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Leptospira infection of rodents captured at the slaughterhouses and their risk to public health in Unguja island, Tanzania

Bakari I. Ngecha[1*], Abdul S. Katakweba[2,3], Ernatus M. Mkupasi[2]

Abstract

Background: Leptospira infection is an invasion of animal or human body with the pathogenic spirochete bacteria of the genus Leptospira resulting to a disease called leptospirosis. This study aimed to investigate Leptospira infection and the carrier status of rodents caught near slaughterhouses at Unguja Island, Tanzania.

Methods: This cross-sectional study was conducted from January to April 2022 at Unguja Island to determine the seroprevalence of Leptospira infection in rodents captured in and around the slaughterhouse's compounds. A total of 302 sera samples from four slaughterhouses were tested for anti-leptospiral antibodies using microscopic agglutination test (MAT) with a panel of 5 Leptospira serovars: Pomona, Lora, Hebdomadis, Grippotyphosa and Sokoine; and were considered positive at MAT titer ≥1:20. Chi-square test and the Fisher exact test were used to assess the statistical association between variables at a p value of < 0.05.

Results: The overall seroprevalence of Leptospira infection in rodents was 10.6% (32/302). Individual rodent species had seroprevalence of 8.5% for Mus musculus, 0.0% for Mastomys natalensis, 20.8% for Rattus norvegicus, 9.3% for Rattus rattus and Cricetomys gambianus at 12.5%. However, the apparent seroprevalence in the individual slaughter facilities was 15.0% (15/100) Kinyasini, 10.4% (5/48) Mfenesini, 9.3% (5/54) Kisakasaka, and 7.0% (7/100) Muwanda.

Conclusion: With over ten percent of rodents testing positive for Leptospira infection, it is crucial to implement rodent control measures to prevent the spread of the disease to those working in or around the slaughterhouses in Unguja Island.

Keywords: Rodents, Leptospira infection, Microscopic Agglutination Test, Seroprevalence, slaughterhouses, Unguja Island, Tanzania

Correspondence: Bakari I. Ngecha (bakari.ngecha@lita.go.tz)

[1]Livestock Training Agency – Morogoro Campus, P. O. Box 603, Morogoro, Tanzania

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Background

Leptospira infection is a term that expresses an invasion of an animal or human body with the pathogenic spirochete bacteria of the genus Leptospira, of which more than 250 leptospires exist in over 30 serologically classified serovars [1], resulting in a disease called leptospirosis [2]. These bacteria have hook-like ends and a helically coiled body [3]. Leptospirosis is a worldwide distributed disease with low incidence in temperate regions and high prevalence in tropical and subtropical regions that have zoonotic, emerging, and re-emerging potential. Most of the infected animals, with or without clinical signs, continuously excrete leptospires into the urine, which contaminates the soil and water in the area [4]. The transmission of Leptospira infection is achieved through contact with bacterially contaminated urine from animals or by contact with Leptospira-contaminated feeds, food, and water [5]. Rodents are the most important reservoirs for different bacterial serovars. Their quantity and broad dispersion make them critical in the maintenance and transmission of bacteria in animals and people [6]. Rodents become infected with Leptospira after interacting with diseased wild animals or entering a polluted environment [7]; vertical transmission, such as transplacental or trans-mammary, and coitus within species are also possible [8]. In most cases, the disease is subclinical in animals. In some circumstances, however, it might be mild, acute, or chronic [9]. Immature animals are more likely to develop a severe form of the disease [4]. Signs may include fever, conjunctivitis, diarrhea,

