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# Serological Evidence of *Leptospira hardjo* Antibodies and The Incidence of Reproductive Disorders in Selected Smallholder Cattle and Goat Farms from Maiduguri, Nigeria

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**ABSTRACT**

The present study investigated the seroprevalence of *Leptospira hardjo* antibodies and their relationship to the burden of reproductive disorders associated with smallholder ruminant production systems in Maiduguri, northeastern Nigeria. We randomly collected 376 blood samples from 11 cattle (n=188) and 10 goats (n.=188) farms in Maiduguri from April to September 2019. The farmers completed structured questionnaires to furnish information regarding herd characteristics and reproductive histories of farm animals. A serum IgG/IgM antibody-capture ELISA test kit with a sensitivity of 96.91%, specificity of 90.40%, positive and negative predictive values of 88.68% and 97.41%, respectively, was used for the detection of *Leptospira hardjo* antibodies from blood serum. The overall seroprevalence of *Leptospira hardjo* was 4.26% (95% CI: 2.17-8.17) in cattle and 2.13% (95% CI: 0.83-5.34) in goats, respectively. *Leptospira hardjo* antibodies were detected in 4 (50%) out of the 8 cattle herds and 3 out of the 7 goat flocks with a history of reproductive disorders. At the present rate of detection of *Leptospira hardjo* antibodies in ruminants with histories of reproductive disorders, the current burden of the disease and its consequences on the reproductive efficiencies could be highly underestimated in the smallholder ruminant production system in the northeastern part of Nigeria.



## Introduction

Leptospira species are host-dependent spirochete of global distribution and significance (Bharti *et al*., 2003). The prevalence of leptospirosis could have a huge impact on reproductive efficiency and cause serious economic losses in the livestock sector (Johnson *et al*., 2004, Walker, 2005; Abiayi *et al*., 2015). Leptospirosis is associated with decreased fertility, reduced milk production, abortion, early neonatal mortality, stillbirth, and retention of fetal membranes in cattle (Faine *et al*., 1999). Although goats are less susceptible to the infection than other species (Leon-Vizcaino *et al*., 1989), different forms of the disease associated with pyrexia, hemorrhagic syndromes, anorexia, and depression have been described in goats (Faine *et al*., 2000). Severe illness in goats could cause marked economic losses due to infertility, neonatal deaths, abortions, and decreased milk production (Lilenbaum el al., 2007).

With the increasing rates of occurrences of leptospirosis globally, the disease has gained the status of an emerging zoonosis (Vijayachari *et al*., 2008). *Leptospira hardjo* infection is distributed worldwide in sheep, goat and cattle herds, and disease is endemic in India, where high seroprevalence rates were recorded among cattle (37%) and goats (29%) (Sharma *et al*., 2014). Another study in India also reported a lower seroprevalence of 9.11% for *Leptospira hardjo* in cattle using the ELISA method (Pandian *et al*., 2015). In Iran, Haji Hajikolaei *et al*. (2007) have reported a prevalence of 4.3% in cattle using microscopic agglutination test (MAT). The disease is endemic in Africa, but its epidemiology is not clearly defined in sub-Saharan Africa (de Vries *et al*., 2014). Machanga *et al*. (1997) reported a prevalence rate of 0.7% in Tanzania, and Wali (1998) found a prevalence rate of 2.5% in Sudan.

A few reports documented the presence of leptospirosis in the south-west (Onyemelukwe, 1993; Agunloye, 2002), and north-central (Ezeh *et al*., 1990; Ezeh *et al*., 1991; Abiayi *et al*., 2011; Isa *et al*., 2014; Abiayi *et al*., 2015) parts of Nigeria. However, it seems there is no available information on the presence of this disease in animals in northeastern Nigeria. It was hypothesized that *Leptospira hardjo* is present in cattle and goats in Maiduguri. The present study aimed to determine the prevalence of *Leptospira hardjo* antibodies in ruminant species

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in smallholder ruminant production systems in Maiduguri, Nigeria and determine its contribution to the total burden of causes of infertility among cattle and goats in the study area

## Materials and methods

*Ethics and consent*

All the protocols used for sample and data collection and laboratory analyses were approved by the postgraduate board of the University of Maiduguri. Each farmer interviewed in this study were contacted by telephone to get their informed consent before inclusion in the study. The study's objectives, including expected outcomes, were explained to the farmers to get their permission before the interviews and sample collection.

