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Serological prevalence of leptospirosis in cattle slaughtered in the Zango abattoir in Zaria, Kaduna State, Nigeria

Emmanuel O. Ngbede[(1)], Mashood A. Raji[(1)], Clara N. Kwanashie[(1)], Emmanuel C. Okolocha[(2)], Victor T. Gugong[(2)] & Sunday E. Hambolu[(2)]

Summary

Leptospirosis is an occupational zoonosis caused by pathogenic leptospires. In this study, the presence and prevalence of antibodies specific to *Leptospira* spp. serovar Hardjo in 142 cattle slaughtered between June and July 2011 was investigated using the enzyme-linked immunosorbent assay (ELISA). Five (3.50%) of the 142 cattle sampled were seropositive for antibodies to *Leptospira* spp. serovar Hardjo. Despite the fact that there was no significant difference ($p>0.05$) in seropositivity between sexes and between breeds sampled, there was a significant difference ($p<0.05$) in sero-positivity between the different age groups examined. Leptospirosis is present in cattle slaughtered in the Zango abattoir; butchers and abattoir workers are exposed to infected animals and are at risk of being infected by *Leptospira* spp. serovar Hardjo.

Keywords

Abattoir, Abattoir worker, Butcher, Cattle, ELISA, Enzyme-linked immunosorbent assay, Leptospirosis, *Leptospira* spp. Hardjo, Nigeria, Public health, Zoonosis.

Sieroprevalenza di leptospirosi in bovini macellati nel macello Zango in Zaria, nello stato di Kaduna, in Nigeria

Riassunto

La Leptospirosi è una zoonosi professionale causata da leptospire patogene. In questo studio, la presenza e la prevalenza di anticorpi specifici per Leptospira *spp. serovar* hardjo *sono state studiate utilizzando l'ELISA, in un totale di 142 bovini macellati tra giugno e luglio del 2011. Cinque (3,50%) dei 142 bovini campionati erano sieropositivi agli anticorpi anti* Leptospira *spp. serovar* hardjo*. Nonostante non vi fosse alcuna differenza significativa* ($p> 0,05$) *nella siero-positività tra i sessi e tra le razze campionate, vi era invece una differenza significativa* ($p<0,05$) *nella siero-positività considerando l' età dei diversi gruppi esaminati. La Leptospirosi è presente nei bovini macellati nel macello Zango e gli operatori delle relative macellerie e mattatoi non solo sono esposti agli animali infetti, ma sono a rischio di essere infettati dalla* Leptospira *spp. serovar* hardjo.

Parole chiave

Bestiame, ELISA, Mattatoio, *Leptospira* spp. Hardjo, Leptospirosi, Macellaio, Nigeria, Operatore di Mattatoio, Salute Pubblica, Zoonosi.

(1) Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, PMB 1069, Zaria, Kaduna State, Nigeria
drngbede@hotmail.com
(2) Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, PMB 1069, Zaria, Kaduna State, Nigeria

Introduction

Leptospirosis is a disease that is caused by infection with pathogenic members of the genus *Leptospira*. The disease is transmissible between animals and humans. There is a growing concern in regard to the under-recognised burden of leptospirosis in developing countries (26). Infections are commonly associated with occupational risk activities (16, 18). Cattle are the maintenance host for *Leptospira* spp. serovar Hardjo (17) which consist of two serologically indistinguishable but genetically distinct species, namely: *Leptospira interrogans* serovar Hardjo and *Leptospira borgpetersenii* serovar Hardjo (11).

Besides being an important cause of bovine abortion, reduced fertility and agalactia, *Leptospira* spp. serovar Hardjo also poses a potential zoonotic threat to humans who are exposed to infected cattle (22). Humans can become infected when they come into contact directly or indirectly with tissue, body fluid or the urine of carrier animals (25). Serological testing is the most widely used method for the diagnosis of leptospirosis and microscopic agglutination test (MAT) is the standard serological test (27). However, the MAT presents some disadvantages, such as the use of live antigens, subjective interpretation of test results; it is a test that requires a well developed and equipped laboratory and cannot detect antibody titres of ≤100 (10). enzyme-linked immunosorbent assays (ELISAs) have therefore been developed (5, 8, 21, 28) for use as an alternative to screening for leptospiral infection, although a separate test is required for each serovar (5, 8) In cattle adapted *Leptospira* spp. serovar Hardjo infection, a significant percentage of animals that are actively infected and are shedding leptospires have antibody titres ≤100 against *Leptospira* spp. serovar Hardjo and they are considered to be seronegative to Hardjo infection (10). Therefore, a low antibody titre detected by MAT does not necessarily rule out a diagnosis of leptospirosis. The MAT measures mainly IgM, the titres of which peak after 10 to 20 days but decline within 6 to 12 months. Consequently, MAT simply demonstrates recent infection (20), whilst the ELISA measures IgG which begins to appear as the IgM peaks after infection and persists for a longer period of time (20). The ELISA is therefore a better guide to longer term status and detection of cattle that are potentially shedding the organism and it is also a more economical and easier means of herd screening against bovine leptospirosis.

