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Sero−prevalence of brucellosis in sheep in North Senatorial District of Kaduna State, Nigeria

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PEER REVIEW

Comments

Brucellosis is a contagious and serious bacterial disease affecting ruminants such as sheep, causing a lot of complications in them including abortion, reduced milk production and sterility. Because of the strong socio−economic and cultural relationships between man and animals in the area of Nigeria under study, this study addresses an important problem that is of high public health significance. This study increases the awareness of people and government to implement control measures to bring down the disease prevalence.
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ABSTRACT

Objective: To determine the current status of *Brucella* antibodies in sheep in Kaduna North Senatorial District of Kaduna State, Nigeria.

Methods: A total of 579 serum samples were collected (114 rams and 465 ewes) and analysed using Rose Bengal plate test (RBPT), serum agglutination test with ethylene diamine tetraacetic acid (SAT−EDTA) and lateral flow assay (LFA).

Results: The prevalence of *Brucella* antibodies was found to be 26.5%, 11.1% and 2.4% using RBPT, SAT−EDTA and LFA respectively. The prevalence was 22.8%, 7.9%, 1.8% and 27.5%, 12.7% and 3.0% for the males and females using RBPT, SAT−EDTA and LFA, respectively. The prevalence rate for animals of less than 1 year of age was 23.8%, 14.3% and 9.5% for RBPT, SAT−EDTA and LFA, respectively, the corresponding prevalence rate for animals within the age bracket of 1 to 3 years was 26.7%, 11.2% and 1.4%, using RBPT, SAT−EDTA and LFA, respectively. Seroprevalence for animals above 3 years old were 24.7%, 12.4% and 3.5%, respectively.

Conclusions: It is evident that sheep in the study area are infected with *Brucella* spp., including *Brucella melitensis* in view of the LFA positivity. This is of public health significance.

1. Introduction

Brucellosis is a contagious systemic bacterial disease primarily of ruminants, which is characterized by several signs like inflammation of the genital organs and fetal membranes, abortion, sterility and formation of localized lesions in the lymphatic system and joints[1,2]. It is a zoonosis that is almost invariably transmitted to people by direct or indirect contact with infected animals or their products[3]. The infection in small ruminants is mostly caused by

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Brucella melitensis (*B. melitensis*). Furthermore, *Brucella ovis* is also an important cause of orchitis and epididymitis in sheep, but it is not recognized as a cause of natural infection in goats[4]. Persistent infection is a common feature of the disease with frequent shedding of the bacterium in reproductive and mammary secretions. Brucellosis in sheep and goats has been reported in various parts of Nigeria[5–7]. 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[4,8,9]. Unconfirmed cases of abortion, stillbirth and retained placenta in livestock, being reported in Nigeria yearly may be caused by members of the genus *Brucella*. Therefore, the prevalence of brucellosis in small and even large ruminants may constitute a significant deterrent to the development of livestock in Nigeria.

The major occupation of the people of Kaduna State is agriculture, producing food and cash crops and rearing livestock[10]. Kaduna State has an estimated population of 1 144 000 cattle, 832 000 sheep and 988 000 goats[10]. Although some seasonal movement of pastoral sheep and goats does take place, the overwhelming majority of small ruminants are sedentary, village based and their patterns of distribution mirror those of human settlement[11]. There are strong socio–economic and cultural relationships between man and his animals, especially small children and women, and there is even a great risk of spread of brucellosis among animals and people in the study area; these animals should be harboring the disease. The aim of this study was to determine the sero–prevalence of brucellosis in sheep in the North Senatorial District of Kaduna State, Nigeria.

2. Materials and methods

2.1. Study area

The study was conducted in the North Senatorial District of Kaduna State, Nigeria. Kaduna State is located in the northwest geopolitical zone of Nigeria. It lies between latitudes 6° and 11° North and longitude 7° and 44° East, and is 608 m above sea level. It has distinct wet and dry seasons and is within the Guinea and part of the Sahel Savannah Zone of Nigeria. The state shares geographical boundaries with Katsina and Zamfara States to the north, Plateau and Bauchi States to the east, Nasarawa State and the Federal Capital Territory to the south, Niger State to the west and Kano State to the northeast. Kaduna State occupies about 48 473.25 km^2, with a human population of over 6 066 562 people according to the census figures of 2006[10].

Four out of the seven local government areas (LGAs) in North Senatorial District of Kaduna State were selected using simple random sampling without replacement. These include, Ikara, Makarfi, Sabon Gari and Soba LGAs. The location of the flock, animal species, breed, age and sex of each animal sampled were recorded. A total of 579 sheep were sampled for the purpose of this study, out of which 114 were rams and 465 were ewes.

