

Seroprevalence And Risk Factors Associated with Leptospirosis in Cattle in Maiduguri and Its Environs Borno State, Nigeria

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Abstract- A cross-sectional study was carried out to determine seroprevalence and risk factors of leptospirosis in Maiduguri and its environs. The study was to determine the seroprevalence of leptospira species in cattle and its risk factors. A total of 270 blood samples comprising of 111 male and 159 female cattle were collected from the animal aseptically. The sera were then screened for the presence of leptospira spp using ELISA. Data obtained, then analysed to know the relationship between the variable tested. The sera tested was 8.9% seropositive to ELISA. There was no statistically significant association between the Sex and Breeds of the animal tested (P value 0.05). but there was statistically significant association between the age of the animal and the leptospira species. (P value 0.05). the study indicated that seroprevalence of leptospirosis higher in Maiduguri and its environs specifically in female cattle and cattle older than 3 years. This can be risk for both human and susceptible animals in the study area

Index Terms- Age, Cattle, Leptospira, Sex, Seroprevalence

I. INTRODUCTION

Bovine Leptospirosis is one of the contagious bacterial zoonotic diseases that occur worldwide, caused by pathogenic serovars of the genus *Leptospira* spp which were historically divided into two species; pathogenic *Leptospira interrogans* and saprophytic *Leptospira biflexa*. (Viana et al., 2021) The organism belongs to the family Leptospiraceae, they are thin, motile, slow-growing and obligately aerobic. They growth optimally at 30°C and measure up to 6-20 µm in length by 0.1 - 0.15 µm in diameter and they are distinguished from other spirochetes because of the presence of unique hook ends (Li et al., 2000). *Leptospira interrogans* serovar hardjo (L. hardjo) is the primary cause of bovine leptospirosis

throughout the world and responsible for most of the losses attributable to the disease (Ellis., et al., 1982).

Leptospirosis is transmitted directly through contact with urine or body fluids of infected animals or indirectly through water or soil contaminated with urine from infected animals. (Suepaul et al., 2011). Leptospirosis is a common global zoonotic disease affecting humans and various animal species especially in sub-tropical and tropical regions of the world. Acute, subacute, and chronic forms of leptospirosis can affect ruminants. In the acute form, symptoms include pyrexia, depression, anorexia, jaundice, and anemia or hemorrhage. Later, the animals become infertile and frequently experience fertility issues, abortions, and stillbirths. It also causes decreased milk yield which results in important economic losses to the livestock industries (Faine et al., 1999). In addition, infected animals can develop chronic renal infection and excrete the organisms in the urine, thereby disseminating leptospirosis to other animals and establishing a potential zoonotic threat to those engaged in animal production and related industries (Gerritsen et al., 1994).

The disease in human is transmitted through direct or indirect contact with water, food or soil contaminated with blood, urine and tissues of infected animals (Evangelista and Coburn, 2010). The carrier animals secrete leptospire in their urine without showing clinical signs of the disease, because the bacteria accumulate in their kidneys. The leptospire are maintained in nature by chronic renal carriage. Therefore, livestock play an important role in the epidemiology of the disease (Fang et al., 2015 and WHO, 2015).

Some infected animals may not show any clinical signs of the disease during their life span (Ellis., et al 1986). The identification of carrier animals is therefore crucial in tackling the Leptospiral infection. The use of culture, which is the most reliable test, has been hindered by some disadvantages. Growing leptospire in vitro is a time-consuming process which can take between three to six months (Smith et al., 1994). Leptospire remain viable in blood samples and in tissues for several days (Quinn. et al., 1994). Because of these disadvantages, immunological tests are more commonly used in the diagnosis of leptospirosis. (Vallee et al., 2015). The most frequently used serological test for this purpose is the microscopic agglutination test (MAT), which is still regarded as an international reference test (Faine. 1997). There is dearth of information on seroprevalence of leptospirosis in cattle in Maiduguri, Borno State, Nigeria. The study was carried out to generate data on epidemiological parameters of leptospirosis, which, are not available in the study area, where a large percentage of the population depend on livestock for sustenance, who are often in very close proximity with their animals, risking disease transmission to and spread among humans. Therefore, this study was conducted to determine the distribution, seroprevalence and risk factors of Leptospiral infection among cattle in Maiduguri and its environs Borno State, Nigeria.

II. MATERIAL AND METHODS

Study Areas

The study was conducted in Maiduguri and its environs. Maiduguri the capital of Borno State is located in the North eastern region of Nigeria. Maiduguri lies between coordinates: 11°50'N 13°09'E and occupies an area of 71,235 square kilometers with estimated population of 822,000 inhabitant making it the biggest city in the state (Britannica, 2018). The climate of Maiduguri is generally hot, dry, windy and dusty for most part of the year and the major occupation of the people is farming, livestock rearing and fishing. The average rainfall is 613 mm per annum which starts usually from June and end in September, with an average temperature range from “25.8°C to 40°C, (NIMET, 2018).

