Seroprevalence of Leptospira spp serovar Hardjo in Nigerian cattle

E. O. NGBEDE1*, M. A. RAJI1, C. N. KWANASHIE1, E. C. OKOLOCHA2

1Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, P.M.B 1069 Zaria, Kaduna State, NIGERIA.
2Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, P.M.B 1069 Zaria, Kaduna State, NIGERIA.

*Corresponding author: drngbede@hotmail.com

SUMMARY

Leptospirosis is a zoonotic disease caused by Leptospira species (mainly serovar Hardjo) that threatens the livestock industries and public health worldwide. Leptospira spp. serovar Hardjo seroprevalence was investigated in apparently healthy more than 1 year old cattle from 8 farms of Zaria, Nigeria, using a commercial ELISA kit. Twenty (8.44%) out of the 237 cattle sampled were seropositive. A high seroprevalence was found in older animals (more than 5 years old) but age influence was not statistically significant. Indigenous breeds appeared significantly more frequently seropositive than exotic and cross breeds. In conclusion, Leptospira spp. serovar Hardjo is present in apparently healthy cattle in farms in Zaria and may contribute to the disease spread in other animals and humans.

Keywords: Cattle, ELISA, leptospirosis, Leptospira spp. serovar Hardjo, seroprevalence, Nigeria, indigenous breed

RESUME

Séroprévalence des leptospires spp de sérovar Hardjo chez les bovins nigérians.

La leptospirose est une zoonose causée par les espèces Leptospira (principalement de sérovar Hardjo) qui menace les industries du bétail et la santé publique partout dans le monde. La séroprévalence des leptospires de sérovar Hardjo a été recherchée dans cette étude sur des bovins apparemment en bonne santé de plus de 1 an issus de 8 fermes de la région de Zaria, Nigeria en utilisant un kit ELISA. Au total 20 (8.44%) des 237 animaux prélevés ont été séropositifs. Une forte séroprévalence a été observée chez les animaux les plus âgés (de plus de 5 ans) mais l’influence de l’âge ne s’est pas révélée statistiquement significative. Les races autochtones sont apparues significativement plus souvent séropositives que les races exotiques et leurs croisements. En conclusion, les leptospires de sérovar Hardjo sont présentes dans le cheptel apparemment sain des fermes de la région de Zaria ce qui peut contribuer à la propagation de la maladie aux autres animaux et aux hommes.

Mots-clés : Bétail, ELISA, leptospirose, Leptospira spp. sérovar Hardjo, séroprévalence, Nigeria, race autochtone

Introduction

The economic impact in the livestock industry and zoonotic nature of leptospirosis makes it a disease of global importance [34]. The disease is caused by pathogenic Leptospira species and considered to be an emerging or re-emerging disease in many countries of the world [38]. Cattle are the maintenance host for Leptospira spp. serovar Hardjo [22] which consist of two serologically indistinguishable but genetically distinct species, Leptospira interrogans serovar Hardjo and Leptospira borgpetersenii serovar Hardjo [14]. Leptospira spp. serovar Hardjo has been reported to be a major cause of economic loss to the livestock industry as a result of reduced fertility, abortion, birth of weak calves, agalactiae [10, 11, 19, 20], non reproductive losses due to septicaemia, nephritis and death [19] and a cause of leptospirosis in humans [24, 31].

Serological testing is the most widely used means for the diagnosis of leptospirosis and microscopic agglutination test (MAT) is the standard serological test [27]. However, MAT has some disadvantages such as the use of live antigens, subjective interpretation of test results, requires well developed and equipped laboratory, and it cannot detect antibody titres of ≤ 100 [13] and produces cross-reaction between Leptospira spp serovar Hardjo and other serovars [36]. ELISA have therefore been developed [4, 9, 29, 37] as an alternative to screen for leptosporal infection, though it requires a separate test for each serovar [4, 9]. In cattle adapted Leptospira spp serovar Hardjo infection, a significant percentage of animals that are actively infected and shedding leptospires have antibody titers ≤ 100 against Leptospira sp serovar Hardjo but they are considered as seronegative to Hardjo infection [13]. Therefore, a low antibody titer detected by MAT does not necessarily rule out a diagnosis of leptospirosis. The MAT measures mainly IgM which titres peak after 10 to 20 days but decline within 6 to 12 months; consequently MAT just demonstrates recent infection [28] while ELISA measures IgG which begins to appear as the IgM peaks after infection and persists for a longer time [28]. ELISA is therefore a better guide to longer term status and detection of cattle potentially shedding the organism and it also provides a cheaper and easier means of herd screening against bovine leptospirosis.

Important factors for farmers are low productivity especially due to low fertility of their animals, abortion,
decreased milk production and high mortality of the young animals. Previous evidences of the disease in the study area were given by DIALLO [12] approximately three decades ago. Therefore, the aim of this study is to assess the seroprevalence status of *Leptospira* spp. serovar Hardjo in cattle population in the study area. This data will provide useful information that will help farmers, veterinarians and medical personnel in the design of preventive measures that will help avoid economic losses and threat to public health.