severe pulmonary hemorrhagic syndrome, hemoglobinuria, and jaundice. Others include poor reproductive performance, abortion, and decreased milk production [10]. In humans, the disease manifests as mild to deadly symptoms such as fever, headache, myalgia, conjunctivitis, dyspnea, crackles, and, in certain cases, significant hemoptysis [11]. Since 1974, when Pro. Semguruka conducted a postmortem examination of a dog at the Faculty of Veterinary Medicine of Sokoine University of Agriculture (SUA), studies on leptospirosis in humans and animals have continued in various settings in Tanzania. The incidence in humans has been reported to increase annually [12]. The recent leptospirosis studies reported a prevalence of 15.5% and 36% in rodents and febrile humans [13, 14], respectively. The very recent case of Leptospira infection in humans in Tanzania occurred in 2022 during an outbreak of Leptospirosis in the Lindi region, which resulted in a few deaths [15]. This outbreak was argued to have occurred due to unsupervised agricultural practices, urbanization, and a lack of sanitation, which made rodents invade and contaminate the environment where humans could be infected when they came in contact with them [16, 17]. In Tanzania, especially Unguja Island, there have been few reports of leptospirosis despite the reports of the leptospirosis burden worldwide [13, 18]. Like those in other developing countries, slaughterhouses in Unguja feature poor sanitation, inappropriate waste disposal, and sewage, which promotes the spread of rodents and poses a danger of Leptospira infection to slaughter facility personnel [19]. This study assessed the Leptospira infection and carrier status of rodents captured around the slaughterhouses in Unguja Island, stressing the possible hazards to animals and people. The results will help develop leptospirosis prevention and control efforts in Unguja.

Methods

Study design and participants

Description of the study area

This cross-sectional field study was carried out over a period of three months, from January to April 2022, at the selected slaughterhouses in Unguja Island (Figure 1).

Study area

Unguja Island is divided into three regions: Kasikazini Unguja, Kusini Unguja, and Mjini Magharibi. However, the data was gathered at the following locations: Mfenesini slaughterhouse in Magharibi A district; Kisakasaka slaughterhouse (6015’26.6’’S 39016’44.1’’E) in Magharibi A district; Kasikazini Unguja at the Muwanda (5054’36.1’’S 39013’33.5’’E) and Kinyasini (5058’33.9’’S 39018’48.5’’E) slaughterhouses in North A district; and Mjini Magharibi area at the Mfenesini slaughterhouse (6002’21.3’’S 39013’33.7’’E) in Magharibi A district. The daily slaughtering activities and quarantine points of imported slaughter animals served as the basis for selecting data collection.

Sample size

The sample size was calculated using the formula n = Z 2 P (1-P)/d2 [20], where n is the required sample size, P is the estimated previous prevalence of leptospirosis, d is the desired absolute precision at 5%, and Z is the standard normal deviation (1.96) at a 95% confidence level. Thus, the expected prevalence of leptospirosis in rodents was 15.5% [13]. The estimated minimum sample size for the study was 201 rodents; however, we were able to collect a total of 302 rodent samples.

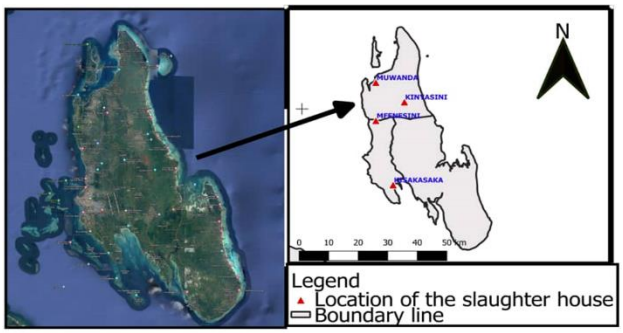

Figure 1: Map of Unguja Island showing the selected and sampled areas

Rodent trapping

Rodents dwelling in the slaughterhouse surroundings were trapped using Sherman traps and locally made wire mesh traps, in which peanut butter in combination with maize flour was used as a bait. The traps were set up every evening and picked up early the following day. A hundred traps were set in each trapping area around the slaughter facility, where five traps were spaced 10 yards apart in a line outside the fences for fourteen consecutive nights.

Sample collection from captured animals

All traps that contained rodents were collected and delivered to the Department of Livestock Development at Maruhubi's Zanzibar Central Veterinary Laboratory for the identification of species, sex (male or female), and age (juvenile or mature) based on morphological appearance as described by Krystufek and Vohralík [21]. Also, blood samples were collected through a heart puncture after anesthetizing them with di-ethyl ether.

