

Bacteriological and Serological Studies on Brucellosis in Sheep and Goats in a Research Farm in Kaduna State, Nigeria


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ABSTRACT	Article History
<p><i>This study aimed at investigating the occurrence of brucellosis among sheep and goats in a Research Farm in Zaria, Nigeria. Whole blood and serum samples from 580 small ruminants (265 from goats and 315 from sheep) were respectively cultured and serologically screened using the Rose Bengal plate test (RBPT) and Serum agglutination test (SAT). Notably, although no Brucella growth were observed from the blood cultures, antibodies were detected by the RBPT and SAT among goats (33.58%, 89/265; 25.66%, 68/265) and sheep (33.65%, 106/315; 6.35%, 20/315) respectively. Females of both species were observed to have a significantly higher seroprevalence from the RBPT compared to males ($P < 0.05$). Our findings raise serious reproductive, economic and public health concerns and we recommend improved surveillance and screening particularly prior to introduction of new animals into the farm.</i></p>	Original Research Article
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INTRODUCTION

Brucellosis is an infectious disease caused by the bacteria of the Genus *Brucella*. These bacteria are primarily passed among animals and they cause disease in many vertebrates like sheep, goats, cattle, deer, elk, pigs, dogs, horses, camels, several other animals and man; hence its zoonotic importance (CDC, 2010).

Small ruminants constitute the bulk of Nigeria's food animals' population estimated at 23 million sheep and 28 million goats (FAO, 2006). Several studies on brucellosis in sheep and goats have been carried out

by several investigators in Nigeria for years (Falade et al., 1974; Falade, 1978, 1981; Okoh, 1980; Bale, 1982; Okewole, et al., 1988; Brisibe et al., 1993 and Bale et al., 2003). The serological evidence of brucellosis in sheep and goats in Nigeria has been recognized as far back as 1934 (Eze, 1977). These studies have shown how brucellosis had been identified as an endemic and problematic disease in Nigeria. However, the infection is not static; it is evident from previous studies that prevalence varies at different times and locations. This is especially apparent where there is no control policy, as is the

case in Nigeria (Cadmus et al., 2006).

This work was carried out in a Livestock Research farm in Zaria, Kaduna State. The research farm among other things serves as a breeding centre for animals and the status of some of these animals is not known. Fertility rate has been observed to be on the decline due to abortion in some pens where the goats were kept. There was a report of an isolation of *Brucella* organism from an aborted foetus from a doe (Personal communication). This study is necessary, considering the economic and zoonotic/public health significance of brucellosis so that preventive and control measures can be instituted. It is important to obtain baseline information so as to determine the health status of these sheep and goats vis-à-vis brucellosis to ensure that animals that are bred and distributed to farmers and consumers are brucella-free and to prevent infection spread to other farm animals and man.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted between 2011-2012 in a Livestock Research Farm in Zaria (110° 7', 110° 12'N and longitude 7° 41'), Kaduna state of Nigeria. The sheep and goats were maintained under a semi-intensive husbandry system and allowed to graze on restricted grasslands or paddocks fenced with barbed wire. Animals reared on the Livestock farm were often purchased from different livestock markets particularly from North Western, North Central and South Western parts of Nigeria. The female animals were kept separately in different pens; the sheep and goats were also kept in separate pens. The sheep and goats had no history of vaccination against brucellosis.

Sample size, sample collection and processing

A total of 580 samples comprising of 315 from sheep and 265 from goats were collected. The sample size was determined using the formula as described by Thrushfield (1997) using prevalence from previous studies carried out by Bertu et al., 2010 (14.5% for sheep and 16.1% for goats). The sheep and goats were selected using random sampling without replacement until desired number of samples was obtained.

Ten millilitres (10 mls) of venous blood was collected

from the jugular vein of each animal and aliquoted into 2 different tubes; one containing ethylenediamine tetraacetic acid (EDTA) and used to attempt cultural isolation of *Brucella* while the second tube was without anticoagulant (to obtain serum) and used for serological screening for *Brucella* antibodies. All samples were transported to the Microbiology laboratory of National Animal Production Research Institute in Zaria for further processing. Information such as sex, breed and age of each animal were also collected and recorded in a log book.

