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Serological evidence of exposure of small ruminants slaughtered in Nsukka, Nigeria to *Leptospira* infection, and haematological alterations associated with it

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Abstract

Leptospirosis is a neglected, globally disseminated zoonotic disease transmitted through contact with contaminated soil, water or urine of infected animals and humans. The clinical manifestations in small ruminants include abortion, stillbirths and the birth of weak offspring. The present study was an abattoir-based survey of the occurrence of *Leptospira* species antibodies in small ruminants slaughtered in Nsukka, Enugu State, Nigeria. The survey was done on 120 small ruminants, from June to August 2025. Blood (5 ml) was collected from the 120 small ruminants and aliquoted into two: one for serology, the other was anti-coagulated and used for haematology. The species, breed and sex of each sampled animal were noted. Serum samples were tested for antibodies against eight Leptospira serovars using the microscopic agglutination test (MAT). Haematological parameters were evaluated on the anti-coagulated blood following standard methods. Chi-square test was used to measure the strength of association between the MAT results and variables such as species, breed and sex. Student's t-test was used to determine differences in haematological values of seropositive and seronegative cases. The occurrence of Leptospira antibodies in slaughtered small ruminants was 80%. The most prevalent serovars recorded were *icterrohaemorrhagiae* (20.1%), followed by *canicola* and *grippotyphosa* (18% each). Leptospira *mini* was the least occurring serovar (5.8%). Twenty-one percent of the small ruminants were seropositive to two serovars, and 7% to three serovars. There was no significant association ($p > 0.05$) between species, breed, and sex of the sampled animals and being seropositive. The mean blood eosinophil count of the seropositive animals was significantly ($p < 0.05$) lower than that of the seronegative animals. The high occurrence (80%) of *Leptospira* spp. antibodies in slaughtered small ruminants in Nsukka as recorded in this study indicates endemic leptospirosis and potential zoonotic risk to abattoir workers and animal handlers. This highlights the necessity for continuous surveillance and public health education on the subject.

Keywords: *Leptospira* species antibody; Small ruminants (goats and sheep); Occurrence; Abattoir-based survey; Nsukka Nigeria; Microscopic agglutination test (MAT).

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Introduction

Small ruminants, particularly goats and sheep, play a significant role in the livelihood and food security of rural and peri-urban households in Nigeria and other developing countries. They provide meat, milk, hides and manure, and serve as financial assets for resource-poor farmers. Their ability to adapt to diverse ecological zones, low maintenance cost, short reproductive cycles and high fertility rates make them a preferred livestock species among smallholder farmers (Chukwudi *et al*., 2020). Despite the socio-economic importance of small ruminant production in Sub-Saharan Africa, this promising sector is faced with numerous constraints, chief of which is disease burden (Erdaw, 2023; Kimeli *et al*., 2025).

Leptospirosis is one of the major constraints to livestock production, health and trade (OIE, 2021). It is a globally disseminated zoonotic disease caused by pathogenic spirochaetes of the genus *Leptospira* (Chávez-Sánchez *et al*., 2025), associated with considerable morbidity and mortality (Costa *et al*., 2015). The pathogens are maintained in nature by various reservoirs, and they are transmitted to incidental hosts, such as humans and domesticated animals, through contact with the urine of infected animals or via contaminated water and soil (Ajayi *et al*., 2020). Leptospirosis is therefore prevalent in tropical and subtropical regions where warm humid conditions are necessary for survival of the causative organism outside the host (Haji Hajikolaei *et al*., 2022).

In goats and sheep, leptospirosis is reportedly associated with disorders of reproduction, such as abortion, stillbirths, neonatal mortality, infertility and decreased productivity (Ajayi *et al*., 2020; Chávez-Sánchez *et al*., 2025). In these small ruminants, subclinical infection is reportedly common, as they often harbour the bacteria asymptomatically, making the disease difficult to detect (Haji Hajikolaei *et al*., 2022). Asymptomatic carriers maintain the disease's epidemiological cycle, thereby increasing the risk of exposure for co-grazing livestock, wildlife and humans in endemic regions (Haji Hajikolaei *et al*., 2022).