2.2. Study animals

Pastoral and village level sheep were used in this study. Flock selection was done by random selection and farmers' consent. There was no record of vaccination against brucellosis in any animal species in the study area for over 20 years.

2.3. Study design and sampling method

The study was carried out between April and May, 2012. Approximately 5 mL of blood was obtained via a jugular venipuncture of apparently healthy sheep, using a 10 mL syringe with 21G needle. The blood was then transferred into well labeled 10 mL plain blood–collecting tubes and placed in a slanting position under shade to allow it to clot. The samples were then transported to the laboratory in leak proof ice–packed containers, where they were further centrifuged at 291 r/min for 5 min to allow for proper separation of serum from the clotted blood. The serum was then decanted into 5 mL plastic tubes, which were properly labelled for the corresponding tubes, then they were stored in the freezer at −20 °C until used.

2.4. Ethical issues

Sheep owners involved in this study were adequately informed the purpose of the study and their agreement were obtained.

2.5. Serological tests

Serum samples were tested for *Brucella* spp. antibodies by Rose Bengal plate test (RBPT) as described by Alton *et al.*[12], serum agglutination test with ethylene diamine tetraacetic acid (SAT–EDTA) as described by Brown *et al.*[13] and lateral flow assay (LFA) according to the manufacturer's instructions. The antigens for the SAT–EDTA and RBPT were obtained from Onderstepoort Biological Products Ltd., South Africa, while the test kit for the LFA was obtained from Bionote INC., Republic of Korea.

2.6. RBPT

Briefly, 30 μL of antigen was placed on a white ceramic tile and the same volume of 30 μL test serum was placed beside the antigen. The two were mixed thoroughly using sterile

applicator stick and rocked gently for 4 min and observed for agglutination. The formation of distinct pink granules (agglutination) was recorded as positive, while the absence of agglutination was recorded as negative.

2.7. SAT–EDTA

Phenol saline with EDTA buffer solution, containing 5 g phenol crystals, 8.5 g sodium chloride, 1.861 2 g disodium EDTA and dissolved in 100 mL of warm distill water was prepared. A 1:10 dilution of the concentrated SAT antigen with the prepared buffer with a pinch of 0.02% safranin O (to provide contrast to the agglutination reaction) was made for each day's work. A 96–well rectangular microtitre plate was set up on the work table. Labeled serum vials were placed on the work table according to positions of the wells already labeled A–H and a corresponding vertical numbering of the wells. A representative entry of the sample details was made in the laboratory record book. Positive and negative were assigned to row 'A', while rows B–H were designated to the test sera. Using automatic micropipette, 40 µL of the buffer solution was measured out into the first well and 25 µL into each of the remaining microtitre wells. This was followed by the addition of 10 µL of test serum into the first microtitre well using a fresh disposable pipette plastic tip for each test, which was later on discarded. A two–fold serial dilution was done by transferring 25 µL aliquots from the first well up to the fifth well. About 25 µL of the aliquot was discarded after the last well. Content of the working dilution of the SAT antigen were mixed gently and 25 µL added to each well. Finally, the contents in the microtitre plate were mixed by gently tapping the edges of the plate for 20 seconds. The microtitre plates were covered to prevent evaporation of the contents in the wells and incubated for 20 h at 37 °C in an incubator.

2.8. LFA

A total of 20 µL of thawed serum was placed into the sample hole of the test device, followed by the addition of 4 drops of the provided diluent. Test results were observed after 20 min by visual inspection for staining of the test and control lines. Tests were scored negative when staining was observed only on the control line, and scored positive when staining was observed on both the test line and control lines.

2.9. Data analysis

Data obtained were presented in form of tables. *Chi–*square (χ^2) test was used to analyse the data with the aid of GraphPad Prism, Version 4.00, 2003. Values of $P<0.05$ were considered significant.

3. Results

Among the 579 sheep sampled in North Senatorial District of Kaduna State, 148 (25.6%), 64 (11.1%), and 14 (2.4%) were positive for RBPT, SAT–EDTA and LFA, respectively (Table 1). Sheep sampled from Sabon Gari LGA had the highest RBPT positive results [65 (11.2%)], while lowest seroprevalence of 13 (2.3%) was obtained in sheep in Makarfi LGA. Furthermore, the sheep in Ikara LGA had the highest seroprevalence of 22 (3.8%) when measured with SAT–EDTA, while those in Makarfi had the lowest value of 3 (0.5%). With respect to LFA, the sheep in Makarfi had the lowest seroprevalence of 0 (0%) and highest value of 8 (1.4 %) was recorded in Soba LGA.

