of 15.5% according to Mgode *et al.* (2021), and d = level of precision that was 5% (0.05). Therefore, the estimated sample size was 201, for every public market, 42 animals were captured, without considering species proportionality because shrews' population is low in market environment.

Rodents and shrews trapping

Rodents and shrews were captured in the public markets using different traps, including Sherman ® LFA live traps (HB Sherman Traps, Inc., Tallahassee, FL, US) and locally−made wire live traps which were baited with peanut butter mixed with maize flour, tomatoes, fried fish, and dried sea fish. Inside each market, 80 traps were set randomly in the evening and checked for captures early the following morning of three consecutive nights (days) before shifting to another market. The capture rodents and shrews were transported to the Zanzibar Department of Livestock Development laboratory at Maruhubi area for identification and blood sample collection. Captured rodents and shrews that died in the traps before taking blood samples were excluded from the study.

Blood collection and identification of captured rodents and shrews

The captured rodents and shrews were anesthetized using diethyl ether (Loba Chemie Pvt, Company Ltd, India) soaked cotton wool before and then characterized morphologically and anatomically including body weight, head-to-body length, tail length, ear length, and hind foot length according to Skinner and Chimimba (2005). Blood was collected through heart puncture using 2ml and/or 5ml sterile syringes and needles and transferred into plain Eppendorf tubes to allow clotting and serum separation at room temperature for at least 30min. Serum collection was maximized by centrifugation of the vials at 3000 rpm for 10-15 minutes to obtain clear serum. The serum was then transferred into other appropriately labeled plain Eppendorf tube and stored frozen at -20℃ at the Zanzibar Department of Livestock Development Laboratory until transported at below 4℃ to the Institute of Pest Management (IPM) Research Laboratory of Sokoine University of Agriculture (SUA), Morogoro, Tanzania using cool boxes with ice packs. The serum samples were frozen at -20℃ at IPM Research Laboratory until used for microscopic agglutination test (MAT) (Machang'u *et al.,* 1997; Mgode *et al.,* 2019; 2021). The small tissue samples from each carcass of rodents and shrews were stored in 70% methylated spirits for ulterior confirmatory of their species of origin and the remaining were safety disposed of in a pit.

Laboratory procedures

The microscopic agglutination test as the gold standard method was used to screen for *Leptospira* spp antibodies of rodents and shrews' serum samples according to Goris *et al.* (2013). The sera were tested for antibodies against five serovars of two species namely *L. kirschneri* (serogroup Ictero-haemorrhagiae serovar Sokoine), *L. kirschneri* (serogroup Grippotyphosa serovar Grippotyphosa), *L. interrogans* (serogroup Pomona serovar Pomona), *L. interrogans* (serogroup Australis serovar Lora) and *L. interrogans (*serovars Hebdomadis). These serovars are mostly frequently in human and animals in Tanzania mainland (Machang'u *et al.* 1997; Assenga *et al*. 2015; Mgode *et al.* 2019; 2021). Pure stocks of cultured leptospira were subcultured into Ellinghausen McCullough-Johnson and Harris (EMJH) medium and incubated at 28−30℃ for 5−7 days. The purity of the Leptospira serovars was frequently checked for growth density and free of fast growing contaminants using a dark field (DF) microscope. The recommended density, estimated to $3x10^8$ leptospira /ml on the MacFarland scale was applied for MAT. The 96 wells of each Microtiter plate were filled with 50µl phosphate-buffered saline (PBS) pH 7.2 except the wells of the second row 2 which were filled with 90µl of PBS. Then, 10µl of sera were added to the wells of row to obtain initial dilutions of 1:10, 1:20, 1:40, and 1:80. Thereafter, serial dilution was performed by mixing and pipetting 50µl from the wells of the second row to the following rows, and finally the remaining 50µl from the last well was discarded. It followed that 50µl volume of well grown live

leptospira was added to all microtiter plate wells to obtain final double dilutions of 1:20, 1:40, 1:80, and 1:160. The plates were gently shaken and then incubated at 30℃ for 2 to 4 hours before being screened for agglutination of the leptospires under DF microscope. The agglutination was appreciated by taking a loopfull of the mixture on a microscopic slide using a wire loop and the cut off titer (positive) was estimated as the one where at least 50% of the spirochetes agglutinated leaving 50% of cells free. This result was compared with a negative control in row 1 of the suspension of PBS and antigens without sera. All sera that agglutinated at titer≥1:20 were recorded as positive for the test.

