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Seroprevalence of *Brucella* infection in small ruminants from two institutional farms and a slaughter slab in Zaria, Nigeria

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Abstract

Brucellosis has continued to become a disease of major economic concern in developing countries. In a study to determine the seroprevalence of brucellosis in small ruminants in Zaria, Kaduna State, Nigeria, 1036 samples comprising 768 goats from institutional farms (n=384) and a slaughter slab (n=384), and 268 sheep all from institutional farms were used. The sera samples obtained from the animals were subjected to the Modified Rose Bengal Plate Test (m-RBPT) followed by Serum Agglutination Test with ethylene diamine tetraacetic acid (SAT-EDTA) for *Brucella* antibodies detection. Milk samples were collected from lactating sheep and goats and subjected to the Milk Ring Test (MRT) for detection of *Brucella* antibodies. Results of the study revealed an overall seroprevalence rate of 6.37%, 8.90% and 12.96% for m-RBPT, SAT-EDTA and MRT, respectively. The seroprevalence in sheep and goats showed significant species difference ($P < 0.05$) for m-RBPT (10.05% vs 5.08%), but insignificant ($P > 0.05$) species difference for SAT-EDTA (9.33% vs 8.72%) and MRT (15.00% vs 11.76%) respectively. The seroprevalence in males and females showed insignificant sex difference ($P > 0.05$) for m-RBPT (6.59% vs 6.21%) and SAT-EDTA (7.76% vs 9.66%). On the other hand, the seroprevalence was higher in young (< 1 year old) than adult (> 1 year old) animals for SAT-EDTA (15.32% vs 8.11%). The seroprevalence in Red Sokoto, Sahel and West African Dwarf goats showed significant breed difference ($P < 0.05$) for m-RBPT (4.59% vs 5.55% vs 8.33%) and SAT-EDTA (6.80% vs 16.67% vs 12.50%). It was concluded that the seroprevalence of brucellosis was higher in sheep, Sahel goats and younger animals. To understand the pattern and dynamics of transmission of brucellosis in different groups of animals, there is the need for further studies to identify the *Brucella* species circulating in small ruminants.

Keywords: Antibodies, Brucellosis, m-RBPT, SAT-EDTA, Small ruminants

Introduction

In Nigeria, small ruminants constitute the bulk of livestock population with about 28 million goats and 23 million sheep (FAO, 2006). The relatively small body size of small ruminants when compared to cattle favours minimal investment in terms of cash and land. Other important characteristics of these species are

their reproductive efficiency as well as their use in ceremonies and religious festivities where they are either loaned, exchanged among relatives and friends or slaughtered for such occasions (Ademosun, 1988; Omoike, 2006).

Brucellosis is one of the most important and highly contagious zoonotic diseases which is prevalent worldwide (Jagapur *et al.,* 2013). The disease is caused by Gram-negative, facultative intracellular coccobacilli of the genus *Brucella* (Young, 2000; Alton & Forsyth, 2004).

In Nigeria, the free movement of livestock practiced by pastoralists, who own about 95% of all food animal population contributes to the spread of diseases, including brucellosis (Ocholi *et al.*, 2004). Mixing of livestock of various species during herding, at market places and at watering points or agricultural shows may stand to increase the risk of infection with *Brucella* organisms should any of the species be infected (Kaltungo, 2012). Also, lending out as well as borrowing breeding sires may also contribute to the spread of the disease (Kaltungo, 2012; Buhari *et al.,* 2015). The lack of understanding of pastoralists and small ruminant keepers in feeding, housing, vaccination, management and disease transmission and prevention may contribute to the spread of the disease in the study area. Pastoralists have also been reported to house or accommodate small ruminants in close proximity to human settlements (Kaltungo, 2018). More worrisome is the practice where unproductive and chronically sick animals which probably may be suffering from brucellosis from these pastoralists' herds are sent to the abattoirs or slaughter slabs for slaughter (Buhari, 2014). This practice may expose the handlers and in-contact animals to the danger of the infection.

