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Cross Sectional Study of Brucellosis in Cattle Slaughtered in Abattoirs within the Transit City of Ilorin, Kwara State, Nigeria

Aiyedun J. O.,[1] Odetunde F. O.,[1], Oludairo O. O.[1], Olorunshola I. D.[2] Daodu O. B.[2], and Nwoha R. I. O.[3]

[1]Department of Veterinary Public Health and Preventive Medicine,
Faculty of Veterinary Medicine, University of Ilorin, Nigeria.
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine,
University of Ilorin, Nigeria.
[3]Department of Veterinary Medicine Micheal Okpara
University of Agriculture, Nigeria.

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ABSTRACT

Brucellosis is one of the most important zoonosis in the world; it is highly contagious and caused by a group of organisms in the genus Brucella. The disease remains endemic in Nigeria and its actual incidence and prevalence are unknown due to both inadequate surveillance and systems of reporting outbreaks. This study was designed to determine the sero-prevalence of bovine brucellosis in Ilorin, Kwara State, a gateway to states, and countries engaging in livestock trades within and around Nigeria. A total of 224 blood samples were randomly collected from cattle slaughtered at the two metropolitan abattoirs in the city. The study employed a combination of 2 serological techniques; the Rose Bengal Plate Test (RBPT) and the Serum Agglutination Tube Test (SAT). Of the 224 cattle screened, 10.71% tested positive with Rose Bengal Antigen, while 11.16% tested positive for Serum Agglutination Tube Test (SATT). Prevalence was higher in cows (11.44%) than in bulls (8.69%). There was no significant relationship between the prevalence of bovine brucellosis and any of the variables examined. However, the overall prevalence (11.16%) observed in the study was significant. Thus, the need for drastic public health interventions/control measures on brucellosis in the livestock industry in Nigeria.

Keywords: Brucellosis, Cattle, Prevalence, Abattoir, Nigeria, SATT, RBPT

Corresponding Author:
email: oludairo@hotmail.com

INTRODUCTION

Brucellosis (Undulant fever, Mediterranean fever, Malta fever, Rock fever, Enzootic abortion, Epizootic abortion, Contagious abortion, Bang's disease) is one of the most important and widespread zoonosis in the world [1, 2]. It is a contagious bacterial zoonosis of livestock caused by a Gram negative coccobacillus organism of the genus *Brucella,* and family *Brucellaceae* (subdivision Alpha 2; class *Alphaproteobacteria*) [2]. Of the ten recognized species of *Brucella*, only *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* are pathogenic to humans [3].

Brucellosis is recognized as a major cause of significant economic losses in livestock due to its primary effect on the reproductive system. These effects include decreased calving percentage, delayed calving, culling for infertility, cost of treatment, decreased milk production, abortions, stillbirth and birth of weak calves among others. It also poses a serious threat to human health leading to loss of working-hours in infected people. Humans are infected through contact with infected animals or by consuming infected food of animal origin [4, 5, 6]. This is particularly serious because the human disease is often debilitating if not correctly cared for at an early stage [6, 7].

Prevalence of bovine brucellosis varies widely across Nigeria, and between herds in the same area, with reported sero-prevalences of 0.2% to 80.0% [8, 9]. In institutional farms, abattoir surveys and other ranches or dairy farms in southern Nigeria, prevalence in cattle ranged between 3.7% and 48.8% (10, 11), while in the traditional nomadic Fulani cattle herds, the prevalence was between 0.4% and 26% [12]. Junaidu *et al* [13] reported a within-herd prevalence of 32.2% on a prison cattle farm and 19.5% seropositive and 25.3% positive milk samples were reported in northern Nigeria.

Despite high and variable prevalence in many countries, brucellosis remains a major neglected zoonosis in some countries like Nigeria. This could be because surveillance for the disease is generally poor. Nigeria is currently the most populous African country with over 170 million in 2012 and an estimated livestock population of 20.49 million cattle, 23.07 million sheep, 28.07 million goats, 6.54 million pigs, 18,200 – 90,000 camels, and 210,000 horses [14]. The knowledge of the incidence and prevalence of the disease would enhance better control measures [7, 15, 16].

Brucellosis remains a problem in Nigeria due to lack of official policy for the control of the disease [17], uncontrolled movement of slaughter cattle within and from neighbouring countries [11, 18], nomadism [19] and poor knowledge and practices concerning the diseases among farmers and other risk groups [20].