Cultural isolation: An aliquot of the whole blood (with anticoagulant) was inoculated onto *Brucella* agar (Oxoid, UK) for recovery of the pathogen as previously described by Alton et al. (1988). The plates were incubated at 37°C for 3-7 days for frequent observations for colonies typical for *Brucella* i.e. circular and 2-4 mm in diameter.

Serological detection: All antigens used for all serological tests (RPBT and SAT) were obtained from Veterinary Laboratories Agency (VLA) Weybridge, Surrey, United Kingdom. Two different serological tests: Rose Bengal Plate and Serum Agglutination tests were used to screen the sera for antibodies to *Brucella*.

Rose Bengal Plate test (RBPT): The method described by Alton et al. (1988) was adopted, briefly, 30 µl of the antigen was placed on a clean white ceramic tile and 30 µl of the serum sample was placed beside (and not into) the antigen. The antigen and serum were mixed thoroughly with a sterile applicator stick and the ceramic tile was rocked gently using the hand and pink distinct granules are observed as agglutination and recorded as positive result after mixing was done for 4 minutes while non-agglutination (clear without pink granules) was recorded as negative.

Serum agglutination Test (SAT): The method of Alton et al. (1988) modified by Bale (2008) was adopted: briefly, five test tubes per sample were set up. The first test tube contained 0.8 ml of phenol saline while 0.5 ml was dispensed into the second, third, fourth and fifth tubes. The test serum (0.2 ml) was added to the first tube and mixed properly and a serial dilution was carried out by transferring 0.5 ml of mixture in the first test tube to the second and so on to the fifth test tube. The final 0.5 ml from the fifth

test tube was discarded and 0.5 ml of the antigen diluted at 1:10 with phenol saline was added into each tube and mixed properly. The test tubes were then incubated at 37°C for 20 hours before the tubes were read and recorded. Sera showing less than 40 i.u i.e titres less than 1:40 (from the fourth and fifth test tubes) were recorded as negative while those from 1:40 and above were recorded as positive. Standards for positive and negative controls were set up along with test serum samples to check for technique and antigen.

DATA ANALYSIS

Results obtained were reduced to contingency tables and Statistical Package for Social Students (SPSS), version 17.0 (SPSS Chicago Inc.) was used to determine Chi-square or Fisher's exact test where appropriate. The seroprevalence of brucellosis was determined by dividing the number of seropositive cases by the total number of animals (sheep or goat) for each category and multiplying by 100. The seroprevalence was subjected to Chi-square or Fisher's exact test for statistical significance between species (caprine and ovine), age groups (<1year, 2-3 years and >3 years), sex (male and female) and serological tests performed. P values less than 0.05 ($P < 0.05$) were considered to be statistically significant at 95% confidence interval.

RESULTS

Interestingly, no *Brucella* isolate was recovered from the 580 blood samples cultured. However, serological evidence of brucellosis was detected by RBPT and SAT in 195 (33.62%, 195/580) and 88 (15.17%, 88/580) of the animals tested respectively (Tables 1 and 2). Higher positive rates of *Brucella* antibodies were detected for goats compared to sheep by SAT (25.6% vs 6.35%) as seen in Table 2 while the rates were nearly similar when RBPT (33.58% vs 33.65%) was used for the screening (Table 1).

Although, few Sahelian goats ($n=17$) compared to the Red Sokoto breed ($n=248$) were sampled, they were all negative for *Brucella* antibodies by both RBPT and SAT (Table 1). Contrasting figures were however observed for the sheep where despite the fewer sampled Balami ($n=5$) and Uda ($n=24$) breeds they had a higher seropositive rates by RBPT of 40% and 25% respectively close to the 34.27% of the

Yankasa breed ($n=286$) (Table 1). A sharp decrease of these rates were however observed for the SAT where none of the Balami sheep were positive while only two (2/24) and eighteen ($n=24$) of the Uda and Yankasa breeds were positive respectively (Table2).