Sample Size Determination

The sample size was calculated using the formula of Thrusfield (2005)

$$n = \frac{1.96^2 \times P_{exp}(1-P_{exp})}{0.05^2}$$

Description

n = required sample size.

d²= allowable error = 5% (0.05²)

P_{exp} = prevalence of 27% (Abiayi *et al.*, 2015)

The sample size = 254

To increase the precision, the number of samples was increased to 270.

Blood Sample Collection and Transportation

Ten milliliters of blood samples were collected aseptically via the jugular vein of each animal, using plain vacutainer tube and needle following proper restraint. Each sample was labeled with unique identification number and information on sex, age, breed, location and the purpose for which the animals are kept were recorded. The samples were then transported to the laboratory on ice packed cooler. The clotted blood samples were centrifuged at 3000 rpm for 5 minutes, the separated sera were collected in a screw capped vial and kept at -20°C until required for test/use, The Enzyme Link Immunosorbent Assay kit was obtained from Biopanda reagents, *Leptospira hardjo* IgG/IgM ELISA Test Kit® United Kingdom. The test was performed based on the manufacturer's instruction, at Immunology Laboratory University of Maiduguri Teaching Hospital.

Serological Test

The sera were tested for antibodies against *Leptospira hardjo* infection with a commercial ELISA KIT. The ELISA is a qualitative test for antibodies to a broad range of *Leptospira* interrogans serovars including: *Hardjo*, *Hardjo bovis*. The ELISA has sensitivity and specificity to detect early and late *Leptospira* infection. Serum sample was pre-diluted 1/30 with assay diluents in a tube for 2 wells that make up 8µl of the sample, the sample was emptied in 232µl of assay diluent. Thereafter, 100µl of the (diluent serum) was added to each well. The strip was covered with a plate sealer and incubated for 30 minutes with shaking at room temperature (15-25°C). After incubation, the plate sealer was removed and the strip washed five times with diluted wash

buffer after ensuring every well was filled. After washing, strips were firmly tapped on absorbent tissue to remove residual wash buffer, 100µl of the conjugate solution was then added to each well. The strip was sealed and incubated for 15 minutes with shaking at room temperature (15-25°C), then washed again after incubation. 100µl of the substrate solution was then added to each well and incubated for 10 minutes at room temperature (15-25°C) in the dark. The reaction was stopped by adding 100µl of stop solution to each well. The result was then read by a microtitre plate photometer (E MaXprecision microplate reader, California, USA) where optical density (OD) at 450nm wavelength were observed.

Interpretation of Results

The test results were expressed as a ratio of sample value (s) related to positive control values (p) using the following formula:

The mean OD values for negative and positive controls should fall within the following ranges

NC mean \leq 0.15

NC mean \geq 0.50

The Elisa results were interpreted using the manufacturers guides based on optical density value of the test samples:

Statistical Analysis

Data generated were presented in tables and analyzed using Chi-square test and the fisher exact test with the aid of the Statistical Package for Social Sciences (SPSS) version 21.0 statistical software. Statistical methods used include descriptive statistic, odds ratio, 95% confidence interval, P value and different was considered significant at value $p \leq 0.05$

III. RESULTS

Sex- Specific Seroprevalence of Leptospiral Infection in Cattle in Maiduguri, the female cattle had higher seroprevalence rate 10.7% than the male 6.3% respectively, though there was no statistically significant association between the sex of the cattle and the *leptospira* infection tested respectively (Table 1).

On age specific, animal greater than 3 years and above had a higher seroprevalence 17.5% than those

less than 3 years old 2.0% respectively. There was statistically significant association between the age of the animal tested and *leptospiral* infection. (Table 2).

Table I Sex- Specific Seroprevalence of Leptospiral Infection in Cattle in Maiduguri and its Environs. Borno State, Nigeria

Sex	No. Tested	No. (%) Positive	OR (95% CI)	p value	Chi-square test (χ^2)
Male	111	7 (6.3)	0.6 (0.18, 1.38)	0.213	1.552
Female	159	17 (10.7)	Ref.		
Total	270	24 (8.9)			

Table II Age- Specific Seroprevalence of Leptospiral infection in cattle in Maiduguri and its environs. Borno State, Nigeria

Age	No. Tested	No. (%) Positive	OR (95% CI)	p value	Chi-square test (χ^2)
\leq 3 years	150	3 (2.0)	0.1 (0.03, 0.33)	<0.0001	19.777
> 3 years	120	21 (17.5)	Ref.		
Total	270	24 (8.9)			

Table III Showed breed-specific seroprevalence of leptospiral infection in cattle in Maiduguri.