Materials and Methods

**EXPERIMENTAL AREA, ANIMALS AND SAMPLING**

The study was conducted in Zaria, a suburban area in Kaduna State, Northern Nigeria, located between latitudes 11°7´ - 11°12´N and longitudes 07°41´E. The area is characterised by a tropical climate; a mean monthly temperature of 13.8-36.7°C and annual rainfall of 1092.8 mm [1]. Eight cattle farms were selected from a list of farms under the ambulatory unit of a Veterinary Unit in Zaria and used for the study based on the willingness of the owners to allow the use of their farms for the study.

Based on the World Animal Health Organisation recommendations [27] that at least 10% of the animals in a farm be sampled, 30% of the animals in each of the farm visited were sampled. The age of animals was determined according to the dentition and only cattle above one year old were sampled. Blood samples were collected from 237 apparently healthy cattle of different sexes, breeds and ages. Venous blood samples (5 mL) were collected from the jugular vein of each cattle into well labelled tubes free of anticoagulant and then transported to the laboratory in an ice packed flask. The blood samples were kept in slanted for 3 hours at room temperature (25-27°C) in the laboratory to allow clotting. They were then centrifuged at 2000g for 5 minutes under room temperature (25-27°C) to allow proper separation of serum from the clotted blood cells. The sera were decanted into 5 mL serum tubes and stored at -20°C until used for the serological test.

**SEROLOGICAL ANALYSIS**

An enzyme-linked immunosorbent assay (ELISA) kit (Linnodee Animal Care, Ballyclare, Ireland) was used to screen the sera for antibodies to *Leptospira spp* serovar

<table>
<thead>
<tr>
<th>Farms</th>
<th>Number of sampled cattle</th>
<th>Leptospirosis seroprevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>24</td>
<td>6 (25.0%)</td>
</tr>
<tr>
<td>B</td>
<td>46</td>
<td>11 (23.9%)</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>1 (6.3%)</td>
</tr>
<tr>
<td>D</td>
<td>20</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>E</td>
<td>39</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>F</td>
<td>20</td>
<td>2 (10.0%)</td>
</tr>
<tr>
<td>G</td>
<td>35</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>H</td>
<td>37</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>237</td>
<td><strong>20 (8.4%)</strong></td>
</tr>
</tbody>
</table>

Table I: Leptospirosis seroprevalence determined using ELISA kit (Linnodee Animal Care, Ballyclare, Ireland) in cattle from the Zaria, Kaduna State, Nigeria.

<table>
<thead>
<tr>
<th>Age</th>
<th>Positive cattle</th>
<th>Seroprevalence</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 years (n = 51)</td>
<td>1</td>
<td>1.96%</td>
<td>NS</td>
</tr>
<tr>
<td>2-5 years (n = 52)</td>
<td>4</td>
<td>7.69%</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 years (n = 134)</td>
<td>15</td>
<td>11.19%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Positive cattle</th>
<th>Seroprevalence</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (n = 35)</td>
<td>2</td>
<td>5.71%</td>
<td>NS</td>
</tr>
<tr>
<td>Females (n = 202)</td>
<td>18</td>
<td>8.92%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breed</th>
<th>Positive cattle</th>
<th>Seroprevalence</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigenous (n = 164)</td>
<td>18</td>
<td>10.98%</td>
<td></td>
</tr>
<tr>
<td>Crosses (n = 62)</td>
<td>0</td>
<td>0.00%</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Exotic (n = 11)</td>
<td>2</td>
<td>18.18%</td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant.

Table II: Leptospirosis seroprevalence determined using ELISA kit (Linnodee Animal Care, Ballyclare, Ireland) in cattle from the Zaria, Kaduna State, Nigeria according to age, sex and breed.
The ELISA kit offers a sensitivity of 94.10%, specificity of 94.80% and a Kappa index (statistical method used for assessing the agreement beyond that expected by chance between diagnostic methods). Statistic ranges from 0 to 1 with a kappa value of about 0.4 to 0.6 indicating moderate agreement while higher values are interpreted as good agreement) of 0.9.

The test was performed as described by SCOLAMACCHIA et al. [30] and according to the manufacturer's recommendations. 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 100 µL 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. The horseradish peroxidase conjugate (100 µL) was added to each well and the plates were incubated at 37°C for 40 minutes, then washed four times with the appropriate buffer. The enzyme substrate TMB-E (100 µL) (3,3',5,5'-tetramethylbenzidine) was added to each well and the plate incubated at room temperature for 10 minutes; after which the reaction was stopped with the addition of the stop solution (50 µL) and the absorbance was read at 450 nm using an ELISA reader. The test results were expressed as a ratio of samples value related to positive control value (S/P) using the following formula: S/P = (Mean sample optical density – Mean negative control optical density) / (Mean positive control optical density – 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.

STATISTICAL ANALYSIS

Data were analysed using the Fisher 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 as significant.