Due to the improper disease surveillance and policies for small ruminant production and the under reporting of small ruminant diseases, there is a dearth of information on the existence of *Brucella* species in small ruminants in institutional farms which farmers consider as reference points when seeking to establish livestock farms. Hence, this study was aimed at determining the seroprevalence of *Brucella* antibodies in small ruminants in selected farms and a slaughter slab in Zaria, Kaduna State, Nigeria using the modified Rose Bengal Plate Test (m-RBPT), Serum Agglutination Test with EDTA (SAT-EDTA) and the Milk Ring Test (MRT) in order to provide data on the level of exposure of animals to brucellosis in the study area.

Materials and Methods

Study area

This study was conducted in two institutional farms and a slaughter slab, all located in Zaria, Kaduna State of Nigeria. Zaria is located in the Northwest geo-political zone of Nigeria. It is situated between latitude 11° 5' 7.9476'' North and longitude 7° 43' 11.8020'' East and is 2,103 feet above sea level (KDSG, 2008). It has distinct wet and dry seasons and is located within the Guinea Savannah and part of the Sudan Savannah zones of Nigeria. Zaria occupies about 563 km^2, with a human population of over 700,000 people according to the 2006 census figures (KDSG, 2008). Daily temperatures range from 14 ^{0}C to 30 ^{0}C with a relative humidity of 12% to 72% (Oyedipe *et al.,* 1982; Mai, 1997). The annual rainfall varies, decreasing from an average of about 1015 mm (Oyedipe *et al.,* 1982; Mai, 1997). The population of livestock in Kaduna State is estimated to be 1,144,000 cattle, 832, 000 sheep and 988,000 goats (KDSG, 2008).

Study design

The study was designed as a purposive one in which the two farms were selected specifically to determine the status of their small ruminants with respect to brucellosis and to ascertain the possible roles of the farms in the spread of the disease. The use of the slaughter slab was based on the assumption that the animals slaughtered there were mostly from pastoralist settings. Also, samples collected from the slaughter slab were to provide the actual picture of the level of exposure of the human population to brucellosis.

Ethical clearance for the study was obtained from the Ahmadu Bello University Committee of Animal Use and Care, with a clearance number ABUCAUC/2017/029.

Sources of samples

Two institutional farms (Farm A abd B) in Zaria were selected for the study. The farms were selected based on the following criteria: the potential for the use of bucks and rams for artificial insemination, supply of breeding sires and females to other farms for breed improvement and the use of intensive or semi intensive management and husbandry practices.

Small ruminants (sheep and goats) were used for the study. Both species were sampled randomly without replacement, giving each individual animal a chance of being selected (Thrusfield, 2005) from the institutional farms irrespective of sex, breed and age. Each sampled animal was aged using dentition

(Hassan & Hassan, 2003), after which the breed and sex of the animal was recorded in a log book with numbers corresponding to those of the blood and/or milk samples collected from that particular animal.

Slaughter slab

The largest slaughter slab for small ruminants in the study area (with an average of 90 animals slaughtered daily) which is located in Dogarawa (latitude $11^0 6' 0''$ North and longitude $7^0 43' 30''$ East) in Sabon Gari Local Government Area (also classified as being part of Zaria) of Kaduna State, Nigeria was selected for the study. Only male goats were sampled, because they were the ones that were predominantly slaughtered there. Animals from the slaughter slab were also sampled across breed and age.

Sampling

One thousand and thirty-six animals were sampled for blood using the formula of Thrusfield (2005).

Five millilitre of blood were collected aseptically from each selected animal from institutional farms via a jugular venipuncture using a 10 ml syringe and 21Guage needle after proper restraint by an assistant. Collection of samples at the slaughter slab was done at the point of slaughter. The age, sex, breed, and location of the flock for each of the study animals were recorded. In case of the slaughter slab samples, only the age and breed were recorded as all animals sampled were males.

