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Research Article

Serological Evaluation of Bovine Brucellosis in the North Senatorial District of Kaduna State, Nigeria

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ABSTRACT

A survey of bovine brucellosis was carried out in the North Senatorial District of Kaduna State, Nigeria. Four Local Government Areas (LGAs) were selected from the senatorial District at random. These LGAs were Ikara, Kubau, Makarfi and Sabon-Gari LGAs. Districts were sampled randomly within LGAs and pastoralists cattle were used for the study. Serum samples obtained from 500 animals, comprising 141 males and 359 females from pastoralist herds were used for the study. Serological tests used were the Rose Bengal Plate Test (RBPT) and Lateral Flow Assay (LFA). A seroprevalence rate of 2.8% was obtained with both the RBPT and LFA. Female cattle were more seropositive (2.4%) than males (0.4%). There was a statistically significant difference (P=0.0154) in the prevalence rates between male and female animals using the LFA. There was, however, no statistically significant difference (P>0.05) in prevalence rate between males and females using the RBPT. Adult cattle (> 5 years) were more seropositive (1.6%) than younger ones (1-5 years and < 1 year of age) with a prevalence rate of 0.8% and 0.4% respectively. The seroprevalence status and poor attitude of farmers as regards bovine brucellosis in the current study calls for stringent measures by the Government and other stakeholders to prevent the spread of brucellosis in animals and even humans.

Key words: Seroprevalence, Brucellosis, Cattle, RBPT, Lateral Flow Assay

INTRODUCTION

Cattle are the most prominent of all domesticated animals in Nigeria (Tewe, 1997). There are many breeds of cattle indigenous to Nigeria and they include Red Bororo, White Fulani, Kuri, Sokoto Gudali, Muturu, Keteku, and Ndama (Adekunle *et al.*, 2002). Brucellosis is a highly contagious, zoonotic, and economically important bacterial disease of animals worldwide (OIE; 2000a). The disease is also called contagious abortion, infectious abortion, and epizootic abortion (Megid *et al.*, 2010). Bovine brucellosis caused by *Brucella abortus* has a worldwide distribution in which cattle, buffaloes, and camels serve as the major reservoirs of the disease (Anon, 2011). It is one of the most important infectious diseases causing reproductive disorders in domestic animals. Brucellosis is an important disease among livestock and humans in sub-Saharan Africa (Radostits *et al*., 2006). The disease in cattle is prevalent and widespread and is caused primarily by *B. abortus*, *B. melitensis* and *B. suis*. *Brucella melitensis* infection is common with a high prevalence in sheep and goats. All domestic animal species are susceptible to brucellosis except cats which are resistant to the infection (Gul and Khan, 2007).

The free movement of animals practiced by the nomadic Fulani herdsmen, who own about 95% of all food animal populations in Nigeria, contributes to the spread of the disease (Ocholi *et al.*, 2004b). Other factors include management system employed in raising animals (Atsanda and Agbede, 2001), especially the herding of different animal species together (Junaidu *et al.*, 2008), grazing animals on the same pastures and the use of the same water sources (Bertu *et al.*, 2010). The age of the animal appears to be a factor in susceptibility or transmission (Cadmus *et al.*, 2010; Junaidu *et al.*, 2011), as well as sex, status of lactation and also, breed (Cadmus *et al.*, 2010), and season (Bertu *et al.*, 2010). Kaltungo (2012) also reported the role of improper disposal of aborted fetuses, addition of newly acquired animals without quarantine, sharing of sires, and mixing of animals during annual vaccination campaigns as some of the factors that predispose to infection with brucellosis in

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small ruminants. The consequences of brucellosis in infected animals are numerous ranging from abortion, premature births, retention of placenta, orchitis, epididymitis (WHO, 2006) while in man, weakness, muscle and joint pain, headache, undulant fever, hepatomegaly, splenomegaly, and other symptoms are observed (WHO, 2006).

The major occupation of the people of Kaduna State is agriculture, producing food and cash crops and rearing of livestock (KDSG, 2008). Cattle provide the major source of animal protein both in the form of meat and milk, and are used by rural farmers for traction in view of the increasing difficulty in sourcing tractors for tilling farms.

The people involved in this study are predominantly peasant farmers and pastoralists with very low levels of educational background and certain livestock practices in the area likely to create increased chances of exposure to brucellosis in both animals and man.

Diagnosis of brucellosis has been based mainly on serological tests which include Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), and Serum Agglutination Test (SAT) which are not without limitations such as having false positive or negative results (Poiester *et al.*, 2010).

Prevalence of bovine brucellosis varies widely across Nigeria, and between herds in the same area (Mai *et al.*, 2012). This study was designed to use RBPT, as well as the Lateral Flow Assay (LFA) in determining the seroprevalence of bovine brucellosis in the study area which was intended to give an insight into the current status of the disease in the North Senatorial District of Kaduna State.

MATERIALS AND METHODS

Study area

This study was conducted in the North Senatorial District of Kaduna State. The District comprises 8 Local Government Areas (LGAs) and is located in the Northwest geo-political zone of Nigeria. It is situated between latitudes 6^0 and 11^0 North and longitude 7^0 and 44^0 East and is 1995ft above sea level (KDSG, 2008). It has distinct wet and dry seasons within the Guinea Savannah and part of the Sahel Savannah zones 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 sq. km, with a human population of over 6,066,562 people according to the 2006 census figures (KDSG, 2008). Daily temperatures range from 14-30^0C with a relative humidity of 12-72%. The rainy season is usually from April through November, with greater variation in the northern part. The annual rainfall varies, decreasing from an average of about 1530 mm in the Southeast to about 1015 mm in the Northeast (Oyedipe *et al.*, 1982; Mai, 1997).