Assessment of features and practices in public markets in Unguja

During market visits, a checklist was used to obtain basic information of public markets, including infrastructures, operations, type of goods sold, and general environmental sanitation. The information was obtained through interview with market staff and complemented by personal observation.

Data processing and analysis

The data were entered into Microsoft Excel 2010 for cleaning and coding. Then the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 26, 2019. Descriptive analyses such as frequency and proportion were computed. Fisher's exact statistical test was used to determine the associations between *Leptospira* serovars, animal species, and public markets. At p-value ≤ 0.05 the association was considered statistically significant. Comparison of features and practices for different public markets was conducted to reveal issues which are related to survival and transmission of *Leptospira* spp and infection of rodents and shrews.

Ethical consideration

The research protocols were reviewed and approved by the Research Ethical Committee at Sokoine University of Agriculture (Ref No. SUA/ADM/R.1/8/767 issued on January 10, 2022). The authorization to conduct research in Zanzibar was issued by the Research Committee from 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/53 and 61B6F85E745B7 respectively issued on December 13, 2021. Also, the Zanzibar Health Research Ethical Committee approved the research protocol again, Ref No. ZAHREC/04/ST/NOV/2021/94 dated on November 30, 2021 before the start of sample collection.

Results

Number of captured rodents and shrews

A total of 210 animals (rodents and shrews) were captured from five public markets. Of the 210 rodents and shrews, 42 (20%) were captured from each market, namely Darajani, Mombasa, Jumbi, Kwerekwe C, and Mkokotoni (Table 1). Out of the 210 captured rodents and shrews, 36 (17.1%) were shrews (*Crocidura* spp) and 174 (82.9%) rodent species. The rodents belong to four species namely, *Rattus rattus, n*=117 (55.7%) followed by *Rattus norvegicus, n*=27 (12.9%), *Mus* spp, *n*=22 (10.5%), and *Cricetomys* spp, *n*= 8 (3.8%).

Table 1

Rodents and shrews species captured from five public markets

Genus/spp	Darajani	Mombasa	Jumbi	Mkokotoni	Kwerekwe C	Total %
R. rattus	22	20	20	27	28	117 (55.7)
R. norvegicus	2	4	8	8	5	27 (12.9)
Mus spp	4	9	5	1	3	22 (10.5)
Cricetomys spp	2	2	4	0	0	8 (3.8)
Crocidura spp	12	7	5	6	6	36 (17.1)
Total (%)	**42 (20)**	**42 (20)**	**42 (20)**	**42 (20)**	**42 (20)**	**210 (100)**

Seroprevalence of leptospira infection among *rodent species and shrews*

The overall seroprevalence of leptospira infection among rodents and shrews was 7.6% (16/210) (95% CI =4.4−12.1), with a prevalence of 8.0% (14/174) in rodent species and 5.6% (2/36) in shrews. Out of 8.0% (14/174) prevalence in rodent species, *Rattus rattus* (5.2%) were shown to have highest prevalence, followed by *Rattus norvegicus* (1.7%), and *Mus* spp (1.1%) (Table 2). Moreover, the seropositivity among rodents and shrews in the public markets was highest in the Darajani market 9 (4.2%), followed by the Mombasa market 5 (2.4%), Jumbi market 1 (0.5%), Kwerekwe C 1 (0.5%), and Mkokotoni market 0 (0.0%).

