LEPTOSPIROSIS AMONG ZEBU CATTLE IN FARMS IN KADUNA STATE, NIGERIA

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Abstract

Objective: This study was conducted to assess the occurrence of *Leptospira* spp serovar Hardjo among Zebu cattle in some livestock producing areas of Kaduna State, Nigeria. **Materials and Methods:** Sera samples were obtained from 164 Zebu breed of cattle above one year of age in seven cattle farms were screened for antibodies to *Leptospira* spp. serovar Hardjo using Enzyme linked immunosorbent assay (ELISA). **Results:** Antibodies to *Leptospira* spp serovar Hardjo were detected in eighteen (10.98%) out of the 164 animals sampled. There was no significant difference ($p>0.05$) in seropositivity between the different age groups or between different Zebu breeds. **Conclusion:** The presence of Leptospirosis among the Zebu breeds of cattle may poses a threat to livestock production and has public health implication due to its zoonotic potential.

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Introduction

Leptospirosis is an economically important zoonotic disease caused by a spirochaete bacterium of the genus *Leptospira* [1]. The cattle mainetained *Leptospira* spp. serovar Hardjo consist of two serologically indistinguishable but genetically distinct species; *Leptospira interrogans* serovar Hardjo and *Leptospira borgpetersenii* serovar Hardjo. Cattle-maintained leptospires of the serovar Hardjo are the major cause of bovine leptospirosis[2]. This infection is responsible for considerable financial loss to the cattle industry as a consequence of agalactia, abortion, stillbirth, birth of weak calves and reduced fertility [3, 4]. The diagnosis of leptospirosis is commonly based on the demonstration of antibodies by serological test. Though in spite of its disadvantages, microscopic agglutination test (MAT) is still the gold standard serological test for the diagnosis of leptospirosis [5]. Other serological test such as enzyme linked immunosorbent assay (ELISA) has been employed as a useful alternative. It is a reliable test and gives good results in diagnosis that has correlation with those of MAT [6] In spite of reports of the occurrence of leptospirosis in cattle worldwide [2] and economic importance due to reproductive problems and overall impaired productivity, few studies have been conducted to assess its occurrence in cattle population in Nigeria and non after the work of Diallo [7] about three decade ago especially in Kaduna State. The intention of this study was therefore, to investigate the occurrence of the disease among Zebu cattle.

Materials and Methods

Blood samples were collected from thirty percent of the total number of zebu cattle in each of seven farms located in Sabon Gari, Giwa and Zaria Local government areas of Kaduna State, Nigeria based on the world Organisation for Animal Health recommendation [5] of at least 10% of animals in a herd (OIE, 2008). The sampling area is located between latitudes 11°7' 11°12'N and longitudes 07°41'E. The area is characterized by a tropical climate; a mean monthly temperature of 13.8-36.7°C and annual rainfall of 1092.8mm [8].

Blood samples were collected from a total of one hundred and sixty four indigenous (Whie Fulani, Sokoto Gudali and Rahaji) breeds of cattle on the seven farms via venipuncture of the jugular vein into anticocoagulant free labelled sample bottles. Only animals above one year of age were sampled. The animals were aged using their dentition. Sera was separated by centrifugation of the clotted blood at 4,000rmp for 5 minutes and stored at -20°C until use.

Enzyme linked immunosorbent assay (ELISA) kit obtained from Linnodee Animal Care, Ballyclare, Ireland was used to screen the sera for antibodies to *Leptospira* spp. serovar Hardjo. The ELISA kit has a sensitivity of 94.10%, a sensitivity of 94.80% and a Kappa index of 0.9. The ELISA was performed as described by the Scolamacchia *et al* [9] and recommended by the manufacturer. Briefly, positive and negative controls were diluted at 1:50 dispensed into duplicate wells on each plate. Sera were also diluted 1:50 in the kit diluents and 100ml was dispensed to each well. The plates were