Serum samples preparation and storage

The clotted blood in the Eppendorf tubes was centrifuged for five minutes at 3500 rpm, after which the serum was aspirated using a tipped micropipette and put into fresh Eppendorf tubes for storage at -20 °C at Maruhubi's Zanzibar Central Veterinary Laboratory before being transported to the Leptospirosis Laboratory of the Institute of Pest Management (IPM) at Sokoine University of Agriculture, Morogoro, for analysis.

Detection of Leptospira infection

Following the guidelines set by the World Organization for Animal Health (WOAH) [22, 23], the microscopic agglutination test (MAT) was conducted in all sera. A panel of five Leptospira serovars, namely Sokoine, Lora, Grippotyphosa, Hebdomadis, and Pomona, which are thought to be the most common in Tanzania, were primarily obtained from rodents and domestic animals [24, 25]. Leptospira infection was considered positive in a microtiter well if the titer was ≥1:20 [24].

Statistical analysis

Proportions were calculated using descriptive statistics, and comparisons were made using the Chi-square and Fisher exact tests supported by SPSS version 23 (IBM SPSS Statistics Inc) in Microsoft Excel 2010. When the value of p was less than 0.05, the results were deemed statistically significant.

Results

Distribution and diversity of the captured rodents

Across four investigated slaughterhouses, 302 rodents were captured over 2,584 trap nights, resulting in an 11.7% trap success rate. The captured rodents were identified as five different species, with Rattus rattus (39.1%) being the most prevalent, followed by Mus musculus (31%), Rattus norvegicus (15.9%), Mastomys natalensis (6%), and Cricetomys gambianus (8%). The distribution of species varied across the different slaughterhouses, with Kinyasini and Muwanda facilities having the highest number of rodents captured (Table 1).

Seroprevalence of Leptospira infection

Table 2 provides a breakdown of seroprevalence across various demographic variables of the rodents. The study found an overall seroprevalence of 10.6% for Leptospira infection among 302 rodents tested using the Microscopic Agglutination Test (MAT) with five different Leptospira serovars. Findings of this study showed slight variations in Leptospira seroprevalence among rodents based on age, sex, species, and slaughterhouse location. Adult rodents and females showed slightly higher rates of infection, with Rattus norvegicus having the highest species-specific seroprevalence. The Kinyasini slaughterhouse had the highest seroprevalence among locations. However, none of these differences were statistically significant.

Titers of tested serovars

The study assessed the seroprevalence of different Leptospira serovars in rodent samples, with antibody titers ranging from 1:20 to 1:160. Hebdomadis and Lora serovars had the highest seroprevalence at 2.98% each, followed by Sokoine at 2.32%, Grippotyphosa at 1.66%, and Pomona at 0.66%. In total, 10.6% of the rodent samples tested positive for Leptospira antibodies, with 32 out of 302 rodents showing titers at or above 1:20 (Table 3).

Table 1. Rodent species collected in respective slaughter facilities (n=302).

Slaughter facility	Species collected					
	Rattus rattus	*Mus musculus*	*Rattus norvegicus*	*Mastomys natalensis*	*Cricetomys gambianus*	Grand Total
Kinyasini	34	39	17	1	9	100
Kisakasaka	25	9	7	6	7	54
Mfenesini	19	21	6	1	1	48
Muwanda	40	25	18	10	7	100
Grand Total (%)	118(39.1)	94 (31)	48 (15.9)	18 (6)	24 (8)	302

Table 2: Distribution of seroprevalence among different demographic groups of the rodents (n=302).

