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Seroprevalence of Brucellosis in Sheep in Maigana and Birnin Gwari Agro-Ecological Zones of Kaduna State, Nigeria

Shu'aibu, G. A.¹; Kabir, J.²; Umoh, J. U.³; Raji, M. A.⁴; Tijjani, A. O.¹ and Umaru, G. A.⁵

1. Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri Borno State, Nigeria. ^{2.} Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria Kaduna State, Nigeria. ^{3.} Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria Kaduna State, Nigeria. ^{4.} Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin Kwara State, Nigeria. ^{5.} Department of Animal Health, College of Agriculture, Jalingo, Taraba State, Nigeria. *Corresponding author: Email: gidado97@gmail.com; Tel No:+2348028383383

SUMMARY

Brucellosis is an ancient and one of the world's most widespread zoonotic diseases affecting public health and animal production. A cross-sectional study using simple random sampling was conducted between May and December, 2016 in Maigana and Birnin Gwari Agro-ecological zones of Kaduna State to determine the sero-prevalence of brucellosis in sheep. In addition the risk factors associated with sero-positivity in sheep were also assessed. A total of 400 sera samples comprised of 141 from males and 259 from females sheep were collected and screened for the presence of Brucella antibodies using Rose Bengal Plate test (RBPT) and competitive enzyme linked immunosorbent assay (cELISA). Sera analysis revealed that, 16.5% and 10.8% were seropositive to Brucella infection by RBPT and cELISA, respectively. There was statistically significant association between sex of the sheep and seropositivity to Brucella infection using RBPT (P < 0.05). Meanwhile, no statistically significant association between the age and breed of sheep and seropositivity to Brucella infection using RBPT and cELISA, respectively (P > 0.05). This study shows evidence of *Brucella* infection with high prevalence mainly among female sheep and the disease can be considered as a potential risk for both susceptible animals and humans in the study area. Therefore, creating awareness about brucellosis, interdisciplinary partnership and complementary effort between veterinary and public health professionals is very important to control the transmission of brucellosis.

Key words: Brucellosis, Kaduna State, Sheep, Sero-prevalence.

INTRODUCTION

Brucellosis is one of the most common zoonotic diseases in the world and is still a serious problem of public health for some Mediterranean countries, Asia and Latin America (Al-Tawfiq and Abukhamsin, 2009). The disease is caused by gram-negative, facultative, intracellular, coccobacilli bacteria, which belong to the genus *Brucella*, which includes *Brucella melitensis* and

B. ovis as well as many other species (Lopes et al., 2010). The natural reservoirs of the species B. melitensis are basically goats and sheep but also affect cattle and swine. However, B. ovis is primarily afflicting sheep (Lopes et al., 2010). The diseases is characterized by reproductive disorders such as abortions, sterility, metritis, mastitis, stillbirth, calves weak at birth, infertility and formation of localized lesions in the lymphatic system and joints (Thakur et al., 2012). It is an infectious disease, almost invariably transmitted by direct or indirect contact with infected animals or their products (Teshale et al., 2006). Brucellosis is transmitted through ingestion of feed, water and grass contaminated by bacteria, aerosol, broken skin, and secretions of infected animals or their products, such as the placenta or aborted materials (Boukary et al., 2010; Alshaalan et al., 2014). Transmission of the Brucella organism to man occur as a result of direct contact, aerosols and consumption of unpasteurized raw milk or other dairy products, especially soft cheese, butter and cream (Boukary et al., 2010; Alshaalan et al., 2014). Brucellosis in sheep and goats has been reported in various parts of Nigeria (Junaidu et al., 2010; Maurice et al., 2013). Apart from the grave zoonotic consequences, the economic losses associated with the disease are enormous including abortion, loss of offspring, reduced milk production and subsequent animal infertility (Xavier et al., 2009; Seleem et al., 2010).