Table 1
Distribution of brucellosis seroprevalence in sheep in four LGAs in North Senatorial District of Kaduna State, Nigeria. *n* (%).

LGA	No. of samples	RBPT	SAT–EDTA	LFA
Ikara	170	39 (6.7)	22 (3.8)	4 (0.7)
Makarfi	40	13 (2.3)	3 (0.5)	0 (0.0)
S/Gari	229	65 (11.2)	20 (3.5)	2 (0.4)
Soba	140	31 (5.4)	19 (3.3)	8 (1.4)
Total	579	148 (25.6)	64 (11.1)	14 (2.4)

S/Gari: Sabon Gari.

There was a statistically significant difference ($P=0.000$ 1, $\chi^2=139.9$) in the rates obtained with the three different serological tests that detected *Brucella* antibodies in sheep.

The number of sheep sampled by sex and their seroprevalence rates for *Brucella* antibodies using RBPT, SAT–EDTA and LFA are presented in Table 2. A total of 114 male and 465 female sheep were sampled.

Table 2
Prevalence of brucellosis in male and female sheep in North Senatorial District of Kaduna State, Nigeria. *n* (%).

LGA	Male				Female			
	Tested	RBPT	SAT–EDTA	LFA	Tested	RBPT	SAT–EDTA	LFA
Ikara	23	6 (5.3)	3 (2.6)	0 (0.0)	147	34 (7.3)	19 (4.1)	4 (0.9)
Makarfi	10	3 (2.6)	0 (0.0)	0 (0.0)	30	10 (2.2)	3 (0.7)	0 (0.0)
S/Gari	51	12 (10.5)	2 (1.8)	0 (0.0)	178	56 (12.0)	20 (4.3)	2 (0.4)
Soba	30	5 (4.4)	4 (3.5)	2 (1.8)	110	28 (6.0)	17 (3.7)	8 (1.7)
Total	114	26 (22.8)	9 (7.9)	2 (1.8)	465	128 (27.5)	59 (12.7)	14 (3.0)

S/Gari: Sabon Gari.

Of the 114 male animals sampled, prevalence of 26 (22.8%), 9 (7.9%) and 2 (1.8%) were recorded based on RBPT, SAT–EDTA and LFA, respectively. The highest prevalence of 12 (10.5%) based on RBPT was recorded in Sabon Gari LGA, while the lowest prevalence of 3 (2.6%) was recorded in Makarfi LGA. Similarly, when measured with SAT–EDTA, the highest prevalence of 4 (3.5%) was recorded in Soba LGA, while the corresponding lowest prevalence of 0 (0.0%) was recorded in Makarfi LGA. In terms of LFA, only Soba LGA recorded a prevalence of 2 (1.8%), while 0 (0.0%) was recorded in the remaining three LGAs.

As for the 465 female animals sampled, a prevalence of 128 (27.5%), 59 (12.7%) and 14 (3.0%) were recorded based on RBPT,

Table 3
Prevalence of *Brucella* antibodies in sheep per different age groups in North Senatorial District of Kaduna State, Nigeria. *n* (%).

LGA	<1 Year				1–3 Years				Above 3 years			
	Tested	RBPT	SAT–EDTA	LFA	Tested	RBPT	SAT–EDTA	LFA	Tested	RBPT	SAT–EDTA	LFA
Ikara	10	2 (4.8)	1 (2.4)	0 (0.0)	84	19 (5.2)	9 (2.5)	2 (0.6)	76	20 (11.8)	11 (6.5)	2 (1.2)
Makarfi	1	0 (0.0)	0 (0.0)	0 (0.0)	19	4 (1.1)	3 (0.8)	0 (0.0)	20	6 (3.5)	1 (0.6)	0 (0.0)
S/Gari	7	2 (4.8)	1 (2.4)	0 (0.0)	198	58 (15.8)	20 (5.5)	1 (0.3)	24	5 (2.9)	2 (1.2)	0 (0.0)
Soba	24	6 (14.3)	4 (9.5)	4 (9.5)	66	17 (4.6)	9 (2.5)	2 (0.6)	50	11 (6.5)	7 (4.1)	4 (2.4)
Total	42	10 (23.8)	6 (14.3)	4 (9.5)	367	98 (26.7)	41 (11.2)	5 (1.4)	170	42 (24.7)	21 (12.4)	6 (3.5)

S/Gari: Sabon Gari.