The highest seroprevalence of *leptospira* was recorded in Wadara breed been 14.6% followed by Sokoto gudali breed which have seroprevalence of 10.4% and the least seroprevalence was observed in Kuri 5.8% and in Red Bororo 5.4% Respectively. There was no statistically significant association between the breeds of cattle and the leptospiral infection

Based on location, the specific seroprevalence of leptospiral infection was higher in Kasuwan shanu 46.3% followed by Gidan madara which had seroprevalence of 19.1%, The least seroprevalence was observed from old Maiduguri with the seroprevalence of 16.7% respectively. There was no statistically significant association between the location of cattle studied and the leptospiral infection Table IV

Table III Breed- Specific Seroprevalence of Leptospiral infection in cattle in Maiduguri and its environs. Borno State, Nigeria

Breed	No. Tested	No. (%) Positive	OR (95% CI)	<i>p</i> – value	Chi-square test (χ^2)
Sokoto Gudali	48	5 (10.4)	0.7 (0.22, 2.06)	0.491	0.474
Red Bororo	37	2 (5.4)	0.3 (0.07, 1.57)	0.221	- 1.440*
Abore	45	0 (0.0)	NA	0.008	2.686*
Kuri	58	5 (5.8)	0.6 (0.18, 1.66)	0.283	1.152
Wadara	82	12 (14.6)	Ref.		
Total	270	24 (8.9)			

Table IV Location- Specific Seroprevalence of Leptospiral infection in cattle in Maiduguri and its environs. Borno State, Nigeria

Location	No. Sampled	Seroprevalence	
		No. Positive	% Positive (95% CI)
Fariya	47	8 (17.4%)	17.0 (6.4, 29.7)
Gidan Madara	53	4 (19.6%)	7.5 (1.9, 15.1)
Kasuwan Shanu	125	7 (46.3%)	5.6 (1.6, 9.6)

Old Maiduguri	45	5 (16.7%)	11.1 (2.2, 22.2)
Total	270	24 (100%)	8.9 (5.6, 12.6)

IV. DISCUSSION

The seroprevalence rate of 8.9% obtained in the present study is of public health significance due to the zoonotic nature of the Leptospiral organisms. The prevalence might be due to lack of vaccination, presence of rodents, introduction of new animals, unhygienic practices for the disposal of aborted fetuses, placenta and vagina discharges which might contain *Leptospira* organisms. The prevalence obtained in this study was also similar to the findings of Balamurugan *et al.* (2012) in different dairy cattle farms in Zaria (Nigeria).

There was no significant association between the sex of the cattle and Leptospiral infection, the female cattle had a higher seroprevalence (10.7%) than male cattle (6.3%). This result agrees with several serological studies on dairy cattle which reported high seroprevalence in female cattle (Park *et al.*, 1992). This may be due to differences in the reproductive system and the way of keeping the female cattle in the herds.

The higher seroprevalence in age groups three years and above (17.5%) seen in this study may suggest a possible development of immunity through earlier contact and re-exposure to infection which might finally result in some tolerance in adult cattle.

Statistically, there is no significant difference between the leptospiral infections amongst the different breed of cattle in this present study. This is in agreement with the work of Parvez *et al.*, (2015) conducted on *L. hardjo* in dairy cattle of Chittagong, Bangladesh, who reported insignificant association. The study demonstrated that cattle herds in the study location have been exposed to the Leptospirosis there was no significant association between location and leptospiral infection. This was not similar than those found in studies that was carried out in Tamaulipas, where 77.9% was reported (Moles *et al.*, 1997). There was no significant association between Leptospiral infection and the location of cattle herds. The disease

is believed to be endemic across the states of the country. Improved management practices, adequate awareness creation on the economic and public health impact of the diseases is advocated in the prevention and control strategy.

V. CONCLUSION

From the findings of this study, it can be concluded that

1. Leptospiral infection caused by *L. hardjo* was prevalent in herds in Maiduguri and its environs, with 8.9% seroprevalence in cattle
2. Age of cattle, history of abortion, presence of wound on the body of animal, introduction of new animal into the herds, and presence of rodents in the herds were significantly associated with Leptospiral infection in the study area
3. Sex, Breed, Location, Husbandry management systems, Sources of water, Hygienic status of the herds/farm were not significantly associated with Leptospiral infection in the study.

VI. RECOMMENDATIONS

Based on the findings of this study. The following recommendations are made:

1. Screening of new animal before introduction into the herds/farms to reduce the spread of infection, proper disposal of aborted foetal materials, vaccination against Leptospirosis in cattle and other ruminants.
2. Public enlightens on the role and control of Rodents in the transmission of Leptospirosis
3. Further study to determine the seroprevalence of Leptospirosis in other local Government Area of Borno State

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