*Study area and population*

The study was conducted in Maiduguri, Nigeria, the state capital of Borno state, which is situated at an altitude of 354m above sea level, between latitude 10.2°N and 13.4°N and longitudes 9.8°E and 14.4°E (Fig. 1). Smallholder cattle and goats on backyard semi-intensive management systems within and around Maiduguri Metropolitan and Jere Local Government areas were sampled in this study. Zebu breeds of cattle, predominantly red Bororo and Wadara, and Sahelian breed of goat are present in the study areas. Age-wise, both young adults and adult cattle and goats of productive age were included in the study.

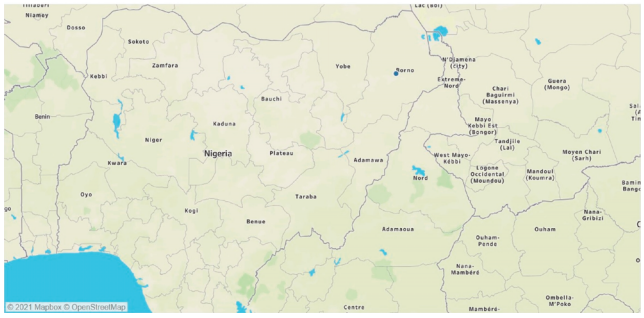


Fig. 1. Map of Nigeria showing the study area.

*Sample size*

The sample size was determined using the formula according to Thrusfield (2005), $n=t^2\times P(1-P)/d^2$; where n= number, $t^2$= 1.96, P= expected prevalence (P for goat =13.1%; P for cattle=8.4% (Agunloye, 2002; Ngbede, 2013), $d^2$= 5% desired absolute precision (=0.05). The calculated sample sizes for goat and cattle were 175 and 118, respectively. However, 188 samples were collected for goas and cattle, respectively, to increase precision.

*Sample Collection*

Blood samples were collected from 11 cattle and ten backyard goat smallholdings from April to September 2019. Five milliliters (5ml) of blood collected by jugular vein-puncture were placed into plain tubes and transported on ice packs to the immunology Laboratory, University of Maiduguri Teaching Hospital for further analysis. Sera were harvested by centrifugation at 1107g for 5 minutes at 25°C and stored at -20°C in identifiable vials until testing.

*Questionnaire survey*

A close-ended structured questionnaire was designed and administered to herd owners via face-to-face interviews to generate data on the farming practices and reproductive histories of animals on their farms. Veterinarians and trained para-veterinary personnel assisted in sample and data collection.

*Serum IgG/IgM antibody ELISA*

The sera were tested for antibodies against *Leptospira hardjo* using *Leptospira hardjo* IgG/IgM ELISA kit (Biopanda Reagents Ltd, Belfast, United Kingdom) according to manufacturer's instructions. The assay has a sensitivity of 96.91%, specificity of 90.40%, the positive predictive value of 88.68% and a negative predictive value of 97.41%. Briefly, each serum sample was pre-diluted with assay diluents (1/30) in a tube for 2 wells makeup 8µl of the sample was emptied in 232µl of assay diluent. To each well, 100µl of diluted serum was added, and the strip was covered with a plate sealer and incubated for 30 minutes with gentle shaking at room temperature (25°C). After incubation, the plate sealer was removed, and the strip was washed five times with diluted wash buffer. After washing, strips were firmly tapped on absorbent tissue to remove residual wash buffer before adding 100µl of the conjugate solution to each well. The strip was sealed and incubated for 15 minutes with shaking at room temperature (25°C) and washed again. After that, 100µl of the substrate solution was added to each well and incubated for 10 minutes at room temperature (25°C) in the dark. The reaction was stopped by adding 100µl of stop solution to each well. The result was then read by a microtiter plate photometer (EMax precision microplate reader, California, USA) at an optical density (OD) of 450nm wavelength. The optical densities of the positive and negative control were ≥ 0.6 and ≤ 0.2, respectively.