SAT–EDTA and LFA, respectively. The highest and lowest seropositive values when measured with RBPT and SAT–EDTA were found in Sabon Gari and Makarfi LGAs, with values of 56 (12.0%) and 20 (4.3%) and 10 (2.2%) and 3 (0.7%), respectively (Table 2). The corresponding highest and lowest values of seroprevalence when measured with LFA were 8 (1.7%) and 0 (0.0%) in Soba and Makarfi LGAs, respectively.

There was no statistically significant difference (*P*=0.7495) in the prevalence rates of brucellosis between the male and female animals.

The number of sheep tested and the prevalence of *Brucella* antibodies in the different age groups are presented in Table 3.

Forty two small sheep of less than 1 year old were tested and a prevalence of 10 (23.8%), 6 (14.3%) and 4 (9.5%) were recorded, based on RBPT, SAT–EDTA and LFA, respectively. Soba LGA recorded the highest prevalence in terms of RBPT, while Makarfi recorded the lowest value (Table 3). Similarly, the seropositivity when tested with SAT–EDTA was highest in sheep from Soba LGA and least in those from Makarfi LGA. Furthermore, 4 (9.5%) of the sheep of this age group were positive with LFA and were all from Soba LGA.

Out of the 367 sheep tested within the age bracket of 1–3 years, prevalence of 98 (26.7%), 41 (11.2%) and 5 (1.4%) were recorded based on RBPT, SAT–EDTA and LFA, respectively. Sabon Gari LGA recorded the highest prevalence in terms of RBPT, while Makarfi LGA recorded the lowest value (Table 3). Similarly, the seropositivity when tested with SAT–EDTA was highest in sheep from Sabon Gari LGA and least in those from Makarfi LGA. Ikara and Soba LGAs recorded the highest prevalence in terms of LFA, while the lowest value was from also from Makarfi LGA.

Furthermore, 170 sheep above 3 years of age were tested and prevalence rates of 42 (24.7%), 21 (12.4%) and 6 (3.5%) were recorded based on RBPT, SAT–EDTA and LFA, respectively. Ikara LGA recorded the highest prevalence in terms of RBPT, while Sabon Gari LGA recorded the lowest value (Table 3). Similarly, the seropositivity when tested with SAT–EDTA was highest in sheep from Ikara LGA and least in those from Makarfi LGA. Soba LGAs recorded the highest prevalence in terms of LFA, while the lowest value was from Makarfi and Sabon Gari LGAs.

There was a statistically significant difference (*P*=0.0045;

χ^2=10.72) in prevalence rates of brucellosis between the three different age groups of the sheep evaluated.

4. Discussion

From the seroprevalence study of brucellosis in four LGAs of the North Senatorial District of Kaduna State, prevalence rates of 25.6%, 11.1%, and 2.4% were recorded using RBPT, SAT–EDTA and LFA, respectively. This is an indication that brucellosis exists in this species of animals. The seropositive differences observed in the various LGAs could be due to the management practices of herdsmen. Sheep are allowed to roam around villages with increasing possibility for street mating and subsequent spread of reproductive diseases, brucellosis inclusive for the village level animals. The migratory habit of the pastoralists could account for infection in small ruminants since they mix freely with cattle, and Maurice *et al.* reported increasing prevalence among them[5].

The study also showed that more animals were seropositive with RBPT and SAT–EDTA as compared with the LFA. The high seroprevalence rate with RBPT may be attributed to its relatively low specificity and very high sensitivity which could be a result reaction to other smooth lipopolysaccharide of *Brucella* species especially *Brucella abortus*. This is because sheep are mostly herded together or kept together with cattle[3,14]. It could also be due to other Gram–negative bacteria like *Vibrio cholerae* O1, *Escherichia coli* O: 157, some strains of *Escherichia hermannii* and *Stenotrophomonas maltophila*, *Salmonella* group N (O: 30), and *Yersinia enterocolitica* O: 9 which has similar lipopolysaccharide O–chains. These organisms have agglutinins capable of reacting with *Brucella* antigens, thus giving a false positive reaction. However, the RBPT may be used as a screening test to ascertain exposure of animals to infection due to *Brucella*[15].

The lower seroprevalence rate obtained in the present study with the SAT–EDTA is in agreement with the work of Bertu *et al.*[7], who reported a prevalence of 9.3% and 5.2% with RBPT and SAT, respectively. This finding may be attributed to the relatively higher specificity of SAT–EDTA than the RBPT. The SAT–EDTA is particularly more

specific because of the addition of 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.

