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Seroprevalence of Leptospira spp serovar hardjo among Cattle Slaughtered at Katsina Central Abattoir, Katsina State, Nigeria

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Abstract

Leptospirosis is a globally distributed zoonotic disease caused by the bacterium Leptospira spp. serovar hardjo, posing a significant threat to livestock industries and public health. This study investigated the seroprevalence of Leptospira hardjo infection among cattle slaughtered at Katsina Central abattoir. A total of 179 blood samples were aseptically collected from the jugular veins of various cattle breeds using anticoagulant-free vacutainers. The samples were centrifuged to separate sera, followed by analysis using an indirect Enzyme-Linked Immunosorbent Assay (ELISA) based on the manufacturer's protocol (ELISA Bovicheck® Lepto HP, Bioveta, Canada). The overall prevalence of Leptospira spp. serovar hardjo was 16.2%. Bulls exhibited a higher prevalence (20%) compared to cows (14.3%), although no significant difference was observed between sexes (p > 0.05). Age-specific prevalence indicated that younger cattle were more exposed; however, statistical associations were not significant (p > 0.05). Breed-specific prevalence revealed higher rates in White Fulani cattle (20.8%) and lower in Red Bororo (12.9%), with no statistically significant association between breed and infection (p > 0.05). This study indicates a high prevalence of Leptospira spp. infectionamong cattle in Katsina Central abattoir. The occurrence of leptospirosis in these cattle poses occupational risks for abattoir workers, endangers livestock productivity, and raises significant public health concerns due to its potential to spread from animals to humans.

Keywords: ***Abattoir, Cattle, ELISA, Katsina State, Leptospira hardjo, Sero-survey, Slaughter***

INTRODUCTION

Leptospirosis is a neglected tropical zoonotic disease of significant public health concern, causing substantial mortality in both humans and animals (Udechukwu *et al.*, 2024). It ranks as one of the most critical global zoonotic bacterial diseases, with an estimated annual burden of approximately 1 million cases and 60,000 deaths (Costa *et al.*, 2015; Bradley and Lockaby, 2023). The prevalence of leptospirosis is increasing worldwide, particularly in tropical and developing countries (Kumar, 2013). The disease is caused by pathogenic species of *Leptospira*, members of the *Leptospiraceae* family within the order *Spirochaetales* (Fraga *et al.*, 2024). Dogs, rodents, cattle, pigs, various wildlife, and other mammals serve as important reservoirs for leptospirosis, often acting as accidental hosts (Solomon *et al.*, 2012).

Cattle specifically serve as maintenance hosts for serovars such as *Leptospira* serovars Hardjo, Pomona, Grippotyphosa, Icterohaemorrhagiae, and *Canicola* (Udechukwu *et al.*, 2024). Transmission to humans occurs primarily through direct contact with infected animals or their urine-contaminated environments (Kumar, 2013). This makes leptospirosis an occupational hazard for individuals frequently handling animals, such as livestock producers, abattoir workers, veterinarians, hunters, game managers, animal control personnel, and scientists (Karpagam *et al.*, 2020; López-Robles *et al.*, 2021; Sykes *et al.*, 2022).

Leptospirosis presents a wide spectrum of clinical symptoms in both humans and animals. In cattle, it is a leading cause of reproductive failures such as dystocia, abortion, stillbirths, and infertility (Tilahun *et al.*, 2013; Robi *et al.*, 2023; Dereji *et al.*, 2024), resulting in significant economic losses. In humans, symptoms range from fever (38-40°C), rigors, headache, retro-

orbital pain, photophobia, muscle pain localized to the calves and lumbar region, conjunctival suffusion, dry cough, nausea, vomiting, and diarrhea. Severe cases may progress to icteric leptospirosis (Weil's disease), characterized by jaundice, renal failure with oliguria, hemorrhagic features, systemic inflammatory syndrome or shock (Sandra, 2024).

The Microscopic Agglutination Test (MAT) is the standard diagnostic tool for leptospirosis; however, it has limitations, including reliance on live antigens, subjective result interpretation, and an inability to detect antibody titers of ≤100 (Dereji *et al.*, 2024). Cross-reactions between serovars further complicate diagnosis. ELISA has been developed as an alternative for screening infections and detects IgG antibodies that persist longer than IgM antibodies, as measured by MAT (Ngbede *et al.*, 2013). This makes ELISA more suitable for identifying chronic infections and monitoring herd-level exposure to bovine leptospirosis at a lower cost and with greater ease (Ngbede *et al.*, 2013). The study aimed to determine the importance of improved diagnostic tools, such as ELISA, for better surveillance and management of leptospirosis in endemic regions.