*Statistical Analysis*

The herd characteristics and the reproductive history of cattle and goat stock were analyzed using EpiTools statistical software and reported as simple proportions. The seroprevalence of *Leptospira hardjo* in cattle and goats and their respective 95%CI were computed with EpiTools statistical software as p=d/n, where p=prevalence, d= number with positive detection of antibodies against *L. hardjo*, and n= number of animals in each category (Thrusfield, 2005). The proportion of positive detection of antibodies against *L. hardjo* in cattle and goat herds with a history of the reproductive disorder is presented on a chart.

## Results

*Questionnaire Survey*

Herd characteristics and reproductive history of cattle and goat farms

In this survey, 11 (47.8%) cattle and 10 (43.4%) goat farms were used. Most of the cattle herds (54.5%) and goat flocks (60%) had 31-60 animals. All the farms included in the survey did not have a previous history or plan for vaccination against *Leptospira hardjo*. The management system in both cattle and goat farms was predominantly semi-intensive (90%), which permitted grazing during the day and provided supplemental feeding and a shade at night (Table 1).

Table 2 shows the proportions of cattle and goat herds with a history of the reproductive disorder in Maiduguri. The

incidence of reproductive disorders was higher in cattle than goat herds. The birth of weak neonates (81.81), retained placenta (72.7%), prolonged calving intervals (63.6%), drop in milk production (63.6%), and early neonatal mortality (45.5%) were the most frequent reproductive disorders reported in cattle herds. Signs of pneumonia or meningitis (80%), abortion (60%), birth of weak neonates (50%), prolonged kidding interval (50%), drop in milk production (50%) and early neonatal mortality (50%) were the most frequent reproductive disorders recorded in goat herds.

*IgG/IgM ELISA*

From the total serum samples analyzed (n.=376), 12 tested positive for antibodies to *Leptospira hardjo*, giving an overall seroprevalence of 3.19% (95% CI: 1.83-5.49). Out of the total number of cattle sampled (n=188), 4.26% (95% CI, 2.17-8.18) and 2.13% (95% CI: 0.83-5.34) of the total number of goats sampled (n=188) were positive for *Leptospira hardjo* antibodies, but there was no significant (p>0.05) statistical difference in the age, breed, and sex distribution of antibodies in cattle

Table 1. Characteristics of cattle herds and goats' flocks tested for *Leptospira hardjo* in Maiduguri, Nigeria.

| Variables | Proportions (%) | |
|---|---|---|
| | Cattle herds (n=11) | Goat flocks (n=10) |
| Number of Animals in the Herd | | |
| 1 – 30 | 4 (36.4) | 3 (30) |
| 31 – 60 | 6 (54.5) | 6 (60) |
| > 60 | 1 (9.1) | 1 (10) |
| History of vaccination against *L. hardjo* | | |
| Vaccinated | 0 (0.0) | 0 (0.0) |
| Unvaccinated | 11 (100) | 10 (100) |
| Introduction of new animals | | |
| 1 – 3 | 5 (45.5) | 8 (80) |
| 4 – 7 | 5 (45.5) | 1 (10) |
| > 7 | 1 (9.0) | 1 (10) |
| Herd management system | | |
| Semi-intensive | 10 (90.0) | 9 (90) |
| Intensive | 1 (9.1) | 1 (10) |
| Extensive | 0 (0.0) | 0 (0.0) |

Table 2. Proportions of cattle and goat herds with a history of reproductive disorder in Maiduguri

| Variable | Proportions | |
|---|---|---|
| | Cattle herds (n=11) | Goat flocks (n=10) |
| Abortion | 3 (27.3) | 6 (60) |
| Retained placenta | 8 (72.7) | 5 (50) |
| Birth of weak neonates | 9 (81.8) | 3 (30) |
| Prolonged calving/kidding interval | 7 (63.6) | 5 (50) |
| Drop in milk production | 7 (63.6) | 5 (50) |
| Brownish discolouration or presence of blood in milk | 1 (9.1) | 0 (0.0) |
| Signs of pneumonia or meningitis | 2 (18.2) | 8 (80) |
| Early neonatal mortality | 5 (45.5) | 5 (50) |

Table 3. The overall seroprevalence of *Leptospira hardjo* antibodies among cattle in Maiduguri, Nigeria (n=188).