The least seroprevalence rate recorded with the LFA is indicative of its very high specificity. This is because LFA detects antibodies only due to *B. melitensis*. Due to the high specificity and simplicity and the fact that LFA does not require refrigeration, it is suggested that this assay should be used for serological survey of brucellosis due to *B. melitensis*. Furthermore, the ability of serological tests to reliably detect brucellosis also depends on the presence of detectable antibodies at the time of examination, some infected animals will inevitably elude detection if antibodies are not in circulation during the time of blood collection[16]. Relatively lower seroprevalence rate for *B. melitensis* in this study may be due to the fact that serum antibody was too little, either because the sheep were only recently being infected, or because some individual animals reacted poorly to the antigenic stimulus.

The prevalence of brucellosis in the screened sheep was 2.4%, comparable with the finding of Falade and Shonekan[17], who reported a prevalence of 2.56% in Ibadan, in western Nigeria. Shehu *et al*. reported a higher prevalence 6.6% in a seroprevalence study of brucellosis in small ruminants in Bauchi and environs[18], while Okoh reported a prevalence of 14.5% in Kano[19]. In an earlier study conducted in Northern Nigeria, Bale *et al*. reported a higher prevalence of 14.1%[20]. The difference in the seroprevalence by different workers could be due to sensitivities and specificities of the different serological methods used by these workers.

From the present study, it was found that more female animals were seropositive than the males. This result may be attributed to the fact that more of the female animals were available for sampling. This is because female sheep are kept longer on the farm for breeding purposes, while the males are mostly slaughtered for festivities, ceremonies and are usually exchanged among relatives as loans. Furthermore, rams are difficult to control when kept in large numbers because of their aggressive nature. The higher prevalence may also be as a result of higher susceptibility of females than the males as reported by Junaidu *et al*[6].

The animals within the age bracket of 1–3 years and above had the highest seroprevalence rate. This result is in agreement with those of Aulakh *et al*. and Abubakar *et al*. who reported that the incidence is higher in sexually mature animals[21,22]. The result of the present study is also related to the fact that sex hormones and meso–Erythritol (in male testicles and seminal vesicles) and erythritol in female allantoic fluid stimulate the growth and multiplication of *Brucella* organisms and tend to increase in concentration with age and sexual maturity. Furthermore, in this study more animals within this age bracket were sampled. Since animals within this age range are actively involved in breeding. The presence of brucellosis in them may result in serious economic loss in terms of reproductive wastages like abortion, still birth, infertility, sterility and reduced milk production. Sheep infected with *Brucella* spp. are capable of spreading the disease since they mix among themselves from different flocks. It is also important to note that animals in this age bracket are more often sold out for slaughter and may pose serious risk for human infections. The fact that sheep below 1 year of age were seropositive to brucellosis may be because they might have been exposed through suckling of their infected dams. They may also have become infected through contaminated pasture or water at grazing and watering points, thereby serving as source of spread of the disease to other animals in the future.

The present study has shown that brucellosis exists in sheep in the study area and that female animals are more affected than their male counterparts. Sheep of breeding age are more affected than the very young ones.

In view of the importance of this disease to the livestock industry and its zoonotic importance, it is recommended that government should institute brucellosis control measures and possible eradication strategies.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Comments

Background

Brucellosis is a contagious and serious bacterial disease affecting ruminants such as sheep, causing a lot of complications in them including abortion, reduced milk production and sterility. In addition, this disease can also be transmitted to humans directly or indirectly. Understanding the prevalence of brucellosis in the small ruminants in a place where agriculture, and rearing livestock is a major occupation is very important.

Research frontiers

This study was performed to understand the sero–prevalence of brucellosis in sheep in the North Senatorial District of Kaduna State, Nigeria using various serologic tests.

Related reports

Many related studies have been conducted in various regions of Nigeria (Falade and Shonekan, 1982; Shehu *et al*, 1999; Okoh, 1980).

Innovations & breakthroughs

This study identified that brucellosis exists in sheep in Nigeria. This increases the awareness and highlights the importance of introducing control measures and possible eradication strategies by the government. The authors also compared various serological tests to understand the sero–prevalence of brucellosis in sheep and identified that LFA is superior to other assays due to its specificity.

Applications

LFA can be used for the serological survey of brucellosis caused by *B. melitensis* due to the high specificity and simplicity and no requirement of refrigeration.

Peer review

Brucellosis is a contagious and serious bacterial disease affecting ruminants such as sheep, causing a lot of complications in them including abortion, reduced milk production and sterility. Because of the strong socio–economic and cultural relationships between man and animals in the area of Nigeria under study, this study addresses an important problem that is of high public health significance. This study increases the awareness of people and government to implement control measures to bring down the disease prevalence.

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