Table 2

Seroprevalence of leptospira infection among rodent species and shrews

Animals captured	Genus/species	Animals Tested	Leptospira Positive	Prevalence (%)
Rodents	***Rattus rattus***	117	9	5.2
	Rattus norvegicus	27	3	1.7
	Mus spp	22	2	1.1
	***Cricetomys* spp**	8	0	0.0
	Total	**174**	**14**	**8.0**
Shrews	*Crocidura* spp.	36	2	5.6
	Total	**36**	**2**	**5.6**
	Total	**210**	**16**	**7.6**

Circulating Leptospira serovars among rodents and shrews antibodies

The serovar Sokoine, ***n*=**11 (5.2%) was most prevalent among rodents and shrews, followed by Lora, ***n*=**4 (1.9%), Pomona, ***n*=**2 (1.0%), and Grippotyphosa, ***n*=**1 (0.5%). Serovar Pomona and Grippotyphosa were not detected in the shrews. Moreover, serovar Hebdomadis was not detected in any of the animals captured (Table 3). Furthermore, serovar Sokoine, ***n*=**11 (5.2%) was predominant in Darajani markets compared to the rest of the public markets. The difference in prevalence of serovars Sokoine in the five public markets was statistically significant (p-value < 0.05)**.** Other serovars that reacted to **rodents and shrews** antibodies among public markets were Lora, ***n*=**4 (1.9%), Pomona, ***n*=**2 (1.0%) and Grippotyphosa, ***n*=**1 (0.5%). However, serovar Hebdomadis was not in any of the public markets (Table 4).

Table 3

Circulating ***Leptospira serovars among rodent's species and shrews***

Leptospira serovars	Animals tested					Total positive (%)	P-value
	Rattus rattus	*Rattus norvegicus*	***Mus* spp**	***Cricetomys* spp**	***Crocidura* spp**		
Sokoine	**5**	**2**	2	0	2	11(5.2)	0.812
Lora	**1**	**1**	0	0	2	4 (1.9)	0.366
Pomona	**2**	**0**	0	0	0	2 (1.0)	0.808
Grippotyphosa	**1**	**0**	0	0	0	1 (0.5)	0.939
Hebdomadis	**0**	**0**	**0**	**0**	**0**	0 (0.0)	-

Antibodies titers of rodents and shrews against Leptospira serovars

The majority of positive sera samples reacted at antibodies titers between 1:20 to 1:80, which is in the range of the cut-off point of significance for rodents and shrews. However, two sera samples of *Rattus rattus* reacted at high MAT titers of 1:160 (Table 5). Furthermore, two samples of *Crocidura* spp reacted to more than one serovar, including cross-reactions between serovars Sokoine and Lora. Hence, they are not included in the overall seropositivity (16 positive rodents and shrews out of 210) (Table 6).

Public market features and practices

The public markets in Unguja Island have many common features and practices such as wet floors, stagnant water, preferable habitats for rodents and shrews (example dark tunnel and holes) and poor drainage system. All markets had electrical power supply and goods were sold on wooden tables. In all the markets, food stuff such as sea food, grains, spices, vegetables and fruits were sold at both retail and wholesale levels. However, the market features and practices differ in some aspects between the markets. These include operation hours, holding capacity (people), types of goods and products sold, selling point, merchandize category, solid wastes collection system, presence of water supply, rodents and shrews control programs, type of floor, vicinity to residences and presence of fencing. Darajani market was the largest, with 6,000 people per day holding capacity and longer duration of service (18hrs), while others had capacity to serve less than 3000 people per day and were operational for 12 hours.