The public health importance of leptospirosis stems from its zoonotic potential. The World Health Organization (WHO) estimated that more than one million human cases and approximately 58,900 deaths occur annually worldwide as a result of leptospirosis; it is believed that the true burden is likely underestimated due to poor surveillance systems, misdiagnosis and inadequate laboratory capacity (Costa *et al*., 2015). Occupational groups, such as abattoir workers, veterinarians, farmers and meat handlers, are at a higher risk due to frequent contact with livestock and contaminated environments (Ajayi *et al*., 2020; Onafruo *et al*., 2024).

Despite the recognised zoonotic potential of leptospirosis, there is a dearth of information on its occurrence in small ruminants in many localities, including Enugu State, Nigeria. The present study investigated the occurrence of antibodies against leptospiral species among goats and sheep in Nsukka, Enugu State, Nigeria, and further evaluated the haematological alterations associated with it.

Materials and Methods

Study location and Sample collection: The study and sample collection were done at the two major abattoirs in Nsukka zone of Enugu State, Nigeria, namely, Ikpa and Obollo-Afor abattoirs (latitude 6° 51′ 24″ N and longitude 7° 23′ 45″ E), between June and August 2025. Samples were collected from a total of 110 goats and 10 sheep presented for slaughter during the sampling visits; sampling took place twice a week during the period. These animals that were sampled were sourced from farms and local markets within the study location. Strict biosafety precautions were observed throughout the sample collection and handling process to prevent contamination and exposure to infectious materials.

Five millilitres of blood was collected from each animal through jugular venipuncture into EDTA bottles and sterile plain tubes for haematology and serology, respectively. The blood samples were properly labeled and packed in an ice box. They were transported to the Department of Veterinary Medicine Laboratory, University of Nigeria, Nsukka, for further processing. Records of sampled animals' species, breed and sex were taken based on the animals' phenotypic characteristics. The blood samples collected in plain tubes were allowed to stand for one hour to clot, and the sera were separated by centrifugation at 1500 x g for 10 minutes. The sera were stored at -20°C and later sent to the Veterinary Pathology Division, National Veterinary Research Institute (NVRI), Vom, using an ice pack for *Leptospira* antibody detection (Miller *et al*., 1990).

Ethical consideration*:* No ethical animal use approval was sought as samples were collected only from slaughtered animals. However, *v*erbal permissions were obtained from the veterinary officers in charge of the abattoirs in the study areas. Before sample collection, consent from the abattoir management and the animal owners was also sought and obtained. All samples were collected in strict adherence to hygiene and safety standards to minimize risks to the researchers and the public.

Detection of antibodies to Leptospirosis: The presence of *Leptospira* antibodies in the serum samples was determined using the microscopic agglutination test (MAT), which involved the serial dilution of the serum samples followed by reactions with *Leptospira* antigens*,* and then, an assessment of the organism's agglutination with dark field microscopy (WOAH, 2023). According to World Organization for Animal Health recommendations, a titre of 1:100 was considered positive. A reference panel of live Leptospira serovars, representing locally and globally prevalent strains was used as antigen, as shown in Table 1. Positive and negative control sera were included in each test run to ensure accuracy and reproducibility.

Haematology: The general principles of haematology and veterinary haematology, as described by Coles (1986) and Schalm *et al.* (1975), were used to evaluate the packed cell volume (PCV), haemoglobin concentration, red blood cell count (RBC), total white blood cell count (WBC), and differential white blood cell count.

Table 1. Strains of pathogenic *Leptospira* (L.) species used as live antigens in the microscopic agglutination test (MAT) during the Leptospira serosurvey in small ruminants in Nsukka, Nigeria.