Infection with host-adapted serovars have been reported to produce subclinical infection with apparently healthy animals serving as chronic carriers and persistent shedders of the organism through their urine, body fluid or tissue. They thereby pose a risk and source of infection to livestock workers, especially butchers and abattoir workers. The aims of this study were to determine the extent of *Leptospira* spp. serovar Hardjo infection in an abattoir in Kaduna State, and the role of cattle as potential zoonotic reservoirs.

Materials and methods

The study was conducted at the Zango abattoir that is located in the village of Samaru, Zaria, Kaduna State, northern Nigeria. It lies between latitude 11°7′N and longitude 07°41′E. The abattoir is owned by the Kaduna State Government and is managed by the Ministry of Agriculture. The area is characterised by a tropical climate, a mean monthly temperature of 13.8°C-36.7°C and annual rainfall of 1 092.8 mm (1). This abattoir serves as the major source of meat for the inhabitants of the Samaru village and the Ahmadu Bello University community.

Based on the abattoir records, 35 to 50 cattle are slaughtered daily with a monthly average of 1 427 cattle. Based on the recommendations of the World Organisation for Animal Health (*Office International des Épizooties*: OIE), 10% (142) of the average number of cattle slaughtered monthly in the abattoir were collected (27). The study population comprised 142 cattle above one year of age that were slaughtered in the Zango abattoir between June and July 2011. The animals were aged using their dentition. Blood samples were

collected twice weekly from the first 15 cattle slaughtered on each day of the visit by venipuncture of the jugular vein. Serum samples were separated by centrifugation of the clotted blood at 4 000 rpm for 5 min and stored at –20°C until use.

An ELISA kit obtained from Linnodee Animal Care, Ballyclare, Ireland, was used to screen the sera for antibodies to *Leptospira* spp. serovar Hardjo. The ELISA has a sensitivity of 94.10%, a sensitivity of 94.80% and a Kappa index of 0.9. It was performed as described by the Scolamacchia *et al.* (23) and in accordance with the recommendations of the manufacturer.

Briefly, positive and negative controls were used diluted at 1:50 and dispensed into duplicate wells on each plate. Sera were also diluted 1:50 in the kit diluents and 100 ml were dispensed to each well. The plates were incubated at 37°C for 40 min in the incubator and then washed four times with the buffer provided with the kit. A total of 100 µl of the conjugate (horse radish peroxidase: HRP) was added to each of the wells and the plates were incubated for 40 min at 37°C, after which the plates were washed four times with the appropriate buffer. Then, 100 µl of the substrate 3,3',5,5'-tetremethylbenzidine ELISA (TMB-E) were added to each well and the plate was incubated at room temperature for 10 min, after which 50 µl of the stop solution was added to each well and the plates read using an ELISA reader at 450 nm. The test results were expressed as a ratio of sample values (S) related to positive control values (P) using the following formula:

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

Cattle sera with an S/P that exceeded 0.12 were considered seropositive, whilst titre plates with negative control sera optical density exceeding 0.25 were considered invalid. Data obtained were presented in the form of tables and analysed using the Chi square test and 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 significant.

Results

Of the 142 cattle samples taken, 5 (3.50%) were seropositive for *Leptospira* spp. serovar Hardjo antibodies (Table I). A total of 81 (57.04%) of the cattle sampled were males whilst 61 (42.96%) were females; of these, 2 males (2.47%) and 3 females (4.92%) were seropositive. There was no significant difference ($p = 0.6515$) in seropositivity

Table I
Demographic information, sex, age, breed and overall serological prevalence of cattle sampled for leptospirosis in Zango abattoir, Zaria, Kaduna State, Nigeria