Table 4

Circulating ***Leptospira serovars among public markets***

Leptospira serovars	**Public market categories**					**Total positive (%)**	**P-value**
	Darajani	Mombasa	**Jumbi**	**Kwerekwe C**	**Mkokotoni**		
Sokoine	**6**	**4**	0	1	0	11(5.2)	**0.008**
Lora	**2**	**1**	1	0	0	4 (1.9)	0.468
Pomona	**1**	**1**	0	0	0	2 (1.0)	0.553
Grippotyphosa	**1**	**0**	0	0	0	1 (0.5)	0.403
Hebdomadis	**0**	**0**	**0**	**0**	**0**	0 (0.0)	-

Table 5

Antibodies titres of rodents and shrews against Leptospira serovars

	Leptospira serovars tested					**Total**
Titres	**Sokoine**	**Lora**	**Pomona**	**Grippotyphosa**	**Hebdomadis**	
1:20	1	0	0	0	0	1
1:40	4	3	1	0	0	8
1:80	5	1	0	1	0	7
1:160	1	0	1	0	0	2
Total	**11**	**4**	**2**	**1**	**0**	**18**

Table 6

Cross-reaction of Leptospira serovars in rodents and shrews antibodies

L. serovars tested	Number of animals tested	Leptospira positive (1:20-1:160 titres)	Serovar prevalence (%)
L. Sokoine	210	11 (1)*	5.2
L. Lora	210	4 (1)*	1.9
L. Pomona	210	2	1.0
L. Grippotyphosa	210	1	0.5
L. Hebdomadis	210	0	0.0

Note. *Two samples of *Crocidura* spp reacted with both serovars Lora and Sokoine (cross-reactions)

Table 7

Summary of features and practices of public markets in Unguja Island

Features	Public Markets				
	Darajani	**Mombasa**	**Kwerekwe C**	**Jumbi**	**Mkokotoni**
Region	Mjini Magharibi	Mjini Magharibi	Mjini Magharibi	Mjini Magharibi	Kaskazini
District	Mjini-Stone Town	Magharibi "B"	Magharibi "B"	Magharibi "B"	Kaskazini "A"
Operation hours	18	12	12	12	12
Capacity (people)	6,000	3,600	4,320	3,950	2,803
Market staffs	304	246	278	210	186
Goods/Products	Seafood, meat, fruits, grains and spices	Vegetables, Seafood, meat, fruits, grains and spices	Vegetables, Seafood, meat, fruits, grains and spices	Vegetables, Seafood, meat, fruits, grains and spices	Seafood and fruits
Selling point	Concrete and wooden table	Concrete and wooden table	Wooden tables and soil	Wooden tables and soil	Concrete and wooden tables
Merchandize category	Retail and wholesale	Retail and wholesale	Retail and wholesale	Retail and wholesale	Retail
Floor	Concrete	Partly concrete and earth floor	Rough and wet earth floor	Rough and wet earth floor	Concrete
Electricity/light	Presence	Presence	Presence	Presence	Presence
Drainage system	Present but not working well	Absent	Absent	Absent	Present and well working
Waste collection frequency	Twice a day	Twice a day	Twice a day	One a day	Twice a day
Water supply	Present	Present	Present	Absent	Present
Rodents and shrews habitat	Drainage system, under wooden table, shops	Wooden table and shops	Wooden table and shops	Wooden tables, shop and farm	Drainage system
Rodent and shrews control	Once in two months	One in three months	Not practice	Not practice	Not practice
Vicinity to residences	Zero distance	Zero distance	Zero distance	Approximately 15m	Approximately 20m
Fencing	Absent	Compete	Absent	Absent	Absent

There were rodents and shrews control programs in Darajani and Mombasa markets but not in the others. Darajani and Mkokotoni markets had concrete floors whereas the remaining markets

had rough earth floors. The summary of important features and practices are shown in Table 7. Some of these features and practices provide suitable conditions for the survival of *Leptospira* spp bacteria and infection of rodents and shrews which being reservoirs of the pathogens pose a risk of environmental contamination and perpetuation of rodent-environment-human infection cycle