MATERIALS AND METHODS

Study Area

This study was conducted in Katsina, within the Sudan Savannah ecological zone, at latitudes 13°00'N and longitudes 7°36'E. The region experiences a short rainy season from June to October, with annual rainfall ranging between 500 and 800 mm. Temperatures range from a minimum of 21°C to a maximum of 35°C. Relative humidity varies between 20% and 40% in January and rises to 60% to 80% in July (Saulawa *et al.*, 2012). This climatic profile supports conditions favorable for the persistence of *Leptospira* spp.

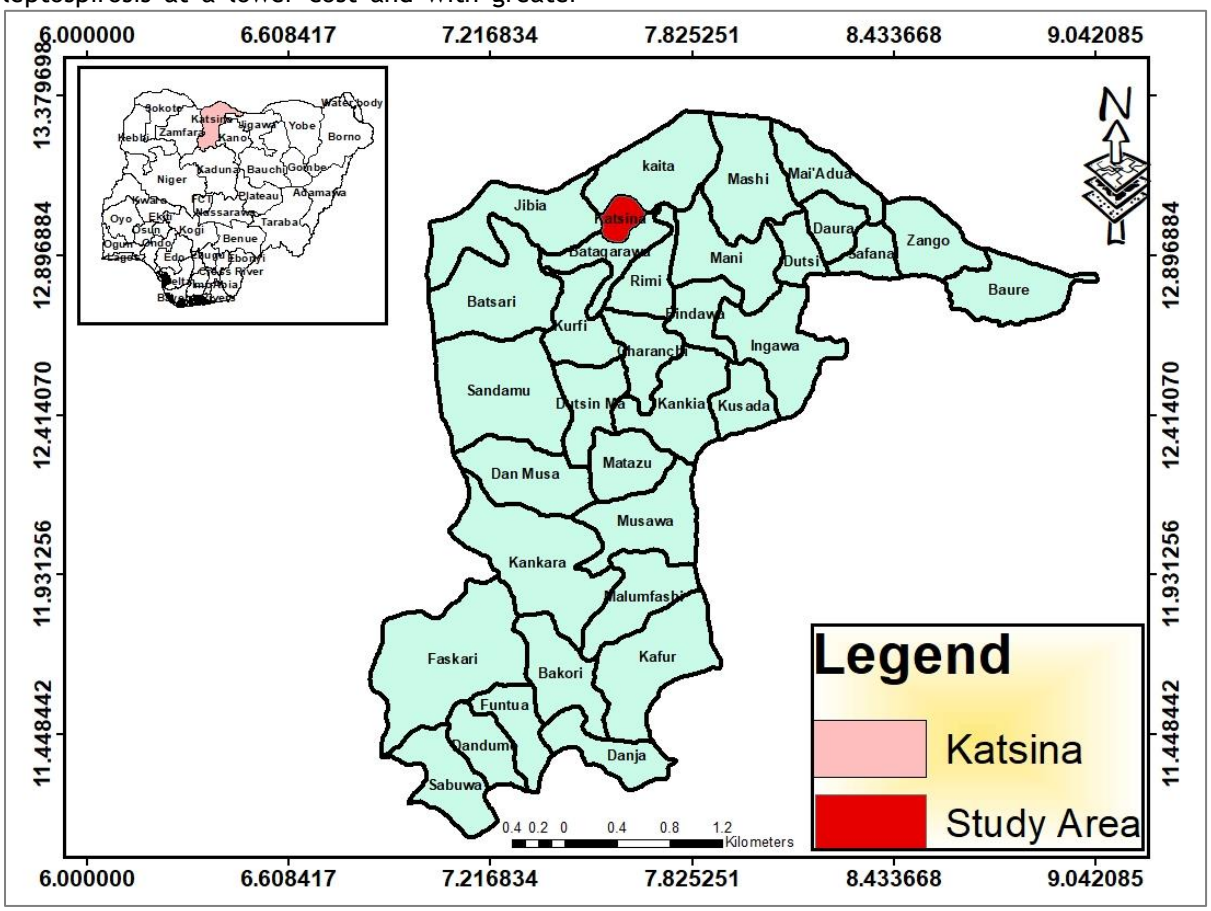

Figure 1: Map of Katsina State showing the 34 LGA's of the State and the Study Area
Source: Saulawa *et al.*, 2012

Study Design

The average daily slaughter rate at Katsina Central Abattoir ranges between 20 and 30 cattle, with approximately 750 cattle slaughtered per month. Consent for conducting the study was obtained from the Manager of Katsina Central abattoir. Following the

recommendations of the World Organization for Animal Health (OIE, 2008), 10% of the monthly average number of cattle slaughtered was sampled throughout the study period. The study population consisted of cattle aged one year or older. Data on the age, sex, and breed of each animal were systematically collected and recorded using a structured data form.

Consent Approval

The official consent of the abattoir authority was soughed before the commencement of the research at Katsina Central abattoir, Katsina LGA, Katsina State.

Inclusion and Exclusion Criteria

All cattle slaughtered inside the slaughter hall of Katsina Central abattoir during the course of the research was included. Cattle slaughtered outside the Katsina Central abattoir slaughter hall were excluded from the study.

Collection of Demographic Data

A comprehensive pre-slaughter antemortem examination was conducted for all the cattle to be slaughtered. Information on the breed of cattle, gender, and age (categorized into two groups: young animals under one year and adults over one year) of each animal was meticulously observed and recorded. The age determination was based on dental eruption and wear patterns of incisor teeth, following established methods (Pace and Wakeman, 2003).

Research assistants at the abattoir labeled the selected animals and closely monitored them throughout the slaughter process, also ensuring that accurate blood samples were taken and demographic data were collected for each of the slaughtered cattle.

Sample Size Determination

The sample size was estimated using Thrusfield's formula (2010). Based on a previous prevalence of 3.5% reported by Ngbede *et al.* (2012), a minimum of 51.9 samples was calculated at a 95% confidence interval with 5% absolute precision. To enhance the likelihood of detecting antigens, a total of 179 samples were collected.

Blood Collection and Processing

Blood samples were aseptically collected from the jugular vein of cattle post-slaughter at Katsina Central abattoir. A 10 ml blood sample was collected from each animal into sterile, anticoagulant-free vacutainers and uniquely labeled to match each individual cow. The tubes were kept at a tilted angle at room temperature for 2 to 4 hours to allow clot formation. They were then transported in ice packs to the Haematology Laboratory at Turai Umar Yaradua Maternity and Child Hospital, Katsina State, where serum was separated by centrifugation at 3000g for 10 to 15 minutes. The harvested sera were transferred into 1.5 mL microfuge tubes (Eppendorf®) and stored at -20°C until further use.

Detection of antibodies to *Leptospira* spp. using ELISA

At the Central Research Laboratory, serum samples were screened for antibodies against *Leptospira hardjo* using an indirect ELISA kit (ELISA Bovicheck® Lepto HP) from Bioveta, Canada. This kit specifically detects antibodies against *Leptospira pomona* and *L.hardjo* in bovine serum. Procedures followed the manufacturer's protocol, where the intensity of color development was proportional to the quantity of antibody present in the serum samples. Briefly, 100 µL of positive control, negative control, and test samples were dispensed into designated wells. The plate was incubated at 23°C for 30 minutes. Each well was washed three times with 300 µL of 1X wash solution; excess liquid was removed after each wash. After drying on absorbent paper, 100 µL of diluted conjugate was added to each well and incubated at 23°C for another 30 minutes. The wells were rewashed as described earlier. Next, 100 µL of substrate solution was dispensed into each well and incubated at 23°C for 15 minutes. Finally, 100 µL of stop solution was added to each well and the Optical density (OD) was measured using an ELISA reader (Optic System IVYMEN® 2100C, USA) at wavelengths between 450-620 nm.

Statistical Analysis

Data collected from this study were entered and stored in Microsoft Excel®. Descriptive statistics were applied to summarize the data. Further analysis was performed using GraphPad Prism® version 8.0.2(263). Categorical variables such as age, sex, and breed were assessed using the Chi-square test to determine associations with Leptospira positivity. Statistical significance was set at $p<0.05$, corresponding to a 95% confidence interval.

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RESULTS

Prevalence of Leptospira infection in Cattle from Katsina Central Abattoir in relation to Sex

The sex-specific prevalence of *L. spp. serovar hardjo infection showed that bulls had a higher prevalence of 20% (12), while cows had* a prevalence of 14.3% (17). However, the Chi-square test revealed no statistically significant association between sex and Leptospiral infection, with a p-value of 0.44 (Table 1).

Table 1: Sex specific Prevalence of *Leptospira spp serovar Hardjo* in Cattle Slaughtered in Katsina Central Abattoir, Katsina State

Sex	Number Tested	Number Positive (%)
Bulls	60	12 (20.0)
Cows	119	17 (14.3)
Total	**179**	**29 (16.2)**
x^2 = 0.58	**df =1**	***p*-value = 0.44**

Prevalence of Leptospira infection in Cattle from Katsina Central Abattoir in relation to Age

Out of 179 sera from cattle screened for antibodies to *Leptospira spp. Serovar hardjo infection was detected using ELISA, yielding a prevalence rate of 38% (29)*. The age-specific prevalence of *L. hardjo* infection showed that young cattle had a higher prevalence of 25.5% (13), while older cattle had a prevalence of 12.5% (16). However, the chi-square test showed no statistical association between age and Leptospiral infection, with a p-value of 0.06 (Table 2).