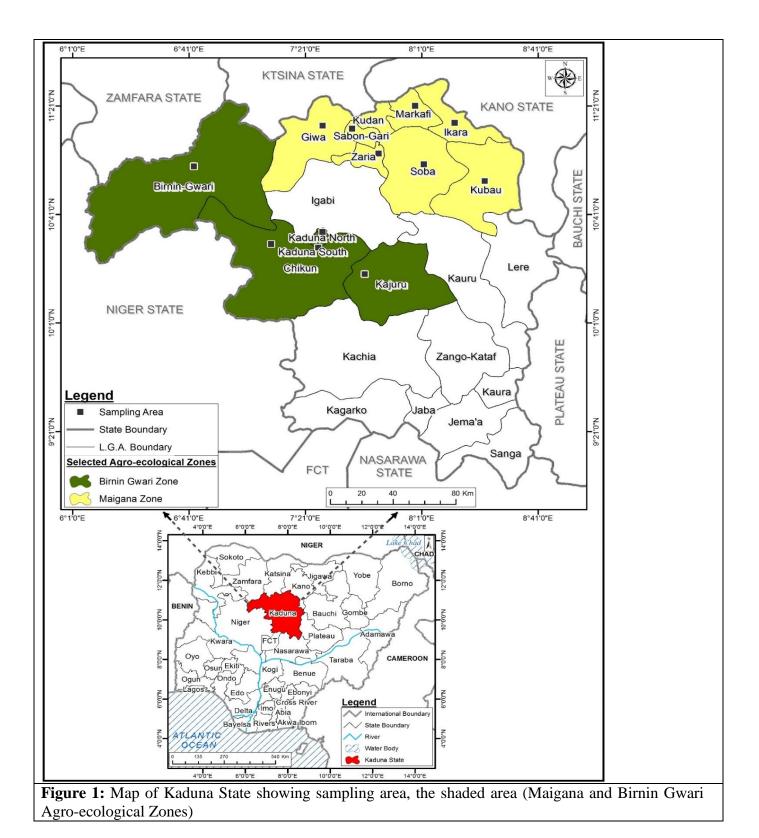
The major occupation of the people of Kaduna State is agriculture, mainly food and cash crops and rearing of livestock (KDSG, 2008). Kaduna State has an estimated cattle population of 3.1 million, 832,000 sheep and 988,000 goats (KDSG, 2008). In view of the relatively smaller population of livestock in relation to the rapidly increasing human population, there is need to understand and control any factor that will limit the productivity of livestock in the State. Infectious diseases that affect reproduction like brucellosis need to be investigated in sheep. The aim of this study was to determine the seroprevalence of brucellosis in sheep and the risk factors associated with the disease in Maigana and Birnin Gwari agro-ecological Zones of Kaduna State.

MATERIAL AND METHODS Study area

The study was conducted in the two agroecological zones of Kaduna State namely Maigana and Birnin Gwari. 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 (Fig. 1). 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 14.6-36 °C and a relative humidity of 12-72% 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 major occupation of the communities in the state with about 80% of the people engaged actively in livestock and crop farming (KDSG, 2008).

Study design

A cross-sectional study consisting of two serological surveys for detecting Brucella species infection was carried out between May and December, 2016 in Maigana and Birnin Gwari agro-ecological zones of Kaduna State. Stratified simple random sampling technique was used to select flocks from each zone with the LGAs forming the first strata and wards the second strata. In each stratum simple random sampling was used proportionate to size. Two Local Government Areas (L.G.As) were selected from Birnin Gwari and four L.G.As from Maigana agro-ecological zones of Kaduna State. Similarly, within each selected LGA, three wards and three flocks of sheep were randomly selected.



Sample size determination

The sample size for this study was determined using the following formula, with an expected disease prevalence of 25.6% (Kaltungo *et al.*, 2015), accepted absolute error of 5%, and a confidence level of 95% by using a simple random sampling design (Thrusfield, 2005):

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

Where n = required sample size, $P_{exp} =$ expected prevalence and d = desired absolute precision.

A minimum of 292 samples was required in this study. However, 400 samples were collected from randomly selected sheep from selected flocks to increase precision.

Sample collection

Five milliliters of blood sample was collected aseptically from the jugular vein of each animal into clean plain vacutainer tubes. Each sample was labeled using codes describing the animal and flock, with a unique identification number and information about sex, age and breeds were recorded for data analysis. The samples were transported on ice packed in coolers to the bacterial research laboratory, department of veterinary public health and preventive medicine and were centrifuged (at 3000 G for 5 min) to obtain clear sera. The harvested sera were stored at -20 °C until tested for evidence of *Brucella* antibodies.

Serological tests

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

Rose bengal plate test (RBPT)

Briefly, 30 μ L 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, and any visible agglutination and/or the appearance of a typical rim was taken as a positive result and negative if there was no agglutination (Alton *et al.*, 1988). *Competitive enzyme linked immunosorbent assay* The reagents in the kit were reconstituted as directed by the manufacturers. Samples, reagents and plate(s) were brought to room temperature $(21^{\circ}C \pm 5^{\circ}C)$ prior to starting the test. Twenty microliter (20µl) of serum sample were added into each of the 80 wells, columns 11 and 12 were left for controls and 20µl of the positive control were added into wells F11, F12, G11, G12, H11 and H12. Similarly, twenty microliter (20µl) of the negative control were pipetted into wells A11, A12, B11, B12, C11 and C12. The remaining wells acted as conjugate control and have no serum added, and 100µl of the prepared conjugate solution was immediately dispensed into all wells. This gave a final serum dilution of 1/6. The plate was then shaken vigorously for 2 minutes, covered with a foil and incubated at room temperature for 30 minutes. The content of the plate was shaken out and rinsed 5 times with washing solution, then thoroughly dried by tapping on absorbent paper towel. Also 100µl of substrate solution were added to each well and incubated for 10 min at room temperature. The reaction was slowed down by adding 100µl of stopping solution to all wells. The optical density (O.D) of the controls and samples were measured at 450nm in a microplate photometer.