Discussion

To the best of our knowledge, this is the first study in Unguja Island to assess the seroprevalence of leptospiral infection in rodents and shrews in public markets according to the existing literatures. The previous study reports the seropositivity of leptospira in rodents, shrews and domestic animals in domestic, peridomestic, and farm areas (Mlowe *et al.,* 2023). The findings of this study have shown that the overall seroprevalence of leptospira infection among rodents and shrews in public markets was 7.6% (95% CI: 4.4−12.1). The finding of this study is in agreement with the previous study reported in Southeast Asia by Cosson *et al.* (2014), with a prevalence of 7.1%. However, the prevalence shown by our study is lower than that reported from Malaysia of 31.6% (Rahman *et al.,* 2018), Bangladesh 13.1% (Krijger *et al.,* 2019), and from Tanzania mainland which showed a prevalence ranging from 15.5% to 25.8% (Mgode *et al.,* 2014; 2019; 2021). Furthermore, the prevalence reported in this study is higher compared to reports from Australia, with a prevalence of 2.9% (Dybing *et al.,* 2017), Tanzania, 1.8% (Machang'u *et al.,* 1997), China, and Ecuador, 3.0% (Zhou *et al.,* 2009; Barragan *et al.,* 2016). The difference in the prevalence of leptospira infection might be due to differences in environmental and climatic factors of the study areas, difference in serovars tested and the species of rodents and shrews captured (Assenga *et al.,* 2015). Sampling sites, methodology and sample size, may also have contributed to the difference in the prevalence of infection (Blasdell *et al.,* 2019; Boey *et al.,* 2019).

This study has shown serovar Sokoine to be the most prevalent of the five serovars tested. A similar finding has been reported in studies conducted in different place in Tanzania, including, Morogoro region (Mgode *et al.*, 2014), Dodoma (Mgode *et al*., 2021), and Kagera (Mgode *et al.*, 2019) indicating that it is the most circulating serovar in rodents and shrews in Tanzania. Other serovars detected were serovar Lora, which has also been previously reported in rodents and shrews in the Morogoro region (Ahmed *et al.,* 2006; Mgode *et al.*, 2014); serovar Grippotyphosa also reported in rodents from Kagera, northwestern Tanzania (Mgode *et al.*, 2019), and Katavi-Rukwa Ecosystem, Tanzania (Assenga *et al.,* 2015).

The findings of this study have shown a higher seroprevalence of leptospira infection (8.0%) compared to shrews (5.6%), however, not significantly different possibly due to the relatively smaller number of shrews captured (Mgode *et al.*, 2019). *Rattus rattus* was an important reservoir of leptospiral infection as demonstrated by the relatively higher seroprevalence of the infection compared to the other rodent species and shrews tested. This finding is in agreement with the study by Katakweba *et al.* (2012) and Katakweba (2018), who concluded that leptospiral infections was most prevalent in *R. rattus* in urban and peridomestic environments because of their close interactions and their ability to adapt quickly to the human environment and thus increased likelihood of human infection. The higher seroprevalence of leptospira infection among *Rattus rattus is* also probably due to their ecology, abundance, and habitat of living in the vicinity of households; thus, most public markets are close to human residences, which allow *R. rattus* to nest in human homes as well as in the markets (Cosson *et al.*, 2014).

Darajani market showed the highest seroprevalence of leptospiral infection in rodents and shrews compared to the other markets. It is also biggest market in Unguja Island with longer opening hours and an overall poor hygiene, which could be additional predisposing factors for rodent and shrews habourage (Wynwood *et al.,* 2014; Rahman *et al.,* 2018).

In addition, the findings have shown a seropositivity of relatively low titers of between 1:20 to 1:80 which suggest that the rodents and shrews have been exposed to the bacteria for a long period of time. This is supported by the observed market features and practices, which favor the population growth of the reservoirs and survival of the bacteria and hence a public health threat. This finding is similar to that of studies by other scholars (Katakweba *et al.,* 2012; Mgode *et al.*, 2014; Mgode *et al*., 2019).

