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Original Article

Seroprevalence of Leptospira infection in slaughtered cattle in Unguja Island, Zanzibar, Tanzania

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Abstract

Background: Leptospirosis is an important disease of global distribution affecting humans and animals in the tropical and subtropical regions caused by pathogenic Leptospira serovars. It's an occupational disease with little information in Unguja Island, Zanzibar.

Methods: A cross-sectional study was conducted in four selected slaughter facilities to determine the seroprevalence for Leptospira infection in slaughtered cattle in Unguja Island, Zanzibar. The blood samples and demographic data from 355 slaughtered cattle were collected and sera were separated for the Microscopic Agglutination Test (MAT) by using five types of Leptospira serovars; Hebdomadis, Sokoine, Lora, Grippotyphosa and Pomona with cutoff titer ≥ 1:40. The Chi-square test at $p < 0.05$ was used to assess the association between the variables and seropositivity of Leptospira infection.

Results: The overall seroprevalence of Leptospira infection in the slaughtered cattle sampled was 13.0% (46/355). The predominant serovars from the tested serogroups were Hebdomadis (3.9%), followed by Pomona (2.8%), Grippotyphosa (2.8%), and Lora (2.3%); while the least reacted was Sokoine (1.1%). The body condition score was the only significant significant variable ($\chi2=103.9038$, $p=0.00001$) associated with Leptospira infection seropositivity.

Conclusion: The study offers the first report on the Leptospira seroprevalence in slaughtered cattle on Unguja Island. This might be a probable source of infection to slaughter facilities workers and other animals encroaching on the area. Therefore, precautions should be observed to prevent infection, especially for slaughter facility workers in Unguja.

Keywords: Seroprevalence, Leptospirosis, Microscopic Agglutination Test (MAT), Slaughter Facilities, Unguja Island, Tanzania

Background

Leptospirosis is a neglected occupational zoonotic infection caused by members of pathogenic Leptospira [1, 2]. It has a cosmopolitan pattern of distribution but is much more prevalent in tropical regions including Tanzania [3]. The infection clinically may manifest in acute, subacute, or chronic with asymptomatic or signs that mimic other febrile diseases [3, 4]. Slaughter animals may asymptomatically maintain Leptospira interrogans in their renal tubules [5, 6] posing an occupational risk to slaughter facility workers following direct contact with the infected animal tissues [7, 8]. Since leptospirosis was reported in Tanzania, several studies have been conducted to determine the Leptospira antibodies in humans and animals; though they have been reported to be conducted in a few regions [9]. The absence of documentation in other regions may pose a public health risk to workers, particularly the slaughter facility workers in those unstudied regions including Unguja Island [10, 11, 12] because of the nescience of leptospirosis. Based on the National Census, the population of Unguja Island has been reported to increase [13]. This population growth together with the increased tourism industry in Zanzibar expands demands for meat; hence bringing a vast potential for increased importation of slaughtered animals from Tanzania mainland [14].

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However, some of the regions where slaughter animals are imported from, have reported the presence of leptospirosis infection for instance Tanga [11], Mwanza [15], Morogoro [16, 17], and Kilimanjaro [18]. This means animals imported for slaughter in Unguja slaughter places may carry Leptospira infection that would be a threat to the slaughter facilities workers and animal products consumers if biosafety measures are not observed. This study determined the seroprevalence and the associated risk factors of Leptospira infection in the slaughtered cattle at the slaughter facilities in Unguja Island, Zanzibar, Tanzania.

Methods

Study design and setting

This was a cross-sectional study conducted between January 2022 and April 2022 in Unguja, a major Island of Zanzibar; a semi-autonomous part of Tanzania with a surface area of approximately 1,600km2 in the Indian Ocean about 30-40km from the coast of mainland Tanzania [19]. Around two-thirds of the 1.8 million population lives in Unguja, with the West region being the most populated [13]. It has an annual average rainfall of 1,500 to 2,000 mm [20]. The study involved four purposively selected slaughter facilities (figure 1); Donge-Muwanda (5054’36.1’’S 39013’33.5’’E), Kinyasini (5058’33.9’’S 39018’48.5’’E), Mfenesini (6002’21.3’’S 39013’33.7’’E) and Kisakasaka (6015’26.6’’S 39016’44.1’’E). The selection bases of the slaughter facilities were daily slaughtering activity, the average number of slaughtered animals, and diverse sources of slaughter animals.