Variable	**Category**	**Total number of samples (%)**	**Number of positive**	**Prevalence (%)**	***P*-value**
Age	Adult	229 (75.8)	26	11.4	0.519
	Juvenile	73 (24.2)	6	8.2	
Sex	Female	177 (58.6)	20	11.3	0.7068
	Male	125 (41.4)	12	9.6	
Species	*M. musculus*	94 (31)	8	8.5	0.133305
	M. natalensis	18 (6.0)	0	0	
	R. norvegicus	48 (15.9)	10	20.8	
	R. rattus	118 (39.1)	11	9.3	
	C. gambianus	24 (8.0)	3	12.5	0.31871
Slaughter facility	Kinyasini	100 (33.1)	15	15	
	Kisakasaka	54 (17.9)	5	9.3	
	Mfenesini	48 (15.9)	5	10.4	
	Muwanda	100 (33.1)	7	7	
Overall seroprevalence		302	32	10.6	

Table 3: Seroprevalence and specific tested Leptospira serovar, and their antibody titers (≥ 1:20) among rodents (n=302)

Serovars	1;20	1;40	1;80	1;160	Leptospira positive	Seroprevalence (%)
Hebdomadis	1	5	3	0	9	2.98
Sokoine	0	3	2	2	7	2.32
Lora	1	2	4	2	9	2.98
Grippotyphosa	0	1	4	0	5	1.66
Pomona	0	0	2	0	2	0.66
Total	2	11	15	4	32	10.6

Discussion

The study found an overall seroprevalence of 10.6% in rodent species trapped in and around the slaughterhouses in Unguja. This finding suggests that, while rodents are natural hosts for various Leptospira serovars, at slaughter facilities, rodents can acquire Leptospira infection by feeding on animal leftovers and fluids from infected slaughter animals, perpetuating contamination of environments and spreading the infection to slaughterhouse personnel [26]. Workers and other in-touch persons at slaughterhouses are thus in danger of developing Leptospira infection if they come into contact with rodent urine-contaminated settings [13]. The study's findings are consistent with previous research conducted on the Tanzanian mainland (10–11%) [27, 28], as well as studies from Cambodia and Malaysia (11–11.1%) [29]. However, this figure is slightly lower than other previous studies conducted on Tanzania's mainland (15.5-28.5%) [13, 24, 27, 30]. The differences in prevalence could be attributed to a variety of reasons, including the predominant rodent species captured in the study area, such as Rattus rattus (n = 118), which has less potential for hosting leptospires in Tanzania [31], and a smaller number of the captured Rattus norvegicus (n = 48), which is an important maintenance host of several Leptospira serovars [1, 32, 33]. Apart from that, variation in rodent seropositivity may be associated with locale ecology, sample collection season (higher during rain), sample size, and laboratory testing methodologies used [29, 34]. Nonetheless, Rattus rattus and Mus musculus, possible leptospirosis reservoirs [35, 36], were widely captured in all slaughter sites. It could be owing to the placement of traps in arid habitats that are conducive to Rattus rattus, as well as their relative abundance in comparison to other rodent species in the study area. A smaller number of Rattus norvegicus (n = 48) and Cricetomys gambianus (n = 24) were captured than Rattus rattus and Mus musculus, but they had a higher proportion of Leptospira seropositivity (20.8% and 12.5%, respectively) (Table 2.2). This could be due to marshes, sewers, and filthy conditions at slaughterhouses, which promote the growth of pathogenic leptospires and are highly preferred by Rattus norvegicus and Cricetomys gambianus [33, 37].