Four LGAs out of the 8 that constitute the North Senatorial District were selected using simple random sampling without replacement. The selected LGAs were Ikara, Kubau, Makarfi, and Sabon-Gari LGAs (Fig 1). Furthermore, three to four districts were selected from each of the selected LGAs using the same sampling method after determining the number of districts in each LGA. From each of the districts selected, at least two herds were chosen from which blood samples from cattle were collected using convenience sampling and farmers' willingness. A minimum of 80 samples were taken per LGA.

Sample size

The sample size was determined using the formula below as described by Thrusfield (2005).

$$n = z^2 pq / d^2$$

Where:
n- Minimum sample size
z- Appropriate value for the standard normal deviation set at 95% confidence interval (1.96).
p- Prevalence of 18.3% obtained in migratory Fulani cattle in Kaduna State (Mbuk *et al.*, 2011).
q- Complementary probability, i.e. 1-p
d- Level of significance at 5%

$$n = (1.96)^2 \times 0.183 \times (1-0.183) / (0.05)^2$$
$$n = 3.84 \times 0.183 \times 0.817 / 0.0025$$
$$n = 229.64$$

The sample size was 230 using this formula, but was increased to 500 for better precision, and a minimum of 80 animals were sampled per LGA.

Study animals

Cattle from pastoralist herds were used for the study. The sampled herds were selected using convenience sampling and based on farmers' willingness. Furthermore, individual animals were randomly sampled within the selected herds.

Sample collection

Blood samples were collected aseptically from each selected animal via jugular venipuncture following proper restraint by an assistant. The age, breed, sex and location of the herd for each of the study animals were recorded. Five millilitres of blood were collected from each animal using a 10 ml syringe and 21 G needle. The collected blood was gently transferred into clean plain sample bottles and labeled accordingly. The samples were transported to the laboratory over ice and were centrifuged at 3,000rpm for 10 minutes for proper separation of serum from the clotted blood. The serum was decanted into 5 ml plastic tubes and labeled appropriately. The samples were stored in the freezer at -20^0C until used (Bertu *et al.*, 2010).

Laboratory examination

Serological tests

Rose Bengal Plate Test

The procedure as described by Bale (2008) was used which involved placing 0.03 ml of the RBPT antigen on each square of white tile and 0.03 ml of the test serum sample alongside (but not onto) the antigen. The antigen and serum were then thoroughly mixed with a sterile applicator stick and the mixture rocked for four minutes.

The test was then read by examining for agglutination. A separate sterile applicator stick was used for each serum sample. The result was interpreted as (a) positive (+ve) for any degree of agglutination and (b) negative (-ve) for no agglutination.

Lateral flow assay

The LFA test was carried out according to the instructions of the manufacturers (Quicking Biotech; Republic of China). The test result was read after 10 minutes by visual inspection for staining of the test and control lines in the test zone of the assay device. The assay was scored negative when no staining of the test line was observed and positive when distinct staining of the test line was observed.

The RBPT antigen was sourced from Onderstepoort Veterinary Institute, Republic of South Africa.

RESULTS

Serology results

Distribution of cattle and their seroprevalence rates for brucellosis in the study area

A total of 500 cattle were sampled from the four LGAs of the North Senatorial District of Kaduna State. Out of these, 177 (35.4%) were from Makarfi, while 130 (26.0%), 104 (20.8%), and 89 (17.8%) were from Kubau, Ikara, and Sabon-Gari LGAs, respectively (Table 1).

A total of 14 (2.8%) of the 500 animals were seropositive for both the RBPT and LFA respectively (Table 2). Nine (1.8%) animals from Ikara LGA were seropositive for *Brucella* antibodies based on the RBPT while 2 (0.4%) each from Kubau and Makarfi, and one (0.2%) from Sabon-Gari were seropositive. Of all the animals tested using the LFA, 6 (1.2%) from Ikara and 2 (0.4%) each from Makarfi and Kubau, and 4 (0.8%) from Sabon-Gari were seropositive for *Brucella* antibodies.

With respect to seropositivity based on districts within Ikara LGA, 1 (0.2%) animal from Ikara District, 4 (0.8%) each from Saulawa and Furana Districts were positive while none from Kurmin-Kogi District was positive by RBPT test. Similarly, 2 (0.4%) of the animals from Ikara District were seropositive using LFA while 1 (0.2%) each from Kurmin Kogi and Furana Districts and 2 (0.4%) from Saulawa District were positive for brucellosis using the LFA. With respect to cattle sampled from Kubau LGA, 1 (0.2%) each from Anchau and Damau Districts were seropositive using both RBPT and LFA tests.

The results for the tests from Makarfi LGA showed that 1 (0.2%) animal each from Gazara and Gimi Districts was seropositive using both RBPT and LFA while none of the animals from Makarfi District was positive using the two tests. Also, using the RBPT, only 1 (0.2%) sample tested positive from Hanwa district while none from the other districts of Basawