Journal of Animal Science and Veterinary Medicine
Volume 4(5), pages 157-162, October 2019
Article Number: C65745341
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2019.158
https://integrityresjournals.org/journal/JASVM
Full Length Research

Seroprevalence of brucellosis in flocks of goats in Kaduna State, Northwestern Nigeria

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Received 25th August, 2019; Acceptedm10th September, 2019

ABSTRACT: Brucellosis is a bacterial contagious disease of animals and humans caused by bacteria of genus *Brucella*. A cross sectional study was carried out to determine the seroprevalence of brucellosis in flocks of goats in Maigana agro-ecological zone of Kaduna State. This study was aimed to determine the seroprevalence of brucellosis and the risk factors influencing the occurrence of *Brucella* specie in goats in Kaduna State. Total of four hundred (400) blood samples were tested for brucellosis using Rose Bengal Plate Test (RBPT) and Competitive Enzyme Immunosorbent Assay (ELISA). Out of the 400 sera samples tested, 48 (12.0%) and 24 (6.0%) were seropositive by RBPT and cELISA respectively. Out of the 147 male goats tested, 10 (6.8%) and 6 (4.1%) were seropositive, while out of 253 female goats tested, 38 (15.0%) and 18 (7.1%) were seropositive using RBPT and cELISA. There was statistically significant association ($p<0.05$) between the sex of goats and RBPT, but there was no statistically significant association ($p>0.05$) between the sex of goats and cELISA. Based on age distribution, the seroprevalence 13.4 and 6.3% by RBPT and cELISA were recorded in the age group 2 to 4 years. Based on breed distribution, the highest seroprevalence 13.4 and 8.1% by RBPT and cELISA were recorded in Sokoto Red. There was no statistically significant association ($p>0.05$) between the age and breed of goats with RBPT and cELISA. The study concludes that brucellosis is prevalent in the flocks of goat in the study area. The high seroprevalence of brucellosis is of economic and public health concern because the pastoralists regard goats' milk to have exceptional medicine potentials. Therefore, brucellosis may be prevented via vaccination of domestic livestock, serologic testing, quarantine of herds, and slaughter of infected animals.

Keywords: Brucellosis, cELISA, goats, RBPT, seroprevalence.

INTRODUCTION

Brucellosis is an important bacterial zoonotic disease that mostly affects cattle, sheep and goats as well as humans and man. The disease is caused by various species of *Brucella* among them *Brucella melitensis* causes brucellosis in sheep and goats most commonly and it has the highest potential for zoonotic infection followed by *Brucella abortus* and *B. suis* (Ninprom et al., 2016). Richey and Harrell (1997) and FAO (2003) described brucellosis as an infectious and contagious bacterial zoonotic disease caused by *Brucella* species. The disease produces non-specific clinical signs including fever, which are often misdiagnosed leading to severe long-lasting disease in humans. *Brucella melitensis* is the aetiological agent of brucellosis in goats, an infectious zoonotic disease with significant economic impact on both the livestock industry and human health. Brucellosis in goats has been controlled in most developed countries; however, the disease remains endemic in under developed countries (FAO, 2010). Clinical manifestations of brucellosis in ruminants are abortion and stillbirths, which usually occur

in the last trimester following infection (Diaz–Aparicio, 2013).