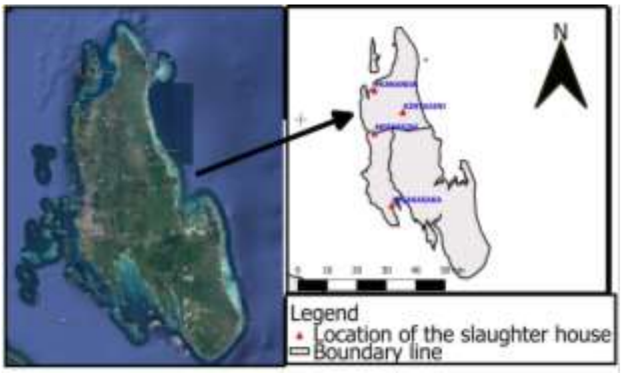

Figure 1: A map showing study areas (slaughter facilities) in Unguja Island

Sample size

The sample size for slaughter cattle (n = 355) was obtained from the formula n = Z2 P (1-P)/d2 [21] to give the study sufficient power to estimate the required prevalence at a precision of 5% from the expected prevalence of 30% [11].

Study tool

Sampling procedure, collection, processing, and storage of sera

The procedure involved systematic random sampling of slaughtered cattle by counting them as they went through the door of the slaughtering house where every third cattle was selected and sampled. The demographic data including; origin, age (adults/yearlings, as recorded by vendors), slaughter facility, and body condition score (based on a 1–5-point BCS system for beef cattle), were also collected against each sampled slaughtered cattle [22]. Then the body condition score for the slaughtered cattle was categorized into two groups; fair (1-2) and average/moderate (3). The whole blood (3-4mls) was aseptically collected in plain vacutainer tubes (Becton, Dickinson and Company, USA) during the slaughtering process after cattle were stabbed. The samples were left to clot stored in a cool box and transported to Zanzibar Central

Veterinary Laboratory under the Department of Livestock Development located at Maruhubi where samples were centrifuged for 3500rpm in 5 minutes, and sera were transferred into new Eppendorf tubes and then stored at -20°C before being transported to Leptospirosis Laboratory at the Institute of Pest Management (IPM) at Sokoine University of Agriculture (SUA), Morogoro where Microscopic Agglutination Test (MAT) procedure was performed.

Antibody Detection

Microscopic agglutination test (MAT) was used to detect Leptospira antibodies in all sera as it was described by Ngugi et al. [6]. It was 10µl of the sera that was mixed with 90µl phosphate-buffered saline (PBS) in each well of the microtiter plates to obtain 100 µl (1:10 dilutions). Then double dilutions of the serum sample were made in all wells by pipetting 50µl of the serum and PBS mixture. 50µl of the fully grown Leptospira serovars was added into all microtiter plate wells containing serum-PBS mixture. Thereafter gently mixed for 30 seconds, then covered and incubated at 30 °C for 2 hours. The serum antigen mixture was examined by dark field microscopy (DF) for the presence of agglutination of the Leptospira, with the reported titers being the highest dilution of serum that results in 50% agglutination [2]. Positive samples were further titrated to detect the endpoint titers [23, 24]. In this study, we used a panel of five live Leptospira serovars which were reported to be prevalent in Tanzania viz were Sokoine, Lora, Grippotyphosa, Hebdomadis, and Pomona [25, 26].

Statistical analysis

Descriptive statistics were used to determine the infection prevalence in Microsoft Excel 2010 (Microsoft Corp, Redmond WA, USA). The chi-square test and Fisher exact test in SPSS version 20 (IBM SPSS Statistics Inc, College Station, TX, USA) were used to determine the associations between exposure variables and Leptospira seropositivity. The results were considered statistically significant at a value of $p < 0.05$.

Results

Socio-demographic characteristics of study participants

A total of 355 slaughtered cattle from four purposely selected slaughter facilities in Unguja Island were sampled (Table 1).

Table 1: Sample collected from the slaughter facilities (N=355)

Slaughter facility	Frequency	Percent
Kinyasini	35	9.9
Kisakasaka	100	28.2
Mfenesini	20	5.6
Muwanda	200	56.3
Total	355	100

The overall seroprevalence of 13.0% (46/355) and the specific seroprevalence of Leptospira infection of the slaughtered cattle at the specific slaughter facility were indicated in (Table 2). The results for the variables included in the study are displayed in Table 3. Out of 46 positive cases, 40(12.7%) were adults. Most of them (39,12.3%) were males. Indigenous breed was 41(12.9%). The body condition score was fair among 35(49.3%), and 21(24.4%) originated from Pangani. Among the tested serovars, serovar Hebdomadis (14, 3.9%) was highly prevalent and serovar Sokoine (4, 1.1%) was the least prevalent (Table 4).

Table 2: Overall seroprevalence and specific seroprevalence for the slaughter facility (N=355)

Slaughter facility	Number of samples	Number of positive	Prevalence (%)	P-value
Kinyasini	35	5	14.3	
Kisakasaka	100	15	15	
Mfenesini	20	7	35	0.01089
Muwanda	200	19	9.5	
Total	355	46	13	

(Cut-off titer ≥ 1:40)

Table 3: Slaughtered cattle variables and their specific Leptospira infection prevalence and p-values (N=355)

Variable	Category	Positive	Prevalence (%)	P-value
Age	Adult	40	12.7	0.6229
	Yearling	6	15	
Sex	Female	7	18.4	0.3056
	Male	39	12.3	
Breed	Crossbreed	4	16.7	0.73585
	Exotic	1	7.7	
	Indigenous	41	12.9	
BSC	Good	11	3.9	0.00001
	Fair	35	49.3	
Origin	Bagamoyo	8	9.3	
	Handeni	8	9.8	
	North B, Unguja	0	0	0.14669
	Kilindi	7	9	
	Muleba	2	13.3	
	Pangani	21	24.4	

Table 1: The Leptospira serovars, their MAT titers, and specific prevalence (N=355)

Serovar	Titers				Prevalence (%)	P-value
	1:40	1.80	1:160	Total		
Hebdomadis	2	8	4	14	3.9	0.207374
Sokoine	0	2	2	4	1.1	
Lora	2	3	3	8	2.3	
Grippotyphosa	7	3	0	10	2.8	
Pomona	3	4	3	10	2.8	