Table 2: Age-specific Prevalence of *Leptospira spp serovar hardjo* in Cattle Slaughtered in Katsina Central Abattoir, Katsina State

Age Group	Number Tested	Number Positive (%)
Adult	128	16 (12.5)
Young	51	13 (25.5)
Total	**179**	**29 (16.2)**
x^2 = 3.63	**df = 1**	***p*-value = 0.06**

Prevalence of Leptospira infection in Cattle from Katsina Central Abattoir in relation to Breed

The breed-specific prevalence of *L. hardjo* infection showed that White Fulani recorded the highest prevalence, followed by Sokoto Gudali and Red Bororo, respectively. However, the Chi-square test showed no statistical association between breed and Leptospiral infection, with a p-value of 0.39 (Table 3).

Table 3: Breed specific Prevalence of *Leptospira spp serovar Hardjo* in Cattle Slaughtered in Katsina Central Abattoir, Katsina State

Breed	Number Tested	Number Positive (%)
Red Bororo	101	13 (12.9)
White Fulani	48	10 (20.8)
Sokoto Gudali	30	6 (20.0)
Total	**179**	**29 (16.2)**
x^2 = 1.9	**df = 2**	***p*-value = 0.39**

DISCUSSION

Generally, the findings of this study suggest a high prevalence of leptospirosis in the study area. This might be likely due to frequent interactions between humans and potential animal hosts. Previous studies have highlighted that human activities, such as livestock farming, working in marshy areas, fishing, or handling rodents, significantly contribute to pathogen transmission (Benacer et al., 2016; Mgode *et al.*, 2021). Livestock, including goats and cattle, can act as reservoir hosts for leptospirosis or acquire *Leptospira* serovars from other animals, such as rodents (Assenga *et al.*, 2015; Mgode *et al.*, 2021; Msemwa *et al.*, 2021).

This study confirmed the presence of Leptospira spp. as the causative agent of leptospirosis in the region, supporting earlier findings by Garba *et al.* (2013). A higher infection rate was observed in cows than in bulls, suggesting that both sexes are vulnerable to the pathogen, as similarly reported by Ngbede *et al.* (2012). Age-related differences were also noted, with younger cattle showing greater exposure to *L. hardjo* compared to adults, a pattern consistent with Agunloye *et al.* (2002). This disparity may reflect differences in immune maturity or repeated exposure in older animals.

Breed-specific differences were observed, with White Fulani cattle showing the highest prevalence of *L. hardjo* infection, followed by Sokoto Gudali and Red Bororo. White Fulani cattle were the most common breed in the study area, raising concerns about their potential role in spreading infections to other animals and humans (Ngbede *et al.*, 2012a). The exchange of *Leptospira* serovars between humans, goats, and cattle highlights the impact of human-

livestock interactions on pathogen transmission, corroborating findings from Mgode *et al.* (2021).

The prevalence rates observed in this study were lower than those reported by Sharma *et al.* (2014), who found higher rates in cattle and goats using MAT and bacterial culture techniques. Similarly, Rebecca *et al.* (2024) reported a prevalence among 131 cattle examined, while Behera *et al.* (2010) found a prevalence in eastern India. Ngbede *et al.* (2012a) reported a significantly lower seroprevalence of *L. hardjo* in cattle from Zaria, Nigeria. These variations could stem from environmental factors, differences in cattle breeds, geographic specificity of the pathogen, or the absence of vaccination programs in certain regions. Additionally, methodological differences such as assay sensitivity and sample types may explain discrepancies across studies. The findings align with Ngbede *et al.* (2013), which highlighted the persistence of *Leptospira* infections among livestock and humans in Nigeria.

CONCLUSION

This research highlights the significant risk of *Leptospira spp.* infection among cattle in a specific region, with a prevalence rate of 16.2% observed in slaughtered cattle. The presence of leptospirosis in animals processed at abattoirs poses a direct threat to abattoir workers, who are at risk of contracting *Leptospira spp.*, particularly *serovarhardjo*. The study suggests that losses due to leptospirosis within Nigeria's cattle population may be underestimated. Even apparently healthy seropositive animals could act as sources of infection, emphasizing the zoonotic nature of the disease. Furthermore, no significant correlation was found between age, gender, or breed type and *Leptospira* infection among animals slaughtered at Katsina Central abattoir. This finding underscores the widespread susceptibility of cattle regardless of demographic factors. In conclusion, this study recommends targeted interventions to prevent the transmission of leptospirosis. Improved livestock management practices and vaccination programs are essential measures. Additionally, understanding environmental and behavioral risk factors is critical for effective control of this zoonotic disease.

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