In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs with the common clinical symptoms such as weakness, lethargy, chill, fever, sweating, decreased appetite, arthralgia, myalgia, weight loss, headache, back pain and psychological symptoms (Wu et al., 2013). Transmission of the *Brucella* organism to humans occurs as a result of consumption of unpasteurized raw milk or other dairy products, especially soft cheese, butter and cream (Alshaalan et al., 2014). Animals may become infected by direct contact with the secretions of infected animals or their products, such as the placenta or aborted materials and by ingestion of feed, water and grass contaminated by bacteria (Alshaalan et al., 2014). Brucellosis can be diagnosed by serological tests especially at herd and flock level. In small ruminants, the rose Bengal plate test (RBPT), buffered *Brucella* antigen tests (BBAT) and the complement fixation test (CFT) are usually recommended for screening flocks and individual animals. Treatment of brucellosis requires administration of effective antibiotics for an adequate length of time (Alshaalan et al., 2014). Several serological studies have shown that brucellosis is prevalent in the livestock and humans in Nigeria (Adamu et al., 2014; Ya'u et al., 2017; Adamu et al., 2018). The major livelihood of the people of Birnin Gwari agro-ecological zone is agronomy, mainly food and cash crops and rearing of livestock (KDSG, 2008). Kaduna State has an estimated cattle population of 988,000 goats (KDSG, 2008). In view of the relatively smaller population of livestock in relation to the rapidly increasing human population, there is need to know and control any factor that will limit the production of livestock in the State. Contagious diseases that affect reproduction like brucellosis need to be examined in goats. The objective of this study was to determine the seroprevalence of brucellosis and the possible risk factors influencing the occurrence of *Brucella* specie in goats in Kaduna State, Northwestern Nigeria. This may provide baseline information that could be used in designing a control strategy against brucellosis in the study area.

MATERIALS AND METHODS

Ethical statement

The experiment was performed according to the care and use of experimental animals' protocol (Ochei and Kolhatkar, 2000) and was approved by the Faculty of Veterinary Medicine Ethics and Research Committee.

Study area

The study was conducted in the Maigana agro-ecological zones of Kaduna State. Kaduna State is located in the center of the Northern Nigeria, specifically North West zone of Nigeria (KDSG, 2008). The State occupies a land area of about 48,473.2 square kilometers and lies between latitude 9° 10' and 11° 30N and longitude 6° 20' and 9° E and it is located at an elevation of 704 meters above sea level. The state shares boundaries with Niger State to the west, Zamfara, Katsina and Kano States to the north, Bauchi and Plateau States to the east and FCT Abuja and Nassarawa State to the south. The State has 23 Local Government Areas. The state has distinct wet and dry seasons and is within the Northern Guinea Savannah zone and part of the Sudan Savannah zone of Nigeria with daily temperatures ranging from 14.6 to 36°C and a relative humidity of 12 to 72% and with the mean annual rainfall of 1,524 mm (KDSG, 2008). Majority of the population consists of small scale farmers. Thus, agriculture is the main sources of livelihood of the communities in the state with about 80% of the people engaged actively in livestock and crop farming (Figure 1).

Study design

A cross-sectional study consisting of two serological tests for detecting Brucella infection and possible risk factors influencing the presence of *Brucella* specie antibodies in the goats was carried out between March and October, 2017 in Birnin Gwari agro-ecological zones of Kaduna State, Nigeria.

Inclusion criteria

Only settled and semi-settled flocks raised extensively were included. Flocks that had a minimum of 10 goats only were included. Flocks within Birnin Gwari agro-ecological zones only were included. Only flocks whose owners consented were studied. Only goats that were older than 6 months were included in the study.

Sampling and sample size determination

Simple random sampling technique was used to select flocks from each of the five local government areas (LGA) in Birnin Gwari agro-ecological zone. In each flock, simple random sampling was used proportionate to size. Sample size for this study was determined using the Thrusfield (Thrusfield, 2005) formula, with an expected diseases prevalence of 8.2% for brucellosis (Dogo and Maikai, 2015) accepted absolute error of 5%, and a confidence interval of 95% (Thrusfield 2005);