S/No.	Genomospecies	Serovars
1	*L. interrogans*	*Icterohaemorrhagiae* *Pomona* *Hardjo* *Canicola* *Bratislava*
2	*L. borypetersenii*	*Tarassovi* *Mini*
3	*L. kirschneri*	*Grippotyphosa*

Data analysis: Data obtained were subjected to Pearson's Chi-square test in Stata version 12, to assess the association between the presence of *Leptospira* antibodies and the variables considered (species, breed, and sex). Student t-test was also used to compare the means of the haematological values between the seropositive and seronegative samples. Significant level was set at $p < 0.05$.

Results

Demographic information on the animals sampled: Table 2 shows the demographic information of the small ruminants surveyed in the study. 91.7% of the small ruminants sampled were goats, while only 8.3% were sheep. 80% were Red Sokoto goats, and 11.7% were West African Dwarf (WAD) goats. 6.7% were Balami breed of sheep, and 1.7% were WAD sheep. 98.3% of the small ruminants sampled were females, while only 1.7% were males.

Occurrence of *Leptospira* species antibodies: The study revealed an occurrence of leptospiral antibodies in 96 out of the total 120 small ruminants sampled, representing 80% of the sample population (Figure 1). Seropositive animals reacted to 8 serovars, viz. *Terrasovi, Pomona, Hardjo, Icterrohaemorrhagiae, Grippotyphosa, Bratislarve, Canicola,* and *Mini,* as shown in Figure 2. The most prevalent serovars were: *Icterrohaemorrhagiae* (20.1%), *Grippotyphosa* (18%), and *Canicola* (18%). *Mini* had the least prevalence (5.8%) [Figure 2]. The frequency of cross-reactivity of goats and sheep sera with different serovars is presented in Figure 3: Of the samples tested, 51% reacted to a single serovars, 21% to two serovars, and 7% to three serovars.

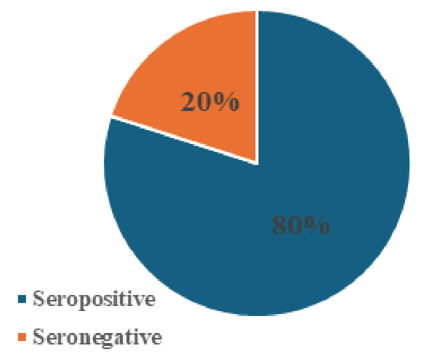

Figure 1. Occurrence of *Leptospira* antibodies in small ruminants surveyed at abattoirs in Nsukka zone, Enugu State, Nigeria.

Table 2. Demographic information on the small ruminants surveyed for *Leptospira* antibodies at abattoirs in Nsukka zone, Enugu State, Nigeria.

Variables	Characteristics	Number of animals	Percentage
Species	Goats	110	91.7%
	Sheep	10	8.3%
Goat breeds	Red Sokoto Goat	96	80%
	WAD Goat	14	11.7%
Sheep breeds	Balami Sheep	8	6.7%
	WAD Sheep	2	1.7%
Sex	Female	118	98.3%
	Male	2	1.7%

WAD = West African Dwarf

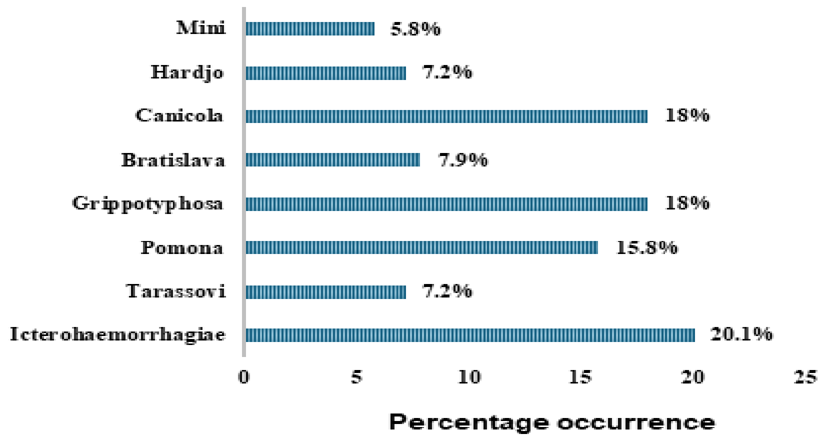

Figure 2. Occurrence of different serovars of *Leptospira* in small ruminants surveyed at abattoirs in Nsukka zone, Enugu State, Nigeria.