Mastomys natalensis, on the other hand, tested negative for all five selected Leptospira serovars. This result is in line with the earlier study's finding that rodents had a 0% prevalence [31], and it is also corroborated marginally by Mgode et al. [13], who discovered Mastomys spp. have a low prevalence of Leptospira infection. This could be due to habitat preference, as they prefer peridomestic areas in shrubs and grasslands that unfavorable to Leptospires life, and a small number of Mastomys natalensis (n = 18) that were trapped [30, 38, 39], or they would have been infected with Leptospira serovars other than those included in the diagnosis. In addition to these factors, the dry season in which this study was conducted has a limited impact on rodent infection rates and abundance [40]. Kinyasini had the highest specific apparent seroprevalence (15%), followed by Mfenesini (10.4%), Kisakasaka (9.3%), and Muwanda (7%), respectively. This would be coupled with the facilities' poor sanitary measures, which resulted in the accumulation of solid waste, attracting stray dogs and rodents. With the exception of the Muwanda slaughterhouse, the remaining areas are flanked by resident farms (Kinyasini and Mfenesini) and overgrown vegetation. Other studies have found that all of these conditions enhance the possibility of Leptospira infection [41]. However, the difference between them was not statistically significant (P = 0.31871). The study found that adults (11.4%) had greater seropositivity than juveniles (8.2%) of the caught rodents, while the difference was not significant (p = 00.519). This result agrees with the results of earlier studies [42, 43], and it could be attributed to the adult rodents' prolonged exposure to Leptospira interrogans. The sex factor revealed that males had a somewhat lower incidence of infection (9.6%, 12/125) than females (11.3%) (20/177). Previous investigations have reported similar findings [44, 45]. This could be linked to female rodents' higher activity and longer life spans, which may have exposed them to Leptospira infection [46]. The Leptospira seroprevalence for the selected serovars had relatively high titers (1:40), with only two Mus musculus sera having a low titer (1:20) in serovar Hebdomadis and Lora, which could be due to chronic leptospirosis infections or non-specific cross-reactivity infections [24]. In contrast, Sokoine serovar had high antibody titers (1:160) in one Mus musculus and one Rattus novergicus, while Lora had strong antibody titers (1:160) in two Rattus norvegicus. This reactive potential of Sokoine demonstrated in our investigation is corroborated by earlier studies in rodents and shrews [24, 25], and it is likely to be an indicator of acute leptospirosis at or around sampling time [47]. This study had some limitations, including the fact that it was restricted to the dry season and did not take seasonal variations into account, that few Leptospira serovars were examined, and that no information was obtained from workers at slaughterhouses.

Conclusion

In conclusion, the presence and spread of Leptospira infection in and around slaughter plants is evidenced by rodent infestations. This study describes the first seroprevalence of Leptospira infection in rodents around the slaughterhouses in Unguja. As a result, slaughterhouse personnel should be made aware of Leptospirosis and its potential for zoonosis. The respective authorities should implement effective Leptospira infection prevention techniques, such as rodent control and the One Health strategy. Future research should focus on slaughter facility workers to determine the status of circulating Leptospira serovars, as they share the same environment as rodents and livestock brought for slaughter.

Abbreviation

ACE: African Center of Excellence, BTD: Biosensors Technology Development, IRPM: Innovative Rodent Pest Management, CDC: Center for Disease Control, CNS: Central Nervous System, EMJH: Ellinghausen-McCullough-Johnson-Harris culture medium, IPM: Institute of Pest Management, MAT: microscopic agglutination test; µm: microliter; NBS: National Bureau of Statistics; OCGS: Office of Chief Government Statistician; OIE/WOAH: World Organization for Animal Health; RPM: revolutions per minute, SUA: Sokoine University of Agriculture; WHO: World Health Organization; ZALIRI: Zanzibar Livestock Research Institute; ZFDA: Zanzibar Food and Drug Authority.

Declaration

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Availability of data and materials

Data will be available by emailing bakaringecha@lita.go.tz

Authors' contributions

Bakari I. Ngecha (BIN) is the principal investigator (PI) who designed the study, collected data from the field, analyzed and interpreted the data, and drafted the manuscript. Ernatus M. Mkupasi (EMM) and Abdul S. Katakweba (ASK) participated in analyzing and interpreting the data, revised the manuscript, and approved it before submitting it to the journal for publication. The authors read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. Permission to conduct this study was granted by the Ethical Clearance Committee (SUA/DPRTC/R/03/2022, March 17, 2022) of Sokoine University of Agriculture, Morogoro, Tanzania, and Zanzibar Research Committee in the Office of the Second Vice President (Reference number: 61E93966AC21B, January 20, 2022) and other respective authorities, including the Office of Chief Government Statistician (OCGS), Ministry of Agriculture, Irrigation, Natural Resources, and Livestock, Zanzibar Livestock Research Institute (ZALIRI), Zanzibar Food and Drug Authority (ZFDA), District Commissioner Officer of Kasikazini A, Kasikazini B, and Magharibi B. Also, the local administrative officers (Shehas) and the slaughter facilities supervisors.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Author Details

[1]Livestock Training Agency – Morogoro Campus, P. O. Box 603, Morogoro, Tanzania.

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

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

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