When the animals were age-stratified twenty five (29.41%) of the 80 animals belonging to the less than one year age group (<1 year) were positive while 40 (31.75%) out of 126 animals between 1-3 years were positive to *Brucella* as measured by RBPT. The differences between various age groups were statistically insignificant ($P > 0.05$) (Table 1). Twenty five out of the 85 goats less than one year (<1 year) were positive representing 29.41%, 33 (26.19%) of goats between 1-3 years were positive while 10 (18.52%) goats greater than three (>3 years) had antibodies to *Brucella* as measured by SAT. This was statistically insignificant ($P > 0.05$) (Table 2).

Seventy seven out of the 211 female goats representing 36.49% were positive while 12 (22.22%) out of 54 male goats had antibodies to *Brucella* as measured by RBPT. The difference between the variables was statistically significant ($P < 0.05$) (Table 1). Fifty two out of 211 female goats i.e 24.64% were positive while 16 (29.63%) out of 54 male goats had antibodies to *Brucella* as measured by SAT. This was statistically insignificant ($P > 0.05$) (Table2).

Eighty one out of the 238 females representing 34.03% were positive while 25 (32.47%) out of 77 male sheep had antibodies to *Brucella* as measured by RBPT. This was not statistically significant ($P > 0.05$) (Table 2). Thirteen out of 238 female sheep representing 5.46% were positive while 7 (9.09%) out of 77 male sheep had antibodies to *Brucella* as measured by SAT. This was not statistically significant ($P > 0.05$) (Table 2).

TABLE 1: Results of *Brucella* antibody detection using Rose Bengal Plate Test (RBPT) in goats and sheep by species, breed, sex and age

Variable	sub-variable	No. sampled	No. positive (%)	Chi-square	P-value
Species	Goat	265	89(33.58)	0.00028	0.9867
	Sheep	315	106(33.65)		
Breeds					
Goats	RSG*	248	89(35.89)	0.9433	0.0010
	Sahelian	17	0		
Sheep	Balami	5	2(40)	0.9433	0.6240
	Uda	24	6(25)		
	Yankasa	286	98(34.27)		
Sex					
Goats	Female	211	77(36.49)	3.926	0.0476
	Male	54	12(22.22)		
Sheep	Female	238	81(34.03)	0.06391	0.8004
	Male	77	25(32.47)		
Age					
Goats	<1 year	85	25(29.41)	0.1297	0.7187
	1-3 years	126	40(31.75)		
	>3 years	54	24(44.44)		
Sheep	<1 year	45	20(44.44)	1.083	0.2981
	1-3 years	118	42(35.59)		
	>3 years	152	44(28.95)		

RSG*-Red Sokoto Goat

TABLE 2: Results of *Brucella* antibody detection using Serum Agglutination Test (SAT) in goats and sheep by species, breed, sex and age

Variable	sub-variable	No. sampled	No. positive (%)	Chi-square	P-value
Species	Goat	265	68(25.66)	41.70	0.001
	Sheep	315	20(6.35)		
Breeds					
Goats	RSG*	248	68(27.42)	0.4994	0.0082
	Sahelian	17	0		
Sheep	Balami	5	0	0.4994	0.7790
	Uda	24	2(8.33)		
	Yankasa	286	18(6.29)		
Sex					
Goats	Female	211	52(24.64)	0.5601	0.4542
	Male	54	16(29.63)		
Sheep	Female	238	13(5.46)	1.288	0.2564
	Male	77	17(9.09)		
Age					
Goats	<1 year	85	25(29.41)	0.2642	0.6072
	1-3 years	126	33(31.75)		
	>3 years	54	10(44.44)		
Sheep	<1 year	45	2(44.44)	2.401	0.3011
	1-3 years	118	5(35.59)		
	>3 years	152	13(28.95)		