The public market attributes in Unguja Island pose a public health risk. The study has revealed the distance from the public markets, where rodents and shrews carrying *Leptospira* spp were found; to residence was 0-20 meters. This close vicinity increases the likelihood of contact between infected rodents and shrews and humans. The types of goods sold at the market and vending milieu support rodents and shrew population and leptospira survival. These public markets characteristics favors possibility of transmission of leptospira to humans and domestic animals. Leptospirosis in humans is a febrile disease which clinically may be easily misdiagnosed for malaria. Given the increase in prevalence of febrile non-malaria cases in Zanzibar and a call for additional diagnostic tests (Baltzell *et al.*, 2013; Ally *et al.,* 2023), the present study highlights a potential source of infection to humans that may be responsible for misdiagnosis and possible inappropriate drug prescription.

Conclusion

The findings of this study have revealed that a number of leptospiral serovars are harbored in rodents and shrews among public markets in Unguja Island. These results strongly suggest that rodents and shrews might be an important reservoirs and source of human leptospirosis; thus, humans, including market workers, sellers, and buyers, are at risk of being infected by leptospira pathogen. The findings of this study, therefore, call for education and awareness creation on leptospira infection and its association with rodents and shrews among market stakeholders, as well as strategies to control rodents and shrews in marketplaces.

Recommendations

Public awareness of diseases spread by rodents and shrews, including leptospirosis are needed for the general population of Unguja Island.

Education on rodent and shrews control techniques is recommended to the communities for the purpose of breaking the chain of rodent-borne disease transmission.

It is important for municipal council officers and market workers to maintain highest level of environmental sanitation especially within the markets, where most of the populations obtain their foods. This will reduce the chance of rodents and shrews to transmit the infection to humans and domestic animals.

Further serological studies of market workers/visitors and molecular characterization of *Leptospira* spp. isolate from environmental samples (water and soils) are needed.

Financial Support
This research was sponsored by Institute of Pest Management (IPM) at the Sokoine University of Agriculture under the African Center of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development Project (ACE II IRPM & BTD, No. 5799/TZ).

Acknowledgments

The authors would like to acknowledge the administration and staffs from the Department of Livestock Development, Zanzibar for their positive cooperation, support, and advice during the study period; the Office of the Second Vice President and Office of the Chief Government Statistician (OCGS) for granting the permission to conduct this research. We would like to thank Mr. Ginethon G. Mhamphi from (IPM-SUA) for assistance on microscopic agglutination test (MAT). We would like to thank market workers and staffs from all municipalities for their cooperation during sample collection. In advance, we would like to thank my supervisors, the late Dr. Georgies F. Mgode and Prof. L.S.

Mulungu, for their positive encouragement, guidance, support, comments, advice, and contribution. Furthermore, we thank the African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development Project (ACE II IRPM & BTD, No. 5799/TZ) at IPM-SUA for their financial support of this research.

References

Ahmed, N., Devi, S. M., De los Á Valverde, M., Vijayachari, P., Machang'u, R. S., Ellis, W. A., & Hartskeerl, R. A. (2006). Multilocus sequence typing method for identification and genotypic classification of pathogenic Leptospira species. *Annals of clinical microbiology and antimicrobials*, 5(1), 1-10.

Ally, A. A., Lupindu, A. M., Machang'u, R., & Katakweba, A. S. (2023). Seroprevalence of leptospirosis among hospitalized febrile patients in Unguja Island. *Journal of Ideas in Health*, *6*(1), 820-827.

Assenga, J. A., Matemba, L. E., Muller, S. K., Mhamphi, G. G., & Kazwala, R. R. (2015). Predominant leptospiral serogroups circulating among humans, livestock and wildlife in Katavi-Rukwa ecosystem, Tanzania. *PLoS neglected tropical diseases*, *9*(3), 1-14.

Azali, M. A., Yean Yean, C., Harun, A., Aminuddin Baki, N. N., & Ismail, N. (2016). Molecular characterization of Leptospira spp. in environmental samples from north-eastern Malaysia revealed a pathogenic strain, Leptospira alstonii. *Journal of tropical medicine*, *2016*, 1-7.

Baltzell, K., Elfving, K., Shakely, D., Ali, A. S., Msellem, M., Gulati, S., & Mårtensson, A. (2013). Febrile illness management in children under five years of age: a qualitative pilot study on primary health care workers' practices in Zanzibar. Malaria journal, 12, 1-10.