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

$$n = \frac{1.96^2 \text{ x } 0.082 \, (1 - 0.082)}{(0.05)^2}$$

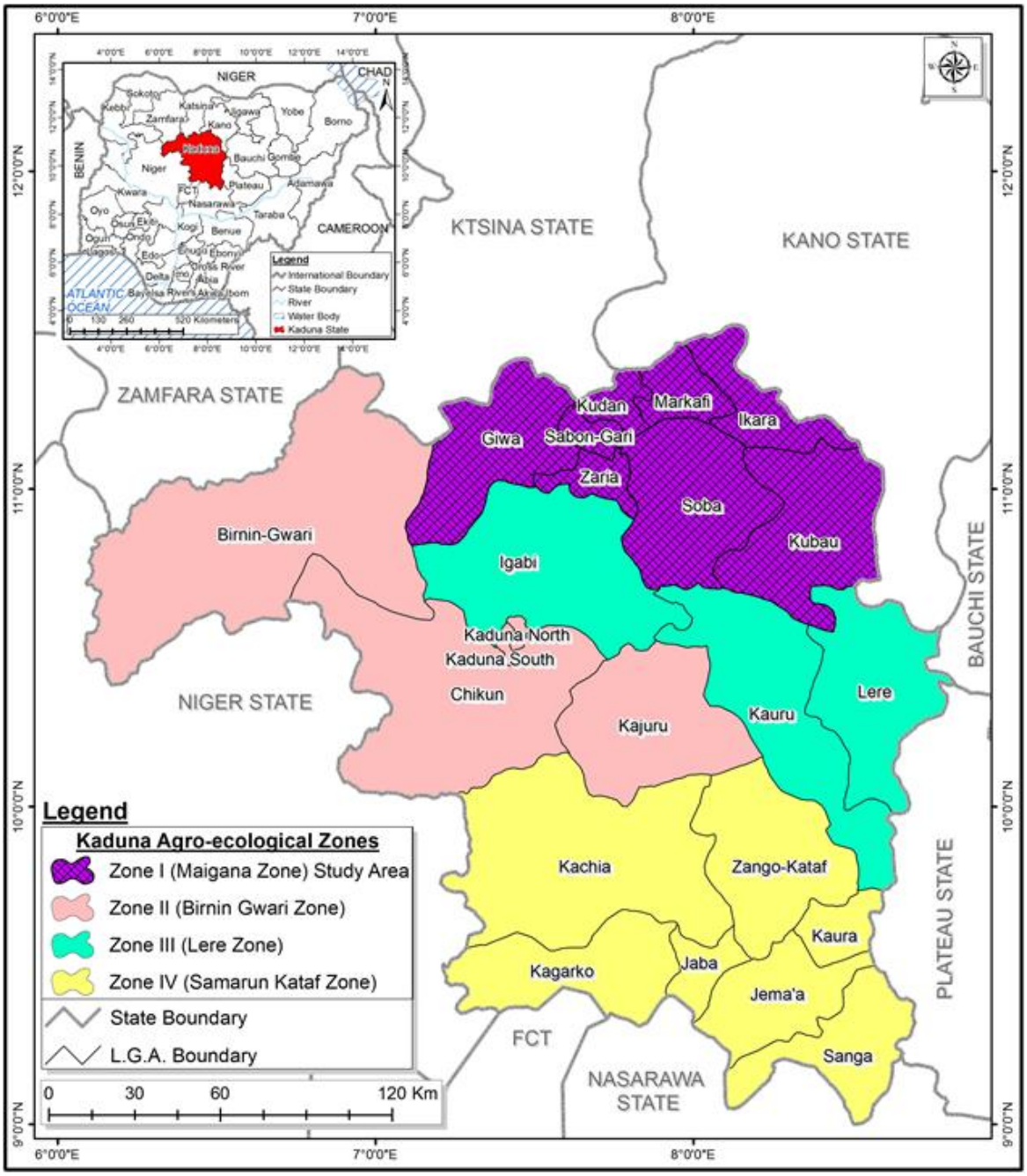

Figure 1. Map of Kaduna State showing the sampling area.
Source: Adapted and modified from the administrative map of Kaduna State.

$$n = \frac{1.96^2 \text{ x } 0.082\ (0.918)}{(0.05)^2}$$

Where: n = required sample size, Pexp = expected prevalence and d = desired absolute precision.

A minimum of 115 samples was required, however 400 samples of goats was randomly selected from their flocks to increase precision of the estimate.

Sample collection

About 5 ml of blood sample from each of the 400 goats were aseptically collected from the anterior jugular vein into a sample bottle and each was labeled. The sex, age and breeds of the animals were documented at the time of blood sample collection. The blood samples were kept in slanted position and allowed to clot. It was then centrifuged at 3000 g for 5 min and the separated sera were stored in

a screw cap sample bottles. The sera samples were kept at −20°C until time of the test.