RSG*-Red Sokoto Goat

DISCUSSION

The gold standard for diagnosis of brucellosis is by isolation of *Brucella*, mostly from culture, or indirectly by the detection of immune response against its antigens (Alton *et al.*, 1988). Although, 580 blood samples were cultured, there was no isolation of any *Brucella* organisms which may be attributed to the slow growth of *Brucella* in primary cultures (Ariza, 1996; Yagupsky, 1999). Besides, blood culture sensitivity is often low and depends on disease stage, *Brucella* species, culture medium, number of circulating bacteria and the culture technique employed (Gotuzzo *et al.*, 1986, Yagupsky *et al.*, 1999). Similarly, *Brucella* is a facultative intracellular organism which is capable of multiplication and survives within host phagocytes. The organisms are phagocytosed by polymorphonuclear leukocytes in which some survive and multiply and are transported to lymphoid tissue and foetal placenta. The inability of the leukocytes to effectively kill virulent *Brucella* at the primary site of infection is a key factor in the dissemination to regional lymph nodes, mononuclear phagocytic system, joints and organs such as the uterus, testes and udder. The ability to survive within macrophages and leukocytes enables the organism to be protected from humoral and cellular bactericidal mechanisms during the periods of haematogenous spread (Nielsen and Duncan, 1990). The localization of brucellae in these tissues and organs reduces the number of circulating organisms in blood and thereby makes isolation of brucellae from blood to be very difficult. While culturing is a specific method, its sensitivity depends on the viability and numbers of *Brucella* within the sample, the nature of sample (foetal organs, foetal membranes, lymph nodes, etc.) and the number of specimens examined from the same animal (Hornitzky and Searson, 1986). The time required for culturing field specimens can be long and tissues or fluids that are only contaminated with a low number of *Brucella* may not be detected. Onoja *et al.* (2008) could not isolate any organisms from a flock of sheep in Zaria which had a history of 2 recent abortions even with the high serological prevalence rate of 76% by Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT). They collected samples from 3 cases of carpal hygroma and from vaginal swabs.

There have been reports on isolation of brucellae in sheep and goats in Nigeria by Okoh (1980), Bale *et al.* (2003) and Ocholi *et al.* (2005). Okoh in 1980 isolated five brucellae organisms from 22 milk samples of ewes in a Sheep Breeding Centre in Kano State of Nigeria where there was history of abortion storm and death of neonate lambs. The *Brucella* species isolated was *Brucella abortus*. Bale *et al.* (2003) isolated ten organisms from 277 milk samples in sheep and 141 goats milk from government farms in different parts of northern Nigeria, four brucellae were isolated from sheep and six from goats. The isolates were biotyped and identified as rough strains of *Brucella melitensis*. Ocholi *et al.* (2005) isolated seven brucellae in a flock of Yankasa sheep in a privately owned farm in Toro near Bauchi, Nigeria where there was report of abortion. They collected milk samples and vaginal swabs and isolated three brucellae from milk samples and four from vaginal swabs from aborting ewes. All the samples were identified and biotyped as *Brucella abortus* biovar 1. It is important to note that the inability to isolate brucellae organisms in this study does not rule out the presence of brucellosis in this farm as difficulties and constraints earlier stated involved in the isolation of this organism may be responsible.

The distribution of RBPT in ovine species (sheep) and caprine species (goats) showed that there was a higher prevalence in sheep (33.58%) than in goats (33.65%) though it was not statistically significant ($P>0.05$). The higher prevalence in sheep may be due to the fact that more samples were collected from sheep (315) than in goats (265). This higher prevalence in sheep than in goats, is similar to the findings of Junaidu *et al.* (2006) where they carried out a serological survey in Sokoto City Abattoir and reported a prevalence of 23.61% by RBPT in sheep and a lower prevalence in (22.93%) goats in a separate study which was carried out in the same Sokoto Metropolitan Abattoir (Junaidu *et al.*, 2010). However, lower prevalences have been reported by other investigators. Bale *et al.* (1982) reported a prevalence of 14.1% and 16.1% in sheep and goats, respectively by RBPT in a serological study of sheep and goats brucellosis in some parts of northern Nigeria. Junaidu *et al.* (2008) in their study carried out in a Prison Farm in Sokoto, Nigeria, reported a prevalence of 22.36% in sheep and 30.97% in goats

using RBPT. Bertu *et al.* (2010) reported a prevalence of 9.3% in sheep and 10.1% in goats using the RBPT in a study conducted in Plateau State. In a serological survey of brucellosis in livestock animals and workers in Ibadan, Nigeria, Cadmus *et al.* (2006), reported prevalence in goats to be 0.86% but none of the sheep sampled was positive to RBPT. It is pertinent to note that the margin between the differences in prevalence rates in sheep and goats using RBPT is small and this indicates that there is no much preference to infection to brucellosis in small ruminants.