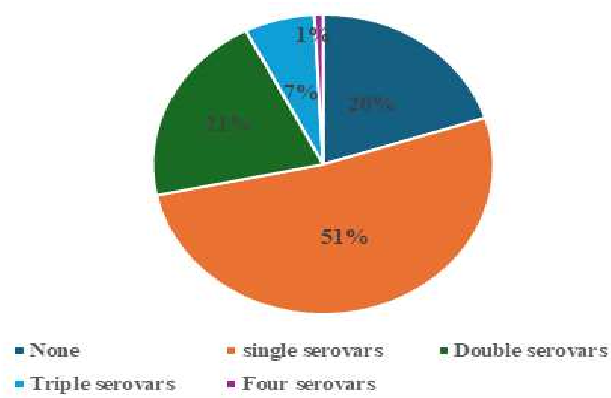

Figure 3. Cross-reactivity of the different serovars of *Leptospira* in small ruminants surveyed at abattoirs in Nsukka zone, Enugu State, Nigeria.

Table 3. Distribution of occurrence of *Leptospira* antibodies in small ruminants surveyed at abattoirs in Nsukka zone, Enugu State, Nigeria, based on species, breed and sex.

Variables	Characteristics	Number of animals	Seropositive (%)	Seronegative (%)	Chi-square	P-value
Species	Goats	110	87 (79.1%)	23 (20%)	0.682	0.67
	Sheep	10	9 (90.0%)	1 (10%)		
Goat breeds	Red Sokoto	96	79 (82.3%)	17 (17.7%)	3.857	0.31
	WAD Goat	14	8 (57.1%)	6 (42.9)		
Sheep breeds	Balami	8	7 (87.5%)	1 (12.5%)		
	WAD Sheep	2	2 (100%)	0 (0%)		
Sex	Female	118	95 (80.5%)	23 (19.5%)	1.144	0.36
	Male	2	1 (50.0%)	1 (50%)		

WAD = West African Dwarf

Species, Breed, and Sex distribution of the occurrence of *Leptospira* antibodies: Table 3 shows the occurrence of *Leptospiral* antibodies across species, breed and sex. Out of the 120 small ruminants sampled, 79.1% (87/110) of the goats and 90% (9/10) of the sheep were seropositive. Based on breed, the occurrence of seropositive cases were: Red Sokoto goats – 82.3%; West African Dwarf goats – 57.1%; Balami sheep – 87.5%; and West African Dwarf sheep – 100%. The sex-based distribution of the seropositive cases were: 80.5% of females and 50% of males. There were no statistically significant associations ($p > 0.05$) between being seropositive or seronegative and the species, breed and sex.

Haematology: A comparison of the haematological profile of seropositive and seronegative cases is presented in Table 4. There were no significant differences ($p > 0.05$) between the seropositive and seronegative cases in their packed cell volume, haemoglobin concentration, red blood cell counts, total white blood cell counts, neutrophil, lymphocyte, monocyte and basophil counts, but the eosinophil counts of the seronegative cases was significantly ($p < 0.05$) higher than that of the seropositive cases (Table 4).

Table 4. Haematological profile of small ruminants surveyed for occurrence of *Leptospira* antibodies at abattoirs in Nsukka zone, Enugu State, Nigeria, showing the mean values for the seropositive and seronegative animals.