| Variables | Examined | Negative | Positive | Prevalence (95% CI) |
|---|---|---|---|---|
| Gender | | | | |
| Male | 75 | 72 | 3 | 4.00 (1.37-11.11) |
| Female | 113 | 108 | 5 | 4.42 (1.90-9.94) |
| Age | | | | |
| Young | 40 | 40 | 0 | 0.00 (0.0-8.77) |
| Adult | 148 | 140 | 8 | 5.41 (2.76-10.30) |
| Breed | | | | |
| Red Mbororo | 84 | 81 | 3 | 3.57 (1.22-9.98) |
| White Fulani | 26 | 26 | 0 | 0.00 (0.00-12.87) |
| Bokoloji | 19 | 17 | 2 | 10.53 (2.94-31.39) |
| Abore | 27 | 24 | 3 | 11.11 (3.85-28.06) |
| Ndama | 11 | 11 | 0 | 0.00 (0.00-25.88) |
| Wadara | 11 | 11 | 0 | 0.00 (0.00-25.88) |
| Ambala | 10 | 10 | 0 | 0.00 (0.00-27.75) |
| Overall | 188 | 180 | 8 | 4.26 (2.17-8.17) |

CI: 95% Confidence Interval.

Table 4. The overall seroprevalence of *Leptospira hardjo* antibodies among goats in Maiduguri, Nigeria (n=188).

| Variables | Examined | Negative | Positive | Prevalence (95% CI) |
|---|---|---|---|---|
| Gender | | | | |
| Male | 76 | 75 | 1 | 1.32 (0.23-7.08) |
| Female | 112 | 109 | 3 | 2.68 (0.92-7.58) |
| Age | | | | |
| Young | 26 | 26 | 0 | 0.00 (0.00-12.87) |
| Adult | 162 | 158 | 4 | 2.47 (0.96-6.18) |
| Overall | 188 | 184 | 4 | 2.13 (0.83-5.34) |

CI: 95% Confidence Interval.

and goats (Tables 3 and 4). The comparative analysis of antibody detection against herd/flock history of reproductive disorders revealed that 4/8 (50%) of cattle herds and 3/7 (42.9%) of goat flocks had a positive detection of antibodies against *Leptospira hardjo*.

## Discussion

The present study shows that most of the farms investigated had histories of reproductive disorders such as abortions, retention of fetal membrane, stillbirths, the birth of weak calves or kids and an increase in the frequency of services per conception. A previous study (Bolin and Kuellner, 2003) has reported similar findings in association with *Leptospira hardjo* infection. Most of the herds investigated (54.5%) had cattle holdings of 31-60 heads per herd, and 60% of the goat flocks kept a similar number of animals per flock. The result showed that of all the farms investigated, none had any vaccination against leptospirosis. Over 45% of all the farms introduce new stock without screening against any infectious agent. This practice may predispose farm animals to infection through the introduction of infected animals to the farm. Herd management factors such as herd size, co-grazing of ruminants and introduction of new animals into the farm, among others, could also predispose animals to infections (Bolin and Kuellner, 2003, Salgado *et al*., 2014). The herd characteristics in the present study are similar to that reported in Chile, where a significant number of herds (84%) were operated at a subsistence level, with animals grazing openly all year round without any control measures against Leptospirosis (Salgado *et al*., 2014). In the present study, most farms investigated (90%) practiced the semi-intensive husbandry system. The farmers reported signs of the paleness of the mucus membrane in up to 3 animals in over 80% of the cattle and goat farms sampled. Other problems reported include retained placenta in 30-40%, drop in milk production in 30-45%, brownish discoloration of blood-tinged urine in 9% (cattle farms only) and neonatal morality in 20-45%, sporadic abortions in 30-40%, the birth of weak kids/calves in 30-70% of the farms investigated. Although these signs may be common to other diseases or conditions, such as Brucellosis, Campylobacteriosis, Trichomoniasis, they also suggest leptospirosis (Balakrishnan, 2012; Lilenbaum and Martins, 2014), which warrants further investigations.