The results of SAT by species revealed that goats (caprine species) had a higher prevalence rate (25.66%) than in ovine species or sheep (6.35%). This was found to be statistically significant ($P < 0.05$). This significant difference may be attributed to the fact that abortion storms and reduction in fertility rates were first observed in the goat pens after introduction of new animals; more so there was isolation of brucella from an aborted foetus in one of the goats prior to the beginning of this study. The higher prevalence seen in goats than in sheep may also be due to the nature of vaginal secretion in goats, Anon (2001) reported that goats have a more copious vaginal discharge than sheep and the excretions from the vagina in goats is prolonged than in cows and ewes and lasts for at least 2-3 months. In sheep, excretion is generally less prolonged, usually ceasing within 3 weeks after abortion or a full-term parturition. The above finding of a higher prevalence rate by SAT in goats than in sheep is similar to the result of Bale *et al.* (1982) where they reported a seroprevalence rate of 1.63% in goats and 0.23% in sheep.

The breed distribution of RBPT in goats showed that Red Sokoto goats had a higher prevalence (35.89%) than sahelians where all were negative and the difference was statistically significant ($P < 0.05$). Ajogi *et al.*, 2002 showed that there is no breed specificity in brucellosis. The difference may be due to a lower number of sahelian goats sampled (17) compared to the Red Sokoto goat (248). This was similar to the breed distribution of SAT in goats where Red Sokoto goats had a higher prevalence (27.42%) than sahelians (0%) and the difference was statistically significant ($P < 0.05$). The above finding is similar to that of Junaidu *et al.* (2010) where they

recorded prevalence rates of 11.83% and 11.65% in red sokoto goats and sahelian respectively using SAT.

The results of RBPT in goats showing prevalence by age indicated that goats belonging to the oldest age group (>3 years) had the highest prevalence rate (44.44%), followed by those between 1-3 years (31.75%), while the least was recorded in animals less than one year (<1 year) with a prevalence of 29.41%. Brucellosis is particularly a disease of sexually matured animals (both males and females), young animals may be infected but do not show any clinical sign and generally show a weak and transient serological response (Anon, 2001). This result is similar to the work of Junaidu *et al.* (2010) which reported the highest prevalence rate in the oldest group of animals (above 24 months) in the study in which they grouped sampled animals into three age groups (0-12 months, 13-24 months and above 24 months).

The result of SAT in goats by age showed that animals less than one year (<1 year) had the highest prevalence rate of 29.41% followed by goats between 1-3 years with 26.19%, while goats greater than three years (>3 years) had the lowest prevalence rate of 18.52%. The differences between these values were not statistically significant ($P > 0.05$). The detection of the highest antibodies in animals less than one year may be attributed to the uptake of antibodies in milk during suckling; also these young animals may have been exposed to other diseases of neonates whose causative agents share similar lipopolysaccharide (LPS) as that of brucellae, example, colibacillosis and salmonellosis. The LPS of brucella are similar to that of *Escherichia coli*, *Salmonella spp*, *Yersinia enterocolitica* 0:9 etc. This finding of the presence of highest antibody titres in the youngest group of goats (<1 year) is similar to the study by Junaidu *et al.* (2010) where they reported the highest seroprevalence of 18.45% in the youngest group of goats (0-12 months).

The age distribution of RBPT in sheep showed that the highest prevalence rate was recorded in animals less than one year (<1 year) (44.44%) followed by animals between 1 and 2 years (35.59%), while the least was recorded in animals greater than three years (>3 years). Young animals (sexually immature) do not favour the proliferation of brucellae organisms

but the presence of high antibody titres suggests that these young animals might have gotten these antibodies while suckling as infants as brucellae organisms can localize in the mammary gland. The localization of brucellae organisms in the mammary gland leads to intermittent shedding of the organisms in the milk in succeeding lactations (Alton, 1990). Junaidu *et al.* (2006) reported the lowest prevalence in the oldest group (>24 months) of sheep (19.58%), they grouped the animals into three (0-12 months, 12-24 months and above 24 months).