Variables	Status	Means	SD	P value
Packed cell volume (%)	Seropositive	34.3	7.66	0.778
	Seronegative	33.7	1.03	
Haemoglobin concentration (g/dl)	Seropositive	9.6	2.45	0.671
	Seronegative	9.9	2.64	
Red blood cell count ($\times 10^6/\mu l$)	Seropositive	11.1	6.91	0.281
	Seronegative	9.5	3.67	
Total white blood cell counts ($\times 10^3/\mu l$)	Seropositive	14.0	6.16	0.968
	Seronegative	14.0	4.95	
Neutrophils (%)	Seropositive	42.1	2.49	0.991
	Seronegative	42.0	1.98	
Lymphocyte (%)	Seropositive	57.0	2.13	0.682
	Seronegative	54.6	2.04	
Monocyte (%)	Seropositive	1.5	2.15	0.685
	Seronegative	1.7	2.60	
Eosinophil (%)	Seropositive	1.2	1.28	0.04
	Seronegative	2.1	1.80	
Basophils (%)	Seropositive	0.05	0.22	0.836
	Seronegative	0.04	0.20	

Discussion

The overall 80% occurrence in the present study of *Leptospira* antibodies in small ruminants slaughtered at abattoirs in Nsukka zone, Enugu state Nigeria is higher than what was reported in most previous studies: 51.5% prevalence in goats in Southwestern Nigeria (Ajayi *et al*., 2020), 57.9% prevalence in slaughtered cattle in North Central states of Nigeria (Abiayi *et al*., 2015), 21.81% in sheep and goats in Uganda (Aliniatwe *et al*., 2024), and 13.5% prevalence in sheep and goats in Mexico (Chávez-Sánchez *et al*., 2025). However, a study in the Bahr El Ghazal region of South Sudan reported 81.8% prevalence in cattle (Onafruo *et al*., 2024), while 66.9% prevalence in cattle was reported in Bor County, South Sudan (Kasiano *et al*., 2025). These variations in prevalence rates may be attributed to various factors, including the detection methods, sample size, management practices, environmental conditions (such as incessant rainfall, flooding, and higher environmental temperatures), and the presence of reservoirs or carriers of *Leptospira* within the environment that facilitate Leptospira dissemination (Ajayi *et al*., 2020).

The MAT test is recognized as the gold standard for diagnosing leptospirosis, as it facilitates the identification of circulating serovars in a given region (WOAH, 2023). Using a reference panel of 8 serovars in the MAT, the present study detected seropositive animals at 1:100 titre. The eight reference panel serovars identified in this study, viz: *Icterohaemorrhagiae*, *Pomona*, *Grippotyphosa*, *Canicola*, *Hardjo*, *Tarassovi*, *Bratislava*, and *Mini*, align with the fact that small ruminants have been regarded as accidental/incidental hosts of *Leptospira* of various serovars (Leon-Vizcaino *et al*., 1987).

The findings in the present study that the most prevalent serovars were *Icterohaemorrhagiae* (20.1% of the animals), *Grippotyphosa* and *Canicola* (each in 18% of the animals) contrasts with the reports by Ajayi *et al*. (2020) and Abiayi *et al.* (2015), who identified L. *hardjo* as the most prevalent serovar among goats in southwestern states of Nigeria and among cattle in the North Central state of Nigeria, respectively. Further, Alinaitwe *et al*. (2024) identified *Tarassovi* in small ruminants in Uganda, Sunder *et al*. (2024) reported *Grippotyphosa* in India, and Chávez-Sánchez *et al*. (2025) reported *Sejroe* in Mexico. These variations in serovar distribution may be attributed to differences in predominant wildlife reservoirs, climatic conditions and management practices across different regions (Cilia *et al*., 2021). Furthermore, it has been reported that serovars can vary geographically and often adapt to specific hosts (Ko *et al*., 2009). It is, however, essential to highlight that the serovars identified in this study hold significant importance, as they are among those responsible for the highest incidence of human Leptospirosis. The *L. interrogans* serovars *Icterohaemorrhagiae*, *Canicola*, *Grippotyphosa*, and *Hardjo* have been recognized as aetiological agents of human leptospirosis of zoonotic origin (Costa *et al*., 2015; Picardeau, 2017). Therefore, the detection of these serovars among slaughtered small ruminants serves as a crucial indicator of potential health risks to the public, particularly for farmers and their households, and individuals involved in abattoir operations, such as butchers, meat handlers and veterinarians, since they are regularly exposed to animal urine, blood and tissues. Abattoir workers are at a higher risk of contracting leptospirosis through cuts, abrasions or mucous membranes during the performance of their duties (Ajayi et al., 2020).