incubated for 40 minutes in the incubator at 37°C, and then washed four times with the buffer provided alongside the kit. 100µL of the conjugate (HRP) was added to each of the wells and the plates incubated at 37°C for 40 minutes, after which the plates were washed four times with the appropriate buffer. 100µL of the substrate (TMB-E) was then added to each well and the plate incubated at room temperature for 10 minutes, after which 50µL of the stop solution was then added to each well and the plates read using an ELISA reader at 450. The test results were expressed as a ratio of samples value related to positive control value (S/P) using the formular;

$$S/P = \frac{\text{Mean sample optical density} - \text{Mean negative control optical density}}{\text{Mean positive control optical density} - \text{Mean negative control optical density}}$$

Cattle whose serum has an S/P greater than 0.12 were considered seropositive while titre plates with negative control sera optical density of above 0.25 was considered invalid.

Data obtained were presented in form of tables and analysed using Fisher's exact test with the aid of the Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago). Values of $p < 0.05$ were considered significant.

Results

A total of one hundred and sixty four (164) Zebu cattle (White Fulani, Sokoto Gudali and Rahaji breeds) were sampled in seven (7) farms. Thirty three (20.12%) were males while one hundred and thirty one (79.88%) were females. Out of the 164 cattle; Twenty six (15.86%) were < 2 years of age,

Twenty three (14.02%) were between 2-5 years of age and one hundred and fifteen (70.12%) were greater than five years of age. Of the 164 cattle; one hundred and thirty one (79.88%) were White Fulani, twenty nine (17.68%) were Sokoto Gudali and four (2.44%) were Rahaji breeds (Table 1).

Prevalence of cattle seropositive for antibodies to *Leptospira* hardjo varied between 11.11% in farm F to 30.30% in farm B. Out of the one hundred and sixty four animals sampled eighteen (10.98%) were seropositive for *Leptospira* hardjo (Table 2).

None of the males sampled was seropositive for *L.* hardjo while eighteen (13.74%) females were seropositive. There was a statistically significant difference in seropositivity of leptospirosis between the sexes. Based on age group; one (3.85%), two (8.70%) and fifteen (13.04%) animals in the age groups < 2, 2-5 and > 5 years were respectively seropositive for *L.* hardjo. There was no statistically significant difference in seropositivity of leptospirosis between different the age groups. Based on individual breeds prevalences; thirteen (9.92%) White Fulani and Five (17.24%) Sokoto Gudali were seropositive for *L.* hardjo while none of the Rahaji breed was seropositive for *L.* hardjo. There was no statistically significant difference in seropositivity of leptospirosis between the different breeds (Table 3).

Table 1: Sex, Age and Breed distribution of zebu cattle in the different farms.

Farms	A	B	C	D	E	F	G	Total (%)
Sex								
Males	2	3	0	8	10	1	9	33 (20.12)
Females	20	30	16	12	27	8	18	131 (79.88)
Age								
<2	5	7	5	1	2	2	4	26 (15.86)
2-5	2	4	5	6	0	1	5	23 (14.02)
>5	15	22	6	13	35	6	18	115 (70.12)
Breeds								
Sokoto Gudali	9	16	0	0	0	2	2	29 (17.68)
Rahaji	2	2	0	0	0	0	0	4 (2.44)
White Fulani	11	15	16	20	37	7	25	131 (79.88)

Table 2: Prevalence rate of leptospirosis in Zebu cattle in the different farms.

Farms	A	B	C	D	E	F	G	Total
Total number of animals sampled	22	33	16	20	37	9	27	164
Number of animals seropositive	6	10	1	0	0	1	0	18
Prevalence (%)	22.27	30.30	6.25	-	-	11.11	-	10.98

Table 3: Sex, Age and Breed prevalence of leptospirosis among the Zebu cattle.