The blood was gently transferred into a clean, sterile and dry plain sample bottle and labeled according to the location, species, sex, breed and animal number to correspond with the number on the log book. The samples were transported to the Bacterial Zoonoses Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria over ice, after which it was centrifuged at 3,000 g for 10 minutes to allow for proper separation of serum from the clotted blood. The serum samples were then decanted into sterile 5 ml plastic serum tubes, labeled appropriately and stored in the freezer at -20^0C until used (Bertu *et al.,* 2010).

Also, 108 milk samples were collected from lactating sheep and goats in the institutional farm based on availability. All the milk samples were obtained from Farm A with none from either Farm B or the slaughter slab. Of these samples collected, 68 (62.96%) were from goats while the remaining 40 (37.04%) were from sheep (Table 3). The milk was collected by disinfecting the teats of lactating does and ewes using 70% alcohol and dried using a clean gauze. Fore milk streams were stripped off to reduce contamination of samples. Five milliliters of milk were taken in clean sterile plain sample bottles and labeled. The milk samples were transported over ice to the Bacterial Zoonoses Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria and refrigerated for 24 hours before testing (FAO, 2010).

Processing of samples

Modified Rose Bengal plate test (m-RBPT): The m-RBPT technique as described by Bale (2008) and modified by Bertu (2014) was used. It involved placing 75µl of test serum on a white clean ceramic tile and 25 µl of the RBPT antigen beside it. The antigen and the serum were then mixed using a sterile applicator stick and gently rocked for 4 minutes. The result of rocking the tile was interpreted as positive (+ve) when any degree of agglutination was observed and negative (-ve) when no agglutination was observed. A separate applicator stick was used for each serum sample.

Serum agglutination test with EDTA (SAT-EDTA): The SAT-EDTA technique as described by Brown et al. (1981) was used. A preparation of phenol saline with EDTA buffered solution containing 5g phenol crystals, 1.8612g disodium EDTA and 8.5g sodium chloride all dissolved in 100 ml of warm distilled water was made. For each day's work, 1:10 dilution of concentrated SAT antigen buffered with a pinch of 0.02% Safranin O was made to provide a contrast to the agglutination reaction. A 96-well "U-bottom" microtitre plate was set up on the working bench and labelled. Test serum vials were also placed on the bench to correspond to the positions of the microtitre wells. Forty (40) microlitres of the buffer solution were dispensed into the first well and 25 µl into each of the remaining wells using an automatic micropipette. Following this, 10 µl of the test serum was added into the first microtitre well using a new disposable pipette tip for each test sample. A two-fold serial dilution was carried out by transferring 25 µl aliquots from the first well up to the fifth well. After the last well, 25 µl of the aliquot were discarded. The content of the working dilution of the SAT antigen was mixed gently and 25 µl were transferred to each well after which the contents of the microtitre plate were mixed by gently tapping the edges of the plate for 20 seconds. A foil paper was then used to cover the microtitre plates to prevent evaporation of the contents. The plates were then incubated for 20 hours at 37^0C in an incubator after which the results were read. A mat of stained cells covering the bottom of the well

surrounded by slightly opaque diluent indicated a positive reaction while a large button of red stained particles in the centre of the well (button-like), surrounded by clear pinkish diluents was indicative of a negative reaction. Similarly, all wells with agglutination titres of 1:40 (equivalent to 50 I.U.) or greater were regarded as positive.

The milk ring test: The procedure as described by the FAO (2010) was used. The test was carried out by placing 1 ml of the test milk in a clean sterile sample bottle after which 1 drop (about 0.1 ml) of the MRT antigen was added to it. The mixture was kept in the incubator for one hour to allow proper antigen-antibody reaction to occur after which it was read.
The results were interpreted and recorded as positive (+ve) when a dark blue cream ring above a white milk column appeared, or as negative (-ve) when the colour of antigen remained homogenously dispersed in milk without any change, and as inconclusive (±) when milk at bottom of tube eventually turned white.

Data analyses
Data were analyzed using SPSS version 17.0 (2009). Chi-square (χ^2) and Fishers exact tests were used to test for association between categorical variables. All data generated were presented as Tables. P-value of < 0.05 was considered significant.

Results
Out of a total of 1036 serum samples tested using the m-RBPT, an overall seroprevalence rate of 6.37% was obtained in this study. With respect to respective study locations, 6.71%, 11.48% and 4.79% were obtained for Institutional Farms A and B and the slaughter slab, respectively (Table 1).
Based on animal species, seroprevalence was higher in sheep (10.07%) than in goats (5.08%). There was a statistically significant difference between species (P<0.05). Based on goat breeds, the West African Dwarf goats had the highest seroprevalence of 8.33% while the Sokoto red goats had 4.59%. Among the sheep breeds, Yankasa sheep had a prevalence of 11.35% while Uda breed had 0.0% seroprevalence. There was a statistically significant difference in the prevalence rates between goat breeds (P< 0.05) while that between sheep breeds was not significant (P>0.05). (Table 1). Seroprevalence based on sex showed that males had slightly higher seroprevalence rates (6.59%) than their female counterparts (6.22%). This difference was not statistically significant (P>0.05) (Table 1).

Table 1: Seroprevalence of *Brucella* infection in goats and sheep by location, species and breed using m-RBPT in selected locations in Zaria, Kaduna State, Nigeria

Variable	Level	No. sampled	No. positive (%)	Pearson's Chi-square (χ^2)	P-value
Location	Farm A	641	43 (6.71)	7.34	0.004
	Farm B	61	7 (11.48)		
	Slaughter slab	334	16 (4.79)		
	Total	1036	66 (6.37)		
Species	Goats	768	39 (5.08)	6.691	0.0353
	Sheep	268	27(10.07)		
Breeds					
Goats	Red Sokoto	588	27 (4.59)	15.43	0.004
	Sahel	108	6 (5.55)		
	WAD	72	6 (8.33)		
Sheep	Yankasa	229	26 (11.35)		
	Balami	11	1 (9.09)		
	Uda	28	0 (0.00)		
Sex	Male	425	28 (6.59)	2.5999	0.2727
	Female	611	38 (6.22)		
Age	Young	111	6 (5.41)	18.42	0.0001
	Adult	925	60 (6.49)		

Key: WAD- West African Dwarf
Young – less than 1 year
Adult- Above 1 year

Table 2: Seroprevalence of *Brucella* infection in goats and sheep by location, species and breed using SAT-EDTA in selected locations in Zaria, Kaduna State, Nigeria

Variable	Level	No. sampled	No. positive (%)	Pearson's Chi-square (χ^2)	P-value
Location	Farm A	641	73 (11.39)	2.543	0.035
	Farm B	61	6 (9.84)		
	Slaughter facility	334	13 (3.89)		
	Total	1036	92 (8.88%)		
Species					
	Goats	768	67 (8.72)	3.279	0.1636
	Sheep	268	25 (9.33)		
Breeds					
Goats	Red Sokoto	588	40 (6.80)	56.71	<0.0001
	Sahel	108	18 (16.67)		
	WAD	72	9 (12.50)		
Sheep	Yankasa	229	23 (10.04)		
	Balami	11	0 (0.00)		
	Uda	28	2 (7.14)		
Sex	Male	425	33 (7.76)	2.643	0.2667
	Female	611	59 (9.66)		
Age	Young	111	17 (15.32)	21.86	<0.0001
	Adult	925	75 (8.11)		

Key**:**
WAD- West African Dwarf

Based on age, seroprevalence was higher in the adult animals (above 1 year of age) (6.48%) than in the young (less than 1 year) (5.41%). This difference was statistically significant (P<0.05) (Table 1).

The standard agglutination test with ethylene diamine tetraacetic acid (SAT-EDTA) revealed an overall seroprevalence rate of 8.9% as well as individual seroprevalence rates of 11.39%, 9.84% and 3.89% in Farm A, B and slaughter slab respectively. The seroprevalence was higher in sheep (9.33%) than in goats (8.72%) but this was not statistically significant (P>0.05).

Based on breed, the highest seroprevalence rate was obtained in the Sahel breed of goats (16.67%) while the least (6. 80%) was obtained in Red Sokoto breed of goats. In sheep, the Yankasa breed had highest seroprevalence (10.04%) while the Balami breed had 0.0% seroprevalence. There was a statistically significant difference in seroprevalence rates between goat breeds (P<0.05) but no significant difference between sheep breeds (Table 2).

Based on sex, seroprevalence was found to be higher in females (9.66%) than in males (7.76%), though not statistically significant (P>0.05). Young animals had higher seroprevalence (15.88%) than adults (8.11%) and the difference was statistically significant (P<0.05). Details are shown in Table 2.

An overall seroprevalence rate of 12.96% was obtained using the MRT. Seroprevalence was found to be higher in sheep (15.00%) than in goats (11.76%). With respect to breed, the Red Sokoto does had the highest seroprevalence (16.22%), while the West African Dwarf does had 0.0% seroprevalence. Among sheep, the Yankasa Sheep had seroprevalence of 22.22% while the Uda and Balami ewes had 0.0% seroprevalence each (Table 3).

Discussion

From this study, seroprevalence was found to be higher using the SAT-EDTA (8.88%) than with the m-RBPT (6.37%). It shows that SAT-EDTA seems to be more sensitive in detecting animals with *Brucella* infection and this finding may be attributed to the increase in specificity of this test due to the addition of ethylenediaminetetraacetic acid (EDTA) which increases the specificity of the test by eliminating nonspecific agglutination reactions, apparently by preventing binding between *Brucella* cell surface components and the Fc portion of IgM (Poester *et al.,*

Table 3: Seroprevalence of *Brucella* infection in goats and sheep by location, species and breed using MRT in selected locations in Zaria, Kaduna State, Nigeria

Variable	Level	No. sampled	No. positive (%)	Pearson's Chi-square (χ^2)	P-value
Location	Farm A	108	14 (12.96)		
	Farm B	0	0 (0.00)		
	Slaughter slab	0	0 (0.00)		
	Total	108	14 (12.96)		
Species	Goats	68	8 (11.76)	1.78	0.075
	Sheep	40	6 (15.0)		
Breeds					
Goats	Red Sokoto	37	6 (16.22)	4.68	0.048
	Sahel	26	2 (7.69)		
	WAD	5	0 (0.00)		
Sheep					
	Yankasa	27	6 (22.22)		
Age					
	Young	0	0 (0.00)		
	Adult	108	14 (12.96)		

Key:
WAD- West African Dwarf

2010). Similar findings were reported by Kaltungo (2012), Baba (2016) and Kaltungo (2018).

The seroprevalences obtained in this study are lower than those reported by other workers including Junaidu *et al.* (2010) who reported a seroprevalence of 10.9% in small ruminants in Sokoto State and Kaltungo *et al.* (2013) who reported prevalence rates of 25.8% and 11.1% using the m-RBPT and SAT-EDTA respectively in the North Senatorial district of Kaduna State, Nigeria. More recent reports include those of Zubairu *et al.* (2014) who reported a seroprevalence of 18.2% using the RBPT in Taraba State and Kaltungo (2018) where a seroprevalence rate of 13.5 % was reported using the m-RBPT in Katsina and Sokoto States, Nigeria.

The lower seroprevalence in this study when compared to previous reports may be linked to differences in study location, serological tests used, age and number of animals sampled. The study location has been known to affect the seroprevalence of brucellosis as it is more endemic in some states/countries than others (Lopes *et al.,* 2010). Also, the serological tests used may affect the seroprevalence of the disease due to difference in sensitivity and specificity of tests and whether a test is a screening test or a confirmatory test (Godfroid *et al.,* 2002). Age has been shown to affect the seroprevalence of brucellosis, with older animals being reported to be more seropositive than younger ones possibly due to their long time of being in the flocks and higher chances of coming in contact with positive animals (Cadmus *et al.*, 2010; Junaidu *et al.*, 2011).