Cross-reactivity of multiple *Leptospira* antibodies was also revealed in this study. 21% of sheep and goats exhibited seroreactivity to two serovars, while 7% demonstrated seroreactivity to three serovars. This finding suggests that co-infection is relatively prevalent among these animals. Comparable observations were reported by Orlando *et al*. (2020) and Stagnoli *et al*. (2025). Balamurugan *et al*. (2021) stated that small ruminants have the capacity to harbour multiple *Leptospira* serovars simultaneously. This phenomenon may be attributed to cross-reactions among different serovars, co-infection with multiple serovars simultaneously and/or sequential exposure to different serovars during the animal's lifetime (Aliniatwe *et al*., 2024).

The present study revealed no significant association between the animals being seropositive or seronegative and variables such as species, breed and sex. This result concurs with the findings of Ajayi *et al*. (2020) in Southwestern Nigeria, Abiayi *et al*. (2015) in North Central States, Nigeria and Assenga *et al*. (2015) in Tanzania. It however, contrasts with the reports of Aliniatwe *et al*. (2024) in Uganda. These differences could be attributed to factors such as differences in environmental exposure, breed susceptibility, management practices, physiological stress associated with pregnancy and lactation, and behavioural differences influencing pathogen contact frequency (Lilenbaum *et al*., 2008; Alder and de la Peña Moctezuma, 2010; Molinari *et al*., 2021). Also, the non-significant differences observed in this study may be partly attributed to the small number of sheep and the large proportion of females sampled. In the study area, goats are reared and consumed more than sheep, as sheep are mainly used for cultural purposes.

Among all the haematological parameters assessed, eosinophil counts were significantly lower in the seropositive animals. The significantly lower eosinophil count recorded in the present study for seropositive cases concurs with the findings of Fish-Low *et al*. (2020), who reported a reduction in eosinophil counts in confirmed leptospirosis cases. Rentko *et al*. (1992) also reported eosinopenia in canine leptospirosis. Eosinopenia has been associated with sepsis (Lavoignet *et al*., 2016) and has been identified as a biomarker in several infectious diseases, including leptospirosis.

Pathogenic *Leptospira* species can induce eosinopenia by promoting apoptosis of eosinophils or inhibiting their release from bone marrow via cytokine-mediated suppression (Fish-Low *et al*., 2020). Additionally, it has been reported that eosinophils may migrate from blood circulation to infected tissues, particularly the liver, lungs and kidneys, resulting in reduced peripheral blood counts (Davido *et al*., 2017) during acute bacterial infection.

Conclusion: The high (80%) occurrence of seroreactors to *Leptospira spp.* antibodies among slaughtered goats and sheep in the sampled abattoirs in Nsukka zone, Enugu State, Nigeria indicates that *Leptospira* spp. is present and circulating in small ruminant herds in the study area. It is noteworthy that all animals sampled had no history of vaccination against Leptospirosis. The identification of zoonotic serovars such as *L. icterohaemorrhagiae, L. canicola and L. hardjo* highlight the public health implications of this disease. It underscores the need for continued surveillance, public health education and targeted control measures.

Conflict of Interest

The authors declare no conflict of interest.

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