Baltzell, K., Elfving, K., Shakely, D., Ali, A. S., Msellem, M., Gulati, S., & Mårtensson, A. (2013). Febrile illness management in children under five years of age: a qualitative pilot study on primary health care workers' practices in Zanzibar. *Malaria journal*, *12*, 1-10.

Barragan, V., Chiriboga, J., Miller, E., Olivas, S., Birdsell, D., Hepp, C., ... & Pearson, T. (2016). High Leptospira diversity in animals and humans complicates the search for common reservoirs of human disease in rural Ecuador. *PLoS neglected tropical diseases*, *10*(9), 1-14.

Blasdell, K. R., Morand, S., Perera, D., & Firth, C. (2019). Association of rodent-borne Leptospira spp. with urban environments in Malaysian Borneo. *PLoS neglected tropical diseases*, *13*(2), 1-17.

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), 1-24.

Chin, V. K., Basir, R., Nordin, S. A., Abdullah, M., & Sekawi, Z. (2020). Pathology and host immune evasion during human leptospirosis: a review. *International Microbiology*, *23(2)*, 127-136.

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), 1-10.

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., ... & Ko, A. I. (2015). Global morbidity and mortality of leptospirosis: a systematic review. *PLoS neglected tropical diseases*, *9*(9), 1-19.

Dybing, N. A., Jacobson, C., Irwin, P., Algar, D., & Adams, P. J. (2017). Leptospira species in feral cats and black rats from Western Australia and Christmas Island. *Vector-Borne and Zoonotic Diseases*, *17*(5), 319-324.

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), 1 – 11.

Katakweba, A. A. S. (2018). Small Mammals in Fenced Houses as Source of Leptospirosis to Livestock Pets animals and Humans in Morogoro Municipality, Tanzania. *Tanzania Veterinary Journal*, *36*, 83-88.

Katakweba, A. A., Mulungu, L. S., Eiseb, S. J., Mahlaba, T. A. A., Makundi, R. H., Massawe, A. W. & Belmain, S. R. (2012). Prevalence of haemoparasites, leptospires and coccobacilli with potential for human infection in the blood of rodents and shrews from selected localities in Tanzania, Namibia and Swaziland. *African Zoology*, 47(1), 119 – 127.

Kothari, C. R. & Gaurav, G. (2014). *Research Methodology.* (Third Edition), New Age International Publishers, New Delhi, India. 401pp.

Krijger, I. M., Ahmed, A. A., Goris, M. G., Groot Koerkamp, P. W. and Meerburg, B. G. (2019). Prevalence of Leptospira infection in rodents from Bangladesh. *International Journal of Environmental Research and Public Health*, 16(2113), 1 – 19.

Machang'u, R. S., Mgode, G. & Mpanduji, D. (1997). Leptospirosis in animals and humans in selected areas of Tanzania. *Belgian Journal of Zoology*. 127, 97 – 104.

Machang'u, R. S., Mgode, G. F., Assenga, J., Mhamphi, G., Weetjens, B., Cox, C., ... & Hartskeerl, R. A. (2004). Serological and molecular characterization of leptospira serovar Kenya from captive African giant pouched rats (Cricetomys gambianus) from Morogoro Tanzania. *FEMS Immunology & Medical Microbiology*, *41*(2), 117-121.

Mgode, G. F., Machang'u, R. S., Goris, M. G., Engelbert, M., Sondij, S., & Hartskeerl, R. A. (2006). New Leptospira serovar Sokoine of serogroup Icterohaemorrhagiae from cattle in Tanzania. *International Journal of Systematic and Evolutionary Microbiology*, *56*(3), 593-597.

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), 1-7.