Brucellosis is regarded as a disease of public health significance as it is zoonotic in nature, thereby making occupationally risk persons like butchers, abattoir workers, livestock owners and rearers, veterinarians as well as other humans susceptible [21, 22].

This study was conducted with the aim objective of establishing the prevalence of bovine brucellosis in Ilorin, Kwara State, North-Central, Nigeria. It was also to provide inputs for evidence-based brucellosis control in the country. These were achieved by conducting a sero-epidemiological survey of *Brucella abortus* in cattle in selected abattoirs in Ilorin and examining the role of certain associated variables in slaughtered cattle

MATERIALS AND METHODS

Study Area

The study was conducted in Ilorin, Kwara

State, North-Central Nigeria. Ilorin is an area lying between latitude 8°25' N to 8°32' N and longitude 4°30' E to 4°41' E. [23]. The city is 50.2 square Km in area and is situated approximately 420 Km from Federal Capital Territory. It is strategically located as the gateway between the Southern and Northern regions of the country which makes it easily accessible to all parts of the country [24].

Study Design

The study is a cross-sectional survey using simple random sampling technique to screen for *Brucella* antibodies in 224 cattle from slaughtered animals at the Olusola Saraki memorial abattoir, Akerebiata and Mandate market abattoir in Ilorin, Nigeria.

Sample size was calculated using the formula by Thrusfield 2005 [25] and a prevalence of 40% to arrive at a sample size of 224 tested using Rose Bengal Plate Test (RBPT) and Serum Agglutination Tube Test (SATT).

The samples were randomly collected in the morning between the hours of 6:30 to 9:30 am in the months of April and May, 2018. A total of 201 female (twenty pregnant) and 23 male animals between the ages of two to six years that were Sokoto gudali, Rahaji and Bunaji breed variants were used for the study.

Blood samples were collected from each animal through the jugular vein directly into labelled sterile plain universal bottle as soon as the animals were slaughtered. The blood samples collected were left for 30 minutes in ambient temperature to separate serum from the clotted blood before transporting directly to the Veterinary Public Health Laboratory, University of Ilorin, Ilorin. The decanted sera were then centrifuged at 3,000 rpm for 20 minutes and clear supernatant serum samples were further transferred into properly labelled plain sample bottles, which were stored at –20° C. The serum samples were later subjected to Rose Bengal Plate test (RBPT) and Serum Agglutination Tube Test (SATT) to investigate the presence as well as the titre level of *Brucella* antibody in each of the sample.

Rose Bengal Plate Test (RBPT)

Rose Bengal Plate Test (RBPT) is a rapid, spot plate agglutination test using a stained Rose Bengal *Brucella* antigen. The test was performed by placing a drop (0.03 ml) of serum sample on a white ceramic tile and a drop (0.03 ml) of the antigen alongside (but not into) the antigen and mixing the antigen and serum thoroughly with sterile toothpick which was then rocked for three minutes.

The tests were read by examining for agglutination under good illumination. Agglutination is the clumping of cells by agglutinating antibodies. The results of RBPT were interpreted as either; negative or no agglutination (-) and positive for any degree of agglutination (+) and doubtful (+/-).

Rose Bengal Plate Test (RBPT) is simple and one of the easiest tests to perform. It is inexpensive, sensitive, specific, gives a rapid result and detects infection at an acute stage than Serum Agglutination test (SAT).

Serum Agglutination Tube Test (SATT)

About 0.8 ml of phenol saline was dispensed into the first test tubes of each row and 0.5 ml of phenol saline into the remaining seven test tubes with the aid of a glass pipette. 0.2 ml of serum sample was added to the first test tube using a glass pipette and the solution was mixed gently without frothing. 0.5 ml was then transferred from the first tube to the next test tube; this was repeated until the last test tube from which 0.5 ml was discarded. After that 0.5 ml of serum agglutination antigen was added to each tube and mixed thoroughly again. Serial dilutions were made at 1:10, 1;20, 1;40, 1;80,

1;160, 1;320, 1;640, 1;1280. The test tubes were then covered with aluminum foil and were placed in a water bath (humidified atmosphere) at 37° C for 24 hours. Standard positive and negative controls were set up along the test tube samples.

The degree of agglutination was read by visual examination of the bottom of the test tubes against a black background with a source of light. A negative reaction had a sedimenting compact button with clear edges while positive reaction had evenly distributed diffused agglutinations at the bottom of the test tubes. Absence of agglutination or antigen sedimentation was read as doubtful.

Statistical Analysis

Results were analysed using GraphPad PRISM 5© for windows, Version 5.0, 2010. Chi-square was used to determine the level of significance between variables.

RESULTS

Out of 224 cattle sampled, twenty-four (24) (10.71%) were positive for Rose Bengal Plate Test (RBPT) while twenty-five (25) (11.16%) for Serum Agglutination Plate Test (SATT) (Table I).

Prevalence

Out of the 224 cattle slaughtered at Ilorin major Abattoirs within the period under review, 24 (10.71%) cattle have *Brucella* antibodies when tested with Rose Bengal Antigen while twenty-five (25) (11.16%) tested positive in Serum Agglutination Tube Test (SATT) (Table I). Analyses of the 2 test techniques employed in the study revealed that the sensitivity of the 2 tests was 96%, the specificity was 100% while the accuracy was 99.6% (Table II).**Age**

Distribution

Out of 175 young cattle sampled, 14 (8%) were positive for *Brucellosis* using Rose Bengal Antigen, while 15 (8.57%) were positive in Serum Agglutination Tube Test. There was no significant difference (P > 0.05) between the positive *Brucellosis* in the adult and the young cattle slaughtered using RBPT (P = 0.1874). Also, the result of SATT showed no significant statistical difference. (P = 0.2478) in sero-prevalence of Brucellosis in the adult and the young cattle slaughtered at the major abattoirs in Ilorin (Table III).

Sex Distribution

Out of the 23 male samples collected, 2 (8.69%) were positive for *Brucellosis* in both RBPT and SATT respectively. 22 (10.94%) out of the 201 female samples analyzed were positive for *Brucellosis* in RBPT while 23 (11.44%) were positive in SATT. There was no significant difference in their sero-prevalence (P = 0.9986) for RBPT and (P = 9961) for SATT (Table III and IV).

Breed Distribution

Out of 25 Bunaji; 14 Sokoto Gudali and 185 Rahaji from which blood samples were collected, 2 (8%) Bunaji and 22 (11.89%) Rahaji were positive for *Brucellosis* using Rose Bengal Antigen. However, Serum Agglutination Tube Test confirmed *Brucella* antibody in 3 (12%) Bunaji and 22 (11.89%) Rahaji. None of the 14 Sokoto Gudali sampled was positive for *Brucellosis.* There was no significant difference in their sero-prevalence (P = 0.9063) and (P = 0.9079) for RBPT and SATT respectively (Table III and VI).

DISCUSSION

The sero-prevalence of Brucellosis*;* in slaughtered cattle during the period of the study was 10.71% using RBPT and 11.16% with SATT. There was no significant difference in the sero-prevalence of Brucellosis*;* in age breed, sex of cattle slaughtered, during the period of study.

Bovine brucellosis was endemic at a relatively high prevalence among slaughtered cattle in two major Ilorin abattoirs with an overall prevalence of 10.71% and 11.16% using RBPT

and SATT respectively. This result corroborates a similar study carried out in selected herd population in the same environs by Aiyedun *et al* [26] with a reported prevalence of 13.3%. However, this study was relatively higher than that of Akinseye *et al.* [27] which reported an overall prevalence of 3.9% in eleven Southern and Northern States; Jajere *et al.* [28] reported a prevalence of 3.5%, 5.0% and 9.0% for RBPT, SAT and MAT in Gombe State, Nigeria; Adamu *et al.* [29] reported a prevalence of 5.3% and 3.8% for both RBPT and SAT in Bauchi State, Nigeria; Tijani *et al* [30] in Yobe State, Nigeria with the prevalence of 5.7%, but much lower than 36.6% obtained in Adamawa [19] and 34.0% in another report by Adamu, *et al.* [4] in Yobe State. Junaidu *et al.* [32] also reported a much higher (19.5%) prevalence of bovine brucellosis in Sokoto, Nigeria.

This disparity in prevalence of bovine brucellosis in these reports could be attributed to the localized coverage of most of these studies, different serological techniques, laboratory variations, management system and geographical location variations and different seasons of sampling [28].