Discussion

The study reports an overall seroprevalence (13.0%) of Leptospira infection in apparently healthy slaughtered cattle at the selected slaughter slabs/facilities in Unguja. The study indicates the occupational hazard to slaughter facilities workers if protective measures are not observed. This is because they are always in contact with infected animals' contaminated fluids and tissues as also reported elsewhere [6]. Several studies have been conducted to reveal the widespread and endemicity of Leptospira infection in cattle in Tanzania and other regions of Africa [27, 28]. However, this is the first study in Unguja that aimed to determine the serological prevalence of infection in slaughtered cattle. The seroprevalence proportion for the current cross-sectional study is higher than 5.6 -7.08% reported in cattle slaughtered in some facilities in Tanzania [29, 27], and 3.5% from Zango abattoir in Nigeria [30]. On the other hand, the current seroprevalence was slightly comparable to 10.33% of dairy cattle from Toluca Valley, Mexico [31]. Also, it was lower than 30.3%, 51% [11, 32] of cattle slaughtered at Tanga City abattoir, Tanzania, 27.8% of Ugandan slaughter cattle [33], and 27.6% of slaughtered cattle in Gauteng province, South Africa abattoirs [28]. The variation in cut-off titers could be attributed to these differences in the seroprevalence of Leptospira infection in different studies; since lowering the cut-off titer may result in a higher estimation of Leptospira-antibody positivity [6, 34]. Also, the differences could be due to the stage of the disease, agroecological location, sample size, and spectrum of serovars used [35, 28]. Of the used reference Leptospira serovars (table 3); Hebdomadis (3.9%) was the most prevalent serovar; similar to the earlier study conducted in Katavi, Tanzania mainland (7.7%) (n = 1103), though its proportion was higher [23]. Pomona was the second serovar in predominance in this study; its proportion (2.8%) was somehow close to that of the previous study conducted in Usambara, Tanzania mainland (2.5%) (n = 80) [36], on the other hand, it was higher than those reported in Tanga 1.3% (n=230) and 1.2% (n=654) [37, 32]. Most of the slaughtered and sampled cattle originated from some districts of Tanzania mainland and only a few from North B, Unguja with none infected. The Leptospira-antibody seropositivity was detected in cattle from all sourced districts of Tanzania mainland. These findings indicate that cattle imported from the Tanzania mainland are likely to be an important source of Leptospira infection in the personnel working at the slaughter facilities in Unguja Island [12, 32, 11, 17]. The Leptospira-antibody seronegativity of slaughtered cattle from North B, Unguja could be due to the narrow spectrum of serovars included in the MAT testing panel and a small number of samples (n=8). Furthermore, the study reported adults slaughtered cattle were less seroprevalent (12.7%) than yearlings (15.0%). This could be due to the intensive management of yearlings at their calfhood that favored the easy spread of infection. Our finding diverged from the previously reported findings which stated higher seropositivity in adult cattle due to longer exposure time and persistence of the antibodies [38]. However, there was no statistically significant difference (p=0.6229) in Leptospira infection seropositivity between the two age groups. Likewise, the breeds of the slaughtered cattle had no statistically significant difference (p=0.735851) in the seroprevalence of Leptospira infection in this study; as was reported in the previous studies [28]. The study also revealed that sex did not influence the infection seroprevalence although females had slightly higher (18.4%) than males (12.3%). This result approves the previous study which reported seroprevalence in cows (4.92%) and bulls (2.47%) [30]. This difference could be attributed to cows being kept longer for breeding than bulls increasing chances of exposure to the infection. The body condition score (BSC) was the most important risk factor for Leptospira-antibody seropositivity. Of which the slaughtered cattle with fair BCS (49.3%) had higher seroprevalence compared to good BCS (3.9%), and there was a statistically significant difference between BCS ($\chi 2$=103.9038, p=0.00001). This might be attributed to the high stocking rate, undernourishment, and tropical animal diseases which compromised the body's immune system [38]. This study had two limitations. First, the study did not determine Leptospira seroprevalence in the slaughter facilities workers therefore; direct risk could not be established. Second, few Leptospira serovars were included in this MAT study; this could aid the underestimation of the seroprevalence of Leptospira infection in the population.

Conclusion

The evidence of seroprevalence of Leptospira infection in the slaughtered cattle in this study signifies the possible occupational risk of Leptospira infection in people working at the slaughter facilities in Unguja Island. Therefore, there is a need for research efforts that will produce relevant information toward achieving optimal general human and animal health in Unguja Island. Finally, the One Health approach should be undertaken to prevent and control Leptospira infection.

Abbreviation

ACE: African Center of Excellence, BTD: Biosensors Technology Development, IRPM: Innovative Rodent Pest Management, BCS: Body condition score, CDC: Center for Disease Control, CNS: Central Nervous System, EMJH: Ellinghausen-McCullough-Johnson-Harris culture medium, GPS: Global positioning system, IPM: Institute of Pest Management, MAT: Microscopic agglutination test, MM: Millimeter, µm: Microliter, NBS: National Bureau of statistics, OCGS: Office of Chief Government Statistician, PPE: Personal protective equipment, OIE: 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

Acknowledgment

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Availability of data and materials

Data will be available by emailing bngecha@yahoo.com

Authors' contributions

Bakari I. Ngecha (BIN) is a principal investigator (PI) who is involved in study design, and data collection from the field and performed laboratory and statistical analysis of the data, manuscript drafting, and writing. Ernatus M. Mkupasi (EMM), Robert S. Machangu (RSM), and Abdul S. Katakweba (ASK) were the supervisors, who were also involved in the study design, result interpretation, and review of the manuscript. All authors read and approved the final manuscript for submission to the journal for publication.

Ethics approval and consent to participate

The 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.

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