Mgode, G. F., Mhamphi, G. G., Katakweba, A. S., Mboera, L. E. and Machang, R. S. (2017). *Leptospirosis In Tanzania: A Neglected Cause of Febrile Illness That Needs Attention of the Health System*. National Institute for Medical Research, Dar es Salaam, Tanzania, 8pp

Mgode, G. F., Japhary, M. M., Mhamphi, G. G., Kiwelu, I., Athaide, I., & Machang'u, R. S. (2019). Leptospirosis in sugarcane plantation and fishing communities in Kagera northwestern Tanzania. *PLoS Neglected Tropical Diseases*, *13*(5), 1-12.

Mgode, G. F., Mhamphi, G. G., Massawe, A. W., & Machang'u, R. S. (2021). Leptospira Seropositivity in Humans, Livestock and Wild Animals in a Semi-Arid Area of Tanzania. *Pathogens*, *10*(6), 696.

Mlowe, G., Makundi, I., Katakweba, A. S., & Machang'u, R. (2023). Seropositivity of Leptospira in rodents, shrews, and domestic animals in Unguja, Tanzania. *Journal of Ideas in Health*, *6*(2).

Motto, S. K., Shirima, G. M., de Clare Bronsvoort, B. M., & Cook, E. A. J. (2021). Epidemiology of leptospirosis in Tanzania: A review of the current status, serogroup diversity and reservoirs. *PLoS Neglected Tropical Diseases*, *15*(11), 1-17.

Mwachui, M. A., Crump, L., Hartskeerl, R., Zinsstag, J., & Hattendorf, J. (2015). Environmental and behavioural determinants of leptospirosis transmission: a systematic review. *PLoS neglected tropical diseases*, *9*(9), 1-15.

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.

Rahman, M. H. A. A., Hairon, S. M., Hamat, R. A., Jamaluddin, T. Z. M. T., Shafei, M. N., Idris, N. & Daud, A. (2018). Seroprevalence and distribution of leptospirosis serovars among wet market workers in northeastern, Malaysia: A cross sectional study. *BioMed Central Infectious Diseases* 18(1), 1 – 5.

Ricardo, T., Bergero, L. C., Bulgarella, E. P., & Previtali, M. A. (2018). Knowledge, attitudes and practices (KAP) regarding leptospirosis among residents of riverside settlements of Santa Fe, Argentina. *PLoS neglected tropical diseases*, *12*(5), 1-19.

Skinner, J. D. & Chimimba, C. T. (2005). *The Mammals of the Southern African Sub-region.* Cambridge University Press, Cambridge. 2pp.

Tilahun, Z., Reta, D., & Simenew, K. (2013). Global epidemiological overview of leptospirosis. *International Journal of Microbiological Research*, *4*(1), 9-15.

URT (2022). *National Population and House Census of Tanzania*. National Bureau of Statistics, Dar es Salaam, Tanzania.

Zhou, J., Huang, X., He, H., Zhang, X., Liu, A., Yang, T., ... & Tan, H. (2009). Epidemiological study on leptospirosa infection of host animals and healthy population in flood areas. *Zhong nan da xue xue bao. Yi xue ban= Journal of Central South University. Medical Sciences*, *34*(2), 99-103

Said, K., Bakari, G. G., Machangâ, R., Katakweba, A. S., & Muhairwa, A. P. (2018). Seroprevalence of canine leptospirosis, in Urban and Periurban, Morogoro, Tanzania. *African Journal of Microbiology Research*, *12*(21), 481-487.

Sato, Y., Hermawan, I., Kakita, T., Okano, S., Imai, H., Nagai, H., ... & Toma, C. (2022). Analysis of human clinical and environmental Leptospira to elucidate the eco-epidemiology of leptospirosis in Yaeyama, subtropical Japan. *PLoS Neglected Tropical Diseases*, *16*(3), 1-24.

WHO (2003). *Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control*. World Health Organization, Geneva. 122pp.

Wynwood, S. J., Graham, G. C., Weier, S. L., Collet, T. A., McKay, D. B., & Craig, S. B. (2014). Leptospirosis from water sources. *Pathogens and Global Health*, *108*(7), 334-338.