Variables	Total no. of animals sampled	No of animals seropositive	Prevalence (%)	p value
Sex				0.0252[a]
Males	33 (20.12)	0	-	
Females	131 (79.88)	18	13.74	
Age				0.3719[b]
< 2	26 (15.85)	1	3.85	
2-5	23 (14.02)	2	8.70	
>5	115 (70.12)	15	13.04	
Breeds				0.4052[b]
Sokoto Gudali	29 (17.68)	5	17.24	
Rahaji	4 (2.44)	0	-	
White Fulani	131 (79.88)	13	9.92	

([a] statistically significant ($p < 0.05$), [b] not statistically significant ($p > 0.05$))

Discussion

Leptospira spp. serovar Hardjo antibodies were detected in the Zebu cattle with a prevalence of 10.98%. Cattle are not routinely vaccinated against leptospirosis in Nigeria [8] and none of the farms sampled had reportedly vaccinated their cattle against leptospirosis. All animals sampled were above one year of age, thereby ruling out cross-reactions or interference by maternal antibodies. Therefore, the presence of *Leptospiral* antibodies in these animals is suggestive of natural exposure to the organism.

Bovine leptospirosis has been previously reported among cattle in other parts of Nigeria [7, 10-13]. Although, the Zebu cattle in the current study were tested for antibodies against *Leptospira* spp. serovar Hardjo as opposed to cultural isolation, some infected animals have been reported to remain so for life and continue to shed the organism [14-17]. It is therefore, likely that the seropositive animals are still shedding the organism.

Though, cows had a higher (13.74%) prevalence of the disease compared to males where none was positive. The presence of statistically significant difference ($p<0.05$) in seropositivity of leptospirosis between the bulls and cows may have resulted from the higher number of females sampled compared to males as both sexes face the same risk of being infected by the organism.

Though, the age group >5 had more seropositive animals (12.50%) compared to the other age groups, this does not necessarily indicate that the older animals are at higher risk of infection by the organism but may be a reflection

of the long duration/persistence of antibodies against the organism and more exposure time. This is supported by the absence of statistically significant difference ($p<0.05$) in seropositivity of leptospirosis between the various age groups indicating that all ages groups face the same risk of being infected by the organism.

White Fulani, Sokoto Gudali, Rahaji, Adamawa Gudali, are the predominant breeds in Nigeria [18]. The most predominant indigenous breeds in the study area are White Fulani and Sokoto Gudali. Most of the cattle in the farms are obtained from nearby cattle markets and herds [19]. The presence of Rahaji breed of cattle among the sample population is a reflection of the diversity of the sources/location of cattle brought to these cattle markets. There was no statistically significant difference ($p>0.05$) in seropositivity across the three breeds indicating they all face the same risk of infection by *Leptospira* species. The low number of the Sokoto Gudali and Rahaji breeds compared to the White Fulani breed which are the most common breed in this area and the country at large may have contributed to the high number of seropositive (13) animals among the White Fulani breed.

Zebu breeds are commonly purchased from herdsmen or cattle marketers for stocking of new farms or restocking of old farms. They are then cross bred with exotic breeds to produce offspring with greater vigour and characteristics desired by the farmer. The presence therefore, of this disease among the Zebu cattle implies an impending economic loss to the farmers

who intends to use these animals for crossbreeding as *Leptospira* spp. serovar Hardjo has been reported to cause agalactia, abortion, stillbirth, birth of weak calves and reduced fertility [3, 4] asides it zoonotic potential.

The findings of this study indicate that the leptospirosis is present among Zebu cattle despite the paucity of reports on clinical cases. Therefore, the close contact and co-habitation that exists between some of the farm workers and cattle may result in the spread of this zoonosis.

The findings of these study suggests the need for enlightenment of livestock health workers especially veterinarians on the need to include leptospirosis among the diseases to be screened for before addition of new animals into a herd.

Conflict of Interest statement

We declare that we have no conflict of interest.

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