The higher seroprevalence in this study in the institutional farms when compared to the one in the slaughter slab may be linked to the fact that animals slaughtered at the slaughter slab were mainly from pastoralists' flocks which are managed extensively while those at institutional farms were managed intensively or semi-intensively in a somewhat closed herd system. The close and continuous contact between animals in a closed herd system may contribute to the spread of several diseases amongst the animals since they utilize the same feeding and watering equipment, and are managed as a single entity, hence making it easier for disease transfer. This agrees with reports of Terefe *et al.* (2017). It is, however, contrary to the findings of Racloz *et al.* (2013) who reported higher seroprevalence in extensively managed animals as a result of multiple social and ecological factors, such as a lack of access to services, poor governance, harsh climatic conditions, herd composition and animal geospatial density

The higher seroprevalence in sheep when compared to goats using both m-RBPT and SAT-EDTA in this study is contrary to the reports of Junaidu *et al.* (2008) who reported a higher prevalence in goats than sheep in Sokoto Prison farm, Nigeria and that of Zubairu *et al.* (2014) in Taraba State, Nigeria. The reason for this higher prevalence in sheep than in goats is not very clear but could be as a result of species behaviour

where ewes are found to always flock together during lambing and also at night which is seldom seen in goats (EC, 2001). Another reason may be the herding of sheep along with cattle during grazing, giving them more room for acquiring the infection from cattle in which the disease has been reported to be on the increase (Ocholi *et al.,* 2004).

It could be as result of the breed susceptibility since, while most goat breeds are susceptible to brucellosis, certain breeds of sheep are less susceptible (EC, 2001). The use of Yankasa breed of sheep as the predominant breed in this study may have accounted for the higher seroprevalence seen in this breed. Also, low number of sheep used for this study compared to goats may be a contributing factor to this finding.

The study revealed a higher seroprevalence in males than females using the m-RBPT and higher seroprevalence in females than males using the SAT-EDTA. This higher seroprevalence in males than females using the m-RBPT is contrary to most reports from other workers where the seroprevalence has been reported to be higher in female animals than in males even with RBPT (Bertu *et al.,* 2010; Kaltungo *et al.,* 2013; Zubairu *et al.,* 2014; Buhari *et al.,* 2016; Kaltungo, 2018). It has, however, been reported that RBPT gives more of false positives than false negatives partly due to its reaction with IgM which is the first antibody type that comes into play during an infection (Poester *et al.,* 2010). It has also been reported to be positive for cross reacting organisms like *Escherichia coli* 0:116 and 0:157, certain *Salmonella* serotypes, *Pseudomonas multophilia* and *Yersinia enterocolitica* 0:9 and 0:16 (Garcia del Pozo *et al.,* 2014). The SAT-EDTA on the other hand has the advantage of being able to eliminate some non-specific reactions (Macmillan & Cockrem, 1985; Kaltungo, 2018).

This higher seroprevalence in females than males is linked to the fact that more females are allowed to attain sexual maturity and are retained much longer in flocks for breeding while the males are sold out. Also, the fact that the female reproductive tract (especially the uterus) habours erythritol to which *Brucella* species have high affinity further explains this finding (Cutler *et al.,* 2005).

This study revealed a higher prevalence in young animals (0- 1 year of age) using the SAT-EDTA when compared to adults (> 1 year of age). These young animals may have acquired the infection in-utero as Grillo *et al.,* 1997 reported the transmission of *B. melitensis* in *utero* when the dams happen to be infected. This leads to persistent infection in their offsprings and they remain latent carriers. The large number of young animals sampled in this study may be the reason for this finding. Pastoralists are usually not very comfortable allowing their young animals to be sampled for blood. Since this study was carried out in institutional farms and slaughter slab, there was no restriction as to which animal was sampled.

In conclusion, the seroprevalence of brucellosis was found to be higher in sheep, Sahel goats and in younger animals. The high prevalence of brucellosis in these groups of animals should be taken into consideration during breeding and in the management of diseases. Efforts should also be made to determine the dynamics of brucellosis spread in these institutional farms to curtail public health hazards.

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Conflicts of Interest

The authors declare no conflict of interest.

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