Several studies on bovine Brucellosis have shown that the age as a risk factor plays a significant role in the infection [27]. However, there was no statistically significant association ($p>0.05$) between the prevalence of bovine Brucellosis prevalence among age groups in this study. This result is in agreement with Akinseye *et al.* [27] and Jergefa *et al.* [33] who reported that the sero-positivity of cattle for *Brucella spp* antibodies has no significant association with age.

In this study however, the observed higher sero-prevalence (20.4% for RBPT and SAAT) among the adult age groups (>2 years) when compared with the younger cattle (<2 years) (8.50% and 8.57% for RBPT and SATT, respectively) though not significantly different ($P>0.05$) has been attributed to the higher exposure of older cattle to Brucellosis infection through sexual transmission and increase in resistance by the younger ones [34, 35].

This study reported that female cattle were more seropositive (10.94% and 11.44% for RBPT and SATT, respectively) to *Brucella spp.* antibodies than male cattle (8.69% for both RBPT and SATT) although the difference is not statistically significant ($P>0.05$).

This result is in tandem with the findings of Adamu *et al.* [29], Jajere *et al.* [28] and Aiyedun *et al.* [26] who all reported that the proportion of bovine brucellosis seropositivity was higher in female than male animals although statistically insignificant ($p>0.05$).

Although Modupe *et al.* [35], Akinseye *et al.* [27], Bekele *et al.* [36], Dinka and Chala [37], Junaidu *et al.* [32] and Kebede *et al.* [38] similarly reported a higher prevalence for females, they were however, significantly different ($P<0.05$).

According to Aiyedun *et al.* [26], this may be because greater percentage of samples were taken from female cattle. Adamu *et al.* [29] concurred that this is so because female cattle are the foci of infection, Modupe *et al.* [35] further buttressed that female cattle especially the sexually matured ones are usually with calves and pregnancy stress-induced lowered immunity.

In this study, there was no statistically significant ($p>0.05$) association between breeds and occurrence of *Brucellosis* in the cattle sampled. Although for RBPT sero-positivity was higher in Rahaji (11.89%) than Bunaji (8%), prevalence with SATT sero-positivity for Rahaji and Bunaji were 11.8% and 12%, respectively but none of the samples collected from Sokoto Gudali were sero-positive.

This result is in contrast with the report of

Junaidu *et al.* [32], who reported a higher prevalence of bovine brucellosis in Sokoto Gudali (29.59%) breeds; Jajere *et al.* [28] who reported the seropositivity of bovine brucellosis to be higher in Sokoto Gudali and White Fulani in comparison with Rahaji and crossbreeds while Aiyedun *et al.* [26] reported that most positive animals sampled were Bunaji cattle.

While the observed insignificance between breed and disease occurrence might be because breeds of cattle in Ilorin, Kwara State were probably not the major factor affecting the occurrence of brucellosis, the observed wide variations in breed prevalence between this study and others could be as a result of large number of various breeds of cattle slaughtered at the two major abattoirs in Ilorin were Rahaji.

Specificity/Sensitivity of Serological Tests

In this study, the RBPT and SATT serological tests utilized both reported similar sero-positivity results with the prevalence of 10.71% for the former and 11.16% for the later. The variation observed in both tests gives credence to the laboratory kits, procedures and protocols used in this study although RBPT is said to be more sensitive than SATT.

Adamu *et al.,* (2016) also reported a little variation in RBPT and SATT serological tests (5.3% and 3.8% for RBPT and SATT, respectively) while Aiyedun *et al.* [26] reported a relatively wide variation (4.2% sero-positivity for RBPT and 3.3% sero-positivity for SAT). This could be as a result of slight differences in the reagents and overall laboratory protocols.

Conclusion

There was a high prevalence rate of bovine brucellosis (11.16%) among cattle slaughtered at major abattoirs within Ilorin during the period of study (April – May 2018). However, there was no statistical relationship between prevalence of bovine brucellosis, age, sex, breed and abattoir of sampling during the period of study. SATT was found to be more sensitive than RBPT.

Recommendations

Further study in other major abattoirs in Kwara State should be conducted for a synchronized evaluation of the status of bovine brucellosis among cattle slaughtered in the State. Improvement of animal health management and monitoring of cross border movement of animals within West Africa is recommended since this high prevalence of *Bovine Brucellosis* could attributed to improper health management as well as cross border movement of animals within West Africa.