Results

Twenty (8.44%) out of the 237 cattle sampled across the eight farms were seropositive for antibodies to Leptospira spp. serovar Hardjo (Table I). Only four (50%) of the eight farms had at least one sero-positive cattle and the leptospirosis seroprevalence varied from 6.25% to 25.0% among the 4 positive farms (Table I).

The frequencies of seropositive animals according to their age, sex and breed were summarized in Table II. It was observed that leptospirosis seroprevalence gradually increased with the age of animals, ranging from 1.96% in young cattle (below 2 years old) to 7.69% in 2-5 years old animals and to 11.19% in older animals (above 5 years old). However, there was no statistically significant difference in seropositivity across the three age groups (p > 0.05). Two (5.71%) out of the 35 bulls sampled were seropositive while 18 (8.91%) out of the 202 cows sampled were seropositive for antibodies to Leptospira spp serovar Hardjo. No significant difference in seropositivity between the sexes was found (p > 0.05). Eighteen (10.98%) of the 164 cattle from indigenous breeds were seropositive for Leptospira spp serovar Hardjo. Among them, 131 belonged to the White Fulani, 29 were Sokoto Gudali and 4 Rahaji (Red Bororo). None of the crosses among 62 cross-breds were seropositive while 2 animals among 11 (18.18%) from exotic breeds (6 Simmental, 4 Brahman and 1 Montbéliarde) exhibited circulating antibodies against the serovar Hardjo. The seroprevalence was statistically different between indigenous breeds, cross-breds and exotic breeds (p < 0.05).

Discussion

Though the clinical manifestation of Leptospira spp. serovar Hardjo infection in livestock often goes unnoticed, interest in the disease as a cause of economic losses in the livestock industry as a result of infertility, agalactiae, abortion and its public health importance as a zoonosis is increasing worldwide as indicated by the rising number of reviews and published reports [7, 23, 33, 38].

The present results demonstrated that Leptospira spp. serovar Hardjo antibodies are present in cattle in Zaria with an overall seroprevalence of 8.44%. Since vaccination against leptospirosis is not routinely carried out on cattle in Nigeria [2] and none of the sampled farms had reportedly used leptospiral vaccine, the presence of circulating antibodies in the cattle suggested a natural exposure to Leptospira spp. serovar Hardjo. Some cattle infected with L. serovar Hardjo have been reported to remain infected and shed the organism continuously or intermittently throughout their life time [5, 17]. It is therefore, likely that the seropositive animals are still shedding the organism, thus serving as a source of infection for other animals and workers in the farms.

The results of this study is in agreement with previous studies in parts of the country suggests endemicity of the disease [2, 12, 14-16] although a lower prevalence rate was found here. The utilisation of MAT for serological screening may have contributed to the higher prevalence in previous studies: it has been reported to produce cross-reactions [36] between members of the same serogroup e.g. serovar Hardjo cross-reacts with other serovars like Baleranca, Medanensis [25] and Szwajizak [6] whereas the ELISA technique used in this study is higher specific for Leptospira spp serovar Hardjo [32, 37].

Although no statistically significant difference in seropositivity according to the age was evidenced, seropositive animals were more frequently found in the group of cattle above 5 years old. This finding is in agreement with the report of WAI’IN et al. [35]. This does not necessarily mean that older animals exhibit a higher risk for contracting the disease but this may rather portray the longer duration/persistent of leptospiral antibodies. The absence of a statistically significant
difference between seropositivity and sex indicates that both sexes have the same risk of being infected by the organism.

Several reasons could have played a role in high prevalence of antibodies to _Leptospira_ spp serovar Hardjo especially among the indigenous (Zebu) breeds of cattle. One of which is the source of the animals. Most of the farms obtain their cattle from cattle markets to stock or use as replacement animals on their farms. Some of the animals sold in the cattle markets in Nigeria originate from neighbouring African countries such as Mali, Republic of Niger, Republic of Chad, Sudan and Cameroon [3, 8, 18] in some of which high prevalence rates of bovine leptospirosis have been reported [26, 30]. The high concentration of cattle, eventually infected in markets leads to frequent contacts between animals and promotes transmission of _Leptospira_ between animals. Decreased productivity characterized by abortion, infertility, increased calving intervals and mastitis is dominant feature of bovine leptospirosis. Such low productivity may cause a farmer to cull such animal from the herd and sell [22] to unsuspecting farmers in the markets that take them and add to their herds. This may also have contributed to the higher percentage of seropositive animals from indigenous breeds.

As a conclusion, _Leptospira_ spp serovar Hardjo is present with a prevalence of 8.44% in cattle in Zaria, Nigeria despite the lack of reports on clinical cases of the disease. It is therefore possible that losses from leptospirosis in the cattle population in Nigeria may be underestimated as there is evidence from a number of countries that serovar Hardjo continues to cause substantial reproductive losses in cattle through abortion and infertility. Infected animals are reported to shed the organism in their urine or aborted materials [5, 17]. Consequently, the possibility exists that these apparently healthy seropositive animals may also serve as source of infection to other animals in the farm and for humans.

References