Variables	Total no sampled (%)	No. positive	Prevalence (%)	*p* value
Sex				0.6515
Males	81 (57.04)	2	2.47	
Females	61 (42.96)	3	4.92	
Age				0.0313
<2	8 (5.63)	0	0	
2-5	110 (77.46)	2	1.82	
>5	24 (16.90)	3	12.50	
Breeds				0.8039
White Fulani	104 (73.24)	4	3.85	
Sokoto Gudali	27 (19.01)	1	3.70	
Rahaji	11 (7.75)	0	0	
Overall prevalence	142	5	3.50	–

between the sexes. Of the seropositive animals, 2 (1.82%) were in the age group of 2-5 years of while 3 (12.50%) were in the age group of >5 years. There was a significant difference in seropositivity between the age groups ($p = 0.0313$). Out of the 142 animals sampled, 104 (73.24%) were of the White Fulani breed, 27 (19.01%) were of the Sokoto Gudali breed whilst 11 (7.75%) were Rahaji. A total of 4 (3.85%) of the White Fulani breed, 1 (3.70%) of the Sokoto Gudali breeds were seropositive for antibodies to *Leptospira* spp. serovar Hardjo while none of the Rahaji breed was seropositive. There was no significant difference ($p = 0.8039$) in seropositivity between breeds (Table I).

Discussion

Leptospira spp. serovar Hardjo antibodies were detected in the cattle sampled with a prevalence of 3.50%. Bovine leptospirosis has been reported among cattle in other areas of Nigeria at a prevalence rate of 11%-23% (4, 9, 12, 13). The presence of antibodies to *Leptospira* spp. serovar Hardjo in these groups of animals suggests natural exposure to the organism, as vaccination against bovine leptospirosis is not routinely practised in Nigeria (4). The low prevalence in this study compared to the above studies might have been due to the small number of samples obtained and area covered in this study. However, the results of this study concur with the findings of Agunloye *et al.* (2) that were conducted about a decade ago in the Ibadan abattoir.

Reports have indicated that some of the cattle slaughtered in the abattoir originate from neighbouring countries, such as Cameroon, Chad, Mali, Niger and Sudan (3, 7, 15). Some of these countries have reported a leptospirosis prevalence of between 32% and 45% (19, 23). The cattle are brought into the country, mixed with other animals at cattle markets and sold to unsuspecting butchers.

The prevalence in cows was higher (4.92%) than that recorded in males (2.47%). The absence of a statistically significant difference ($p>0.05$) in seropositivity of leptospirosis between the bulls and cows indicates that both sexes face the same risk of being infected by the organism.

There was a statistically significant difference ($p<0.05$) between leptospirosis seropositivity between the various age groups. The group aged over five years had more seropositive animals (12.5%) compared to the other age groups; this does not necessarily indicate that the older animals are at greater risk of infection by the organism but this may be a reflection of the long duration and persistence of antibodies in the animals and a longer period of exposure.

White Fulani, Sokoto Gudali, Rahaji and Adamawa Gudali are the predominant cattle breeds in Nigeria (24). The predominant indigenous breeds in the study area are White Fulani and Sokoto Gudali. The presence of the Rahaji breed among the sample population is a reflection of the diversity of the source/ location of cattle brought to these cattle markets. However, there was no statistically significant difference ($p>0.05$) in seropositivity across the three breeds which indicates that they all face the same risk of infection by *Leptospira* species. The most predominant breed in the study area was the White Fulani.

Infected animals are reported to shed the organism in their urine, aborted material discharge, body fluid and tissues (7, 14). The possibility therefore exists that these apparently healthy seropositive animals may be shedding *Leptospira* spp. serovar Hardjo and they serve as source of infection to other animals and humans. Carrier animals can transmit the disease to other animals in markets due to the frequent contacts between the animals. These cattle markets also serve as source of replacement animals for cattle farms across the country. Therefore, the sources of cattle may have contributed to the prevalence levels and, by implication, the spread of the disease in the cattle and human populations.

Conclusions

The presence of leptospirosis among animals slaughtered in the abattoir is an occupational hazard for the butchers and abattoir workers. These groups of individuals are exposed to

urine, body fluid, foetus and tissue of potentially infected animals and they do not wear adequate personal protective equipment, such as gloves, face masks and boots. These individuals are therefore at risk of being infected by *Leptospira* spp. serovar Hardjo. This has serious implications as the clinical manifestation of leptospirosis in humans produces signs similar to malaria and typhoid which are endemic in the study area and leptospirosis is not currently considered in differential diagnosis when humans present signs of either of these diseases. This may result in misdiagnosis, resulting in complications and under-reporting of the disease and, consequently, an increase in morbidity and mortality.

In conclusion, leptospirosis is present in cattle slaughtered in the abattoir and they are a potential reservoir for transmission to butchers and abattoir workers. There is need to advise butchers and abattoir workers on the need to use personal protective clothing to avoid direct contact with matter from potentially infected animals. It is important that public health workers be educated on the presence of the disease in animals in this environment and on its zoonotic implications.

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