Sero-epidemiological survey of other major zoonotic diseases should be conducted on livestock within Nigeria and encouraged for neighbouring countries for the overall improvement of public health. Systematic, detailed epidemiological studies on butchers and abattoir workers in the study area should be carried out to explore the risk factors associated with the occurrence and perpetuation of brucellosis.

Enforcement of the control of livestock movement, as well as adequate quarantine measures should be ensured. Farmers, butchers and consumers of meat and other animal product such as milk, kilishi, suya, and yogurts should be educated and well informed about the health implications of bovine brucellosis.

Proper meat inspection should be done without fear or economic favour to ensure the slaughter of only wholesome animals for human consumption.

Public enlightenment programmes through seminars, radio and television jingles, and the use of postal and other information dissemination techniques should be embarked upon, in order to create public awareness on bovine brucellosis.

Table I:Prevalence of *Brucella* antibodies among Cattle Slaughtered in Abattoirs in Ilorin metropolis using Rose Bengal Plate test (RBPT) and serum agglutination tube test (SATT).

Test	Positive (%)	Negative (%)	OD (95% CI)	p- value
¹RBPT	24 (10.71)	200 (89.29)	1	
SATT	25 (11.16)	199 (88.84)	0.96 (0.53 – 1.73)	1.00

Key: ¹Reference value OD- Odd ratio 95% CI- Confidence interval at 95%

Table II: Specificity, sensitivity and accuracy of RBPT and SATT for evaluation of *Brucella* antibodies among cattle slaughtered in two major abattoirs in Ilorin Between April – May 2018

	SATT positive	SATT negative	Total	
RBPT positive	24^a	0^b	24	Sensitivity= $^{100a/(a+c)}$= 96%
RBPT negative	1^c	199^d	200	Specificity=$^{100d/(b+d)}$= 100%
Total	25	119	224	Accuracy= $^{100(a+d)/(a+b+c+d)}$= 99.6%

Table III: Distribution of *Brucella*Antibodies Among Cattle Slaughtered in Ilorin Abattoirs Between April – May 2018

Features	Number of sera	SATT positive (%)	OD (95% CI)	p value
Age (years)				
<2	175	15 (8.6)	0.37 (0.15 – 0.87)	0.036
¹>2	49	10 (20.4)	1	
Gender				
Female	201	23 (11.4)	1.36 (0.30 – 6.17)	1.000
¹Male	23	2 (8.7)	1	
Breed				
Rahaji	185	22 (11.9)	0.99 (0.27 – 3.58)	1.000
SokotoGudali	14	0 (0.0)	º0.22 (0.01 – 4.62)	0.54
¹Bunaji	25	3 (12.0)	1	

Key: ¹ Reference value, º Odds Ratio (OD) calculated by adding 0.5 to each value 95% CI- Confidence interval at 95%

Table IV:Age Distribution of *Brucella*Antibodies Among Cattle Slaughtered in Two Major Ilorin Abattoirs Between April – May 2018

Age Group (Years)	Number of Cattle Sampled	RBPT Positive Samples	Percentage (%)	χ2	SATT Positive Samples	Percentage (%)	χ2
YOUNG							
<2years	175	14	8	P>0.1	15	8.57	P>0.2
OLD							
>2years	49	10	20.40		10	20.40	

P < 0.05

Table V:Sex Distribution of *Brucella* Antibodies Among Cattle Slaughtered in Two Major Ilorin Abattoirs Between April – May 2018

Gender	Number of Cattle Sampled	RBPT Positive Samples	Percentage (%)	χ2	SATT Positive Samples	Percentage (%)	χ2
Male	23	2	8.69	P>0.9	2	8.69	P>0.9
Female	201	22	10.94		23	11.44	

P < 0.05

Table VI: Breed Distribution of *Brucella* Antibodies Among Cattle Slaughtered in Two Major Ilorin Abattoirs Between April – May 2018

Breed	Number of Samples	RBPT Positive Samples	Percentage	χ2	SATT Positive Samples	Percentage	χ2
Bunaji	25	2	8	P>0.9	3	12	P>0.9
SokotoGudali	14	0	0		0	0	
Rahaji	185	22	11.89		22	11.89	

P < 0.05

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