Interpretation of test results

The resulting colouration was interpreted by visual reading whereby the appearance of white colouration indicates the presence of *Brucella* antibodies whereas yellowish colouration indicates that there are no *Brucella* antibodies in the tested samples. Sixty percent (60%) of the mean of the optical density (OD) of the 4 conjugate control wells was calculated. Any test sample giving an OD equal to or below this value was regarded as being positive.

Statistical analysis

Data generated were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0, statistical methods used include descriptive statistics to determine percentages. Prevalence was calculated using number of positives divided by the total number of samples tested and expressed as a percentage. Relationship between disease positivity and factors were determined using Chi-square (χ^2) and Fisher's Exact Test to test for association. Strength of association was calculated using Odds Ratio (OR) at 95%, Confidence Interval (CI).

RESULTS

Of the 400 sheep tested from Maigana and Birnin Gwari agro-ecological zones of Kaduna State, 67 (16.8%) and 43 (10.8%) were seropositive to Brucella infection by RBPT and cELISA respectively. Of the 141 male samples out of the 400 sheep tested, 16 (11.3%) and 14 (9.9%) were seropositive to Brucella infection using RBPT and cELISA, respectively. While out of the 259 female samples tested, 51 (19.7%) and 29 (11.2%) were seropositive using RBPT and cELISA, respectively. There was statistically significant difference in the sero-prevalence rates between the male and female sheep screened using RBPT (P < 0.05). While no statistically significant difference in the sero-prevalence rates between the male and female sheep using

cELISA, (P > 0.05) (Table I) was discussed. Based on age distribution, the highest seroprevalence was recorded in the age bracket of 2 to 4 years, with 48 (17.9%) out of 268 samples tested were positive and was followed by the sheep less than 2 years old, with sero-prevalence of 2 (20.0%) out of 10 samples tested. The least was recorded in the sheep older than 4 years, with sero-prevalence of 17 (13.9%) out of 122 samples tested using RBPT and cELISA respectively. Sero-prevalence of 11.5% was recorded in sheep older than 4 years and the sero-prevalence of 10.8% was recorded in the age bracket of 2 to 4 years, while there was no recorded seroprevalence in sheep less than 2 years using cELISA. There was no statistical significant difference in the sero-prevalence rates among different age group of sheep tested (P>0.05) (Table II). The highest sero-prevalence was recorded among Balami with 27 (18.2%) out of 146 samples tested followed by the seroprevalence of 34 (17.2%) out of 198 Yankasa samples tested. The least sero-prevalence was recorded among Ouda with the sero-prevalence of 6 (10.7%) out of 56 samples tested using RBPT.

TABLE I: Sero-prevalence of brucellosis in sheep in Maigana and Birnin Gwari agro-ecological zones of
Kaduna State based on sex distribution using RBPT and cELISA

Sex	No.	RBPT	RBPT	OR	95% CI	cELISA	cELISA	OR	95% CI
	Tested	+ve (%)	-ve (%)			+ve (%)	-ve (%)		
Male	141	16	125	0.545	0.306-	14 (9.9)	127 (90.1)	0.874	0.446-
		(11.3)	(88.7)						
Female	259	51 (19.7)	208 (80.3)	1*	0.969	29 (11.2)	220 (88.8)	1*	1.715
Total	400	67 (16.8)	333 (83.2)			43 (10.8)	357 (89.2)		

* = Reference

TABLE II: Sero-prevalence of brucellosis in sheep in Maigana and Birnin Gwari agro-ecological	
zones of Kaduna State based on age distribution using RBPT and cELISA	

Age	No. Tested	RBPT +ve (%)	RBPT -ve (%)	OR	95% CI	cELISA +ve (%)	cELISA -ve (%)	OR	95% CI
< 2 years		2 (20.0)	8 (80.0)	0.648	0.553-1.538	0 (00)	10 (100)	>999	0.000
2-4 years	268	48 (17.9)	220 (82.1)	0.742		29 (10.8)	239 (89.2)	1.068	0.543-2.103
>4 years	122	17 (13.9)	105	1*		14 (11.5)	108	1*	