Serological tests

Serological tests were conducted using Rose Bengal Plate Test (RBPT) antigen and competitive enzyme-linked immunosorbent assay (cELISA) in the Bacterial Research Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. Both the RBPT antigen and cELISA kits were obtained from Animal and Plant Health Agency, (APHA) New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom.

Sample analysis

The samples were analyzed for evidence of *Brucella* species antibodies by Rose Bengal plate test (RBPT) and competitive enzyme-linked immunosorbent assay (cELISA). The RBPT was done using the commercial RBPT antigen in accordance with the method described by Alton et al. (1988). Briefly, 30 μL of plain serum were dispensed on a white glossy ceramic tile and mixed with an equal volume of RBPT antigen using sterile applicator stick. The mixture on the tile was then rocked gently at room temperature for 4 min, any visible agglutination and/or the appearance of a typical rim was taken as a positive result, while negative if there was no agglutination (Alton et al., 1988). Sera samples from goats were tested for the presence of antibodies against *Brucella* specie by cELISA kit following the manufacturer's instructions. The lack of colour development indicated that the sample tested was positive. A positive/negative cut-off point was calculated as 60% of the mean of the optical density (O.D) of the 4 conjugate control wells. Any test sample given an O.D equal to or below this value was regarded as being positive. The O.D value for measurement using ELISA plate reader is 450nm.

$$\text{Binding Ratio} = \frac{\text{Mean of 6 negative control wells}}{\text{Mean of 6 positive control wells}}$$

The results were considered valid if the following apply;

1. The mean O.D of the 6 negative control wells is greater than 0.700 (the optimal mean negative O.D is 1.000).
2. The mean O.D of the 6 positive control wells is less than 0.100.
3. The mean O.D of the 4 conjugate control wells is greater than 0.700 (the optimal mean conjugate control O.D is 1).
4. The binding ratio is greater than 10

Data analysis

Data obtained from the studies were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 statistical software (SPSS Inc., Chicago, IL, USA) and Chi-square analysis and odds ratio (OR) for comparison and determination of association between the *Brucella* specie infections and other variables (such as age, sex, species and the breeds of the animals tested).

RESULTS

Out of 400 goats tested by RBPT and cELISA from Kaduna State, 48 (12.0%) and 24 (6.0%) were positive to *Brucella* infection. This comprised 10 (6.8%) and 6 (4.1%) out of 147 males and 38 (15.0%) and 18 (7.1%) out of 253 females goats respectively. There was statistically significant association ($p<0.05$) between the sex with seropositivity of goats brucellosis in the study area using RBPT (Table 1). For the age distribution of *Brucella* infection in goats tested, the highest seroprevalence 13.4 and 6.3% were observed in age band 2 to 4 years, this was followed by seroprevalence 9.5 and 6.3% in goats greater than 4 years, while the least seroprevalence 9.8 and 3.9% were observed in goats less than 2 years by RBPT and cELISA, respectively. There was no statistically significant association ($p>0.05$) between the age with seropositivity of goats brucellosis in the study area (Table 2). On breed distribution, the highest seroprevalence 13.4 and 8.1% were observed in Sokoto Red, this was followed by seroprevalence 11.4 and 6.0% observed in Sahel goats, and the least seroprevalence was observed in West African Dwarf (WAD) 9.2% using RBPT but there was no positive sample observed by cELISA. There was no statistically significant association ($p>0.05$) between the breeds and sensitivity to RBPT and cELISA for detecting *Brucella* (Table 3).

DISCUSSION

Brucellosis in animals is a zoonotic disease which should be diagnosed at the earliest stage in order to prevent damages that could arise from infection with *Brucella* organism. In this study, the overall seroprevalence of brucellosis in goats were 12.0 and 6.0% based on RBPT and cELISA. The seroprevalence of brucellosis in goats obtained was higher than the works reported in Nigeria (Ogugua et al., 2014; Dogo et al., 2016), in Egypt (Al-Habaty et al., 2015) and in Kenya (Nakeel et al., 2016). But the seroprevalence was lower than the works reported in Nigeria (Junaidu et al., 2010; Kaltungo et al., 2013; Zubairu et al., 2014; Ya'u et al., 2017), in Ethiopia (Adugna et al., 2013) and in Sudan (Zein and Adris, 2015). The high rates of brucellosis in sheep and goats may be due to free grazing and movement of these flocks which contribute to the wide distribution of brucellosis in these animals and to other animal species and due to non-vaccination against brucellosis (Al-Habaty et al., 2015). The seroprevalence was higher in females than males goat tested, though

Table 1. Seroprevalence of brucellosis in goats in Kaduna State based on sex distribution.

Sex	No. Tested	RBPT +ve No. (%)	OR	95% CI		cELISA +ve No. (%)	OR	95% CI	
				lower	upper			lower	upper
Male	147	10 (6.8)	0.413	0.199	0.856	6 (4.1)	0.556	0.215	1.433
Female	253	38 (15.0)	1*			18 (7.1)	1*		
Total	400	48 (12.0)				24 (6.0)			

RBPT (P value = 0.015); cELISA (P value = 0.218); 1.0 = reference.

Table 2. Seroprevalence of brucellosis in goats in Kaduna State based on age distribution.

Variables (age)	N (%)	RBPT +ve N (%)	OR (95% CI)	P-value	cELISA +ve N (%)	OR (95% CI)	P-value
≤ 2 Years	51 (12.8)	5 (9.8)	0.963 (0.305 - 3.042)	0.533	2 (3.9)	1.652 (0.321 -8.496)	0.803
2–4 Years	254 (63.5)	34 (13.4)	0.677 (0.312 - 1.471)		16 (6.3)	1.003 (0.380 - 2.644)	
> 4 Years	95 (23.8)	9 (9.5)	1*		6 (6.3)	1*	
Total	400	48 (12.0)			24 (6.0)		

1.0 = reference.

Table 3. Seroprevalence of brucellosis in goats in Kaduna State based on breed distribution.

Variables (Breeds)	N (%)	RBPT +ve N (%)	OR (95% CI)	P-value	cELISA +ve N (%)	OR (95% CI)	P-value
Sahel	149 (37.3)	17 (11.4)	0.790 (0.296 - 2.104)	0.644	9 (6.0)	00 (000)	0.776
Sokoto Red	186 (46.5)	25 (13.4)	0.655 (0.256 - 1.676)		15 (8.1)	00 (000)	
WAD	65 (16.3)	6 (9.2)	1*		0 (00)	1*	
Total	400	48 (12.0)			24 (6.0)		

1.0 = reference.

there was no statistically significant association. These findings agreed with the reports of Junaidu et al. (2010) and Kaltungo et al. (2013). The higher seroprevalence in the females may be due to the fact that female animals are kept for a comparatively longer period within the breeding flocks compared to male animals and so increases the risk of exposure to infections (Dinka and Chala, 2009) but it could also be due to high concentration of erythritol in the placenta and foetal fluids of female which stimulates the growth of the Brucella organisms (Radostits et al., 2004). The observed higher seroprevalence among goats' aged 2 to 4 years and goats older than 4 years agreed with earlier reports that young animal tend to be more resistant to *Brucella* infection and frequently eliminate the infection while sexually matured animals are more susceptible (Junaidu et al., 2013). Moreover, the older animals have high contact through sexual transmission. There was no statistically significant association between the breeds of goats and sensitivity of RBPT for detecting *Brucella.*

Conclusion and recommendation

Brucella species are present in goats at seroprevalence rates of 12.0 and 6.0% in goats using RBPT and cELISA respectively in Birnin Gwari agro-ecological zones of Kaduna State, Nigeria. Brucella species were demonstrated at higher prevalence in older goats (6.3%) than in younger ones (3.9%). Based on the findings, the following recommendations were made: Further serological survey on brucellosis in Kaduna State should be carried out in areas that were not included in this study to give a better picture on the occurrence of brucellosis in the State.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to the technical staff of Bacterial Research Laboratory, department of Veterinary Public Health and Preventive Medicine Ahmadu Bello University, Zaria. The livestock owners were also appreciated for their cooperation during sample collection.

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