The present study also showed that the seroprevalence of *Leptospira hardjo* was 4.3 % in cattle and 2.1 % in goats in Maiduguri. This finding indicates an active immune response to natural exposure for *Leptospira hardjo* infection in these animals. This finding is because there was no prior vaccination against this infection in all the herds tested. *Leptospira hardjo* infection has previously been reported in Nigeria (Ezeh *et al*., 1990; Ezeh *et al*., 1991; Onyemelukwe, 1993; Agunloye, 2002; Abiayi *et al*., 2011; Ngbede *et al*., 2013; Isa *et al*., 2014; Abiayi *et al*., 2015); and the result of the present study confirms the findings of Ngbede *et al*. (2013) suggesting the persistence of Leptospira infection among livestock and humans in Nigeria. There is, therefore, the need to assess the burden of this global abortifacient disease across the Nigerian livestock farming areas to curb its potential consequences on the fertility of animals and the threat to human health.

The prevalence of *Leptospira hardjo* observed in cattle and goats in the present study were lower than 8.44% previously reported by Ngbede *et al*. (2013) in Zaria, Nigeria. In one report from studies that used ELISA in zebu cattle in Kaduna State, Nigeria, Ngbede *et al*. (2012) also reported a prevalence of 3.5%, which is like the finding in the present study. Previously, Agunloye (2002) reported a prevalence of 13.1% in goats using a microscopic agglutination test (MAT) in Ibadan, southwestern Nigeria. From the above, it appears that there are variations in the prevalence of Leptospira among species and different geographic locations across the country. The variations in prevalence may be likely due to different sensitivities of the assay techniques or the type of samples used. It is known that MAT was the gold standard for diagnosing leptospirosis, but it has a drawback as it only detects the IgM, which is the early phase of the infection and not the IgG antibodies (Sharma *et al*., 2014).

The prevalence of *Leptospira hardjo* infection in the present study is like the findings in some parts of the world. For example, Haji Hajikolaei *et al*. (2007) reported a prevalence of 4.3% in cattle in Iran using MAT. However, in endemic areas of the world, such as Andaman Island, India (Sharma *et al*., 2014) reported higher prevalence rates of leptospirosis in cattle (37%) and goats (29%) using MAT and bacterial culture from kidney tissues. Another study in India also reported the seroprevalence of 9.11% of *Leptospira hardjo* in cattle using ELISA (Pandian *et al*., 2015). In some countries of Africa, Machanga *et al*. (1997) reported a prevalence rate of 0.7% using culture in Tanzania, while Wali (1998) reported a prevalence rate of 2.5% using culture from kidney tissue in Sudan. The variations in prevalence of Leptospira across the globe are linked to differences in assay techniques, nature of samples, climate, degree of urbanization and interdependence of humans and animals. These factors may be critical for the transmission of *Leptospira hardjo* infection observed globally since its epidemiology is not clearly defined (de Vries *et al*., 2014).

The presence of *Leptospira hardjo* infection and other infectious agents such Brucella abortus, Campylobacter fetus, and Trichomonas vaginalis is a direct indication of infertility in herds with high seroprevalence rates (Vamshi *et al*., 2012). Therefore, the detection of *Leptospira hardjo* antibodies in the present study is significant as it provides information on the infection status in cattle and goats in Maiduguri, Nigeria. Since leptospirosis affects a wide range of animal hosts (Arteaga-Tronsco *et al*., 2015) and given that cattle and goats are commonly reared together in Nigeria (Mshelia *et al*., 2014), this may allow for the ease of transmission of infection among these and other animal species. The result further showed that the rate of detection of *Leptospira hardjo* antibodies was high

in cattle (50%) and goat (42.9%) farms with histories of occurrence of reproductive disorders. This finding is like a previous report on bovine venereal campylobacteriosis in this same study area (Mshelia *et al*., 2010).

## Conclusion

The detection of *Leptospira hardjo* antibodies among smallholder ruminants with histories of reproductive disorders suggests that the burden of disease and its potential consequences on the reproductive efficiencies of smallholder ruminant production system in the northeastern part of Nigeria could be highly underestimated. The result of our study suggests the contribution of leptospirosis to the total burden of the causal agents of the reproductive disorders in Maiduguri. Further studies are required to elucidate the specific role of leptospirosis in the reproductive disorders of ruminants in the region.

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## Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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