The results of SAT in sheep by age indicated the highest prevalence in animals greater than three years (>3 years) with a seroprevalence of 8.55% followed by animals less than one year (<1 year) with a prevalence of 4.4% and the least was reported in animals between 1-2 years (4.24%). The difference was statistically insignificant ($P>0.05$). The reason for the above finding may be due to already established findings that susceptibility to *Brucella* infection is more in sexually matured animals than the young. Nicolleti (1980) reported that the susceptibility of livestock to *Brucella* infection is influenced by the age (young animals are less susceptible to *Brucella* than older animals) and sexually mature, pregnant animals are more susceptible to infection with the organism than sexually immature animals.

The result of RBPT in sheep and goat by sex indicated that prevalence rates were higher in females than males in both species. The female goats (does) had a higher prevalence of 36.44% than male goats (bucks) (22.22%). This was statistically significant ($P<0.05$). Similarly, a higher prevalence rate was recorded in female sheep (34.03%) than in the males; though this was not statistically significant ($P>0.05$). This is similar to the findings of Onoja *et al.* (2008), where they reported a prevalence rate of 0.85% in rams and 69.2% in ewes in a study carried out in a flock in Zaria. Though brucellosis is known to be neither breed nor sex specific (Ajogi *et al.*, 2002), the detection of higher antibody titres in female animals than in males for both sheep and goats using the RBPT suggests the presence of suitable factors such as erythritol, which aid the growth of brucellae organisms. Erythritol, a sugar alcohol synthesized in the ungulate placenta and stimulates the growth of virulent strains of brucellae organisms, has been

credited with the preferential localization of the bacteria within the placenta of ruminants (Smith *et al.*, 1962).

The sex distribution of SAT in goats and sheep showed that males had a higher prevalence than females. The male goats had a prevalence of 29.63%, while the female goats had 24.64%, the same trend was observed in the sheep where the males had a higher prevalence of 9.09% than females (5.46%). The differences between the prevalences in males and females in both sheep and goats were not statistically significant as P values were greater than 0.05. Brucellosis is known to be neither breed nor sex specific (Ajogi *et al.*, 2002). The detection of a higher prevalence rate in males may be attributed to the type of management system. The bucks and rams are kept in separate pens and are allowed to mate the does and ewes only during breeding as they do not graze on the same pasture. It is possible that the bucks and rams were infected during mating and since they are fewer in number than the does and ewes, the possibility of repeated mating by a buck or ram increases the probability of dissemination or spread of brucellae organisms among the males than the females. The does have not been bred since the isolation of brucellae was done prior to this study. Adamu *et al.* (2012) reported a higher seroprevalence in male than female goats in a study they carried out on farmer awareness on caprine abortion and the presence of *Brucella abortus* and *Brucella melitensis* in selected flocks in an arid zone of Nigeria. Many studies and previous works share contrary reports to the above finding (Onoja *et al.*, 2008, Junaidu *et al.*, 2010).

The comparison between results of RBPT and SAT showed that a higher percentage of positive animals (33.62%) were recorded in RBPT than in SAT (15.17%). This disparity may be due to the higher sensitivity of RBPT than SAT. It is generally agreed that RBPT is a screening test which is highly sensitive but heterospecific (Olascoaga, 1976). Infection due to organisms, such as *Vibrio cholera*, *Yersinia enterocolitica* 0:9, *Pasteurella spp*, *Salmonella* or some other members of the Brucellaceae family could give false positive results than SAT and CFT (Bale, 1982). Olascoaga (1976) reported that the RBPT measures IgG₁ while SAT measures predominantly IgM and little of IgG. This probably shows that the predominant immunoglobulin is IgG because of more

positive results in RBPT as SAT may be negative in chronic infections because IgM was no longer present.

Recommendations drawn from this study are aimed at ensuring that safe and healthy animals are sold or distributed to other farms and breeders thereby reducing disease outbreaks in animals and humans. It is important that the Livestock farm enlightens and educates herdsman and other animal handlers about the zoonotic implication of brucellosis in the farm and new animals should be screened for brucellosis before introduction into the herd or flock.

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