(86.1)		(88.5)		
67 (16.8)	333	43 (10.8)	357		
((83.2)		(89.2)		
	67 (16.8)	(86.1) 67 (16.8) 333 (83.2)	67 (16.8) 333 43 (10.8)	67 (16.8) 333 43 (10.8) 357	67 (16.8) 333 43 (10.8) 357

* = Reference

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While using cELISA, the highest sero-prevalence was recorded in among Yankasa, with 23 (11.6 %) out of 198 samples tested, followed by Balami with the sero-prevalence of 15(10.3%) out of 146samples tested. The least sero-prevalence was observed among Ouda, with 5 (8.9%) out of 56 samples tested by cELISA. There was no The sero-prevalence of 16.8% and 10.8% obtained in this study by RBPT and cELISA respectively, were found to be lower than 26.5% recorded from Kaduna State (Kaltungo et al., 2015), 35.2% and 23.8% recorded from Bauchi State (Ya'u et al., 2017), 24.0% recorded from Libya (Ahmed et al., 2010). However, the seroprevalences was higher than 11.1% recorded from Taraba State (Zubairu et al., 2014) and 9.4% recorded from Abuja (Aworh et al., 2017), 2.5% and 1.5% recorded from Sudan (Abdallah et al., 2.3% recorded 2015), and from Bangladesh (Rahman et al., 2011). The high sero-prevalence rates recorded in this study may be due to free grazing and movement of these flocks which contribute to the wide distribution of brucellosis in these animals and other animal species and due to non-vaccination against brucellosis (Al-Habaty et al., 2015). In this study, the sero-prevalence of brucellosis recorded were higher in female than male sheep though it was not statistically significant. These findings are similar with the reports of other workers in Nigeria which associated brucellosis with sex, for examples Junaidu et al. (2010), Farouk et al. (2011), Kaltungo et al.

statistically significant difference in the seroprevalence rates between the different breed of sheep studied using both RBPT and cELISA (P>0.05) (Table III).

Adamu et al.

DISCUSSION

(2013), Adamu et al. (2014), Akinseye et al. (2016) and Hashimu et al. (2017). In Africa and other countries Egypt (Al-Habaty et al., 2015), Sudan (Abdallah et al., 2015) and Bangladesh (Belal and Ansari, 2013). These could 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 highest sero-prevalence of brucellosis were detected in age bracket of 2 to 4 years old compared to the rest of the age groups. This observed difference is similar with the earlier reports in Nigeria; Junaidu et al. (2010), Dogo and Maikai (2015), Senein and Abdelgadir (2012) in Sudan, but in contrast with Bashitu et al. (2015) in Ethiopia. The sero-prevalence was higher in Yankasa and Balami breeds than in Uda breed. This is in contrast to the finding of Junaidu et al. (2008) who reported higher sero-prevalence in Ouda breed in Nigeria, but agreed with the findings Tsegay of et al.

TABLE III: Sero-prevalence of brucellosis in sheep in Maigana and Birnin Gwari agro-ecological zones of Kaduna State based on breed distribution using RBPT and cELISA

Zones or	Zones of Raduna State based on breed distribution using RDT T and eLEDST											
Breed	No.	RBPT	RBPT	OR	95% CI	cELISA	cELISA	OR	95% CI			
	Tested	+ve (%)	-ve (%)			+ve (%)	-ve (%)					
Balami	146	27 (18.5)	119	0.529	0.206-1.360	15 (10.3)	131	0.856	0.296			

			(81.5)				(89.7)		2.478
Yankasa	198	34 (17.2)	164 (82.8)	0.579	0.230-1.458	23 (11.6)	175 (88.4)	0.746	0.270 2.061
Ouda	56	6 (10.7)	50 (89.3)	1*		5 (8.9)	51 (91.1)	1*	
Total	400	67 (16.8)	333 (83.2)			43 (10.8)	357 (89.3)		

* = Reference

(2015) in Ethiopia. This might be due to the large number of different breeds of small ruminants owned by these communities, which graze together in communal grazing lands or use the same watering points. This study concludes that *Brucella* antibodies is present in sheep in Maigana and Birnin Gwari agro-ecological zones of Kaduna State. The high sero-prevalence of 16.8% and 10.8% recorded is of public health concern due to habitual nature of rural people in taking raw milk and the close interaction between humans, sheep, and goats in the study area. It is recommended that the Veterinarians in Kaduna State make an effort to examine cases of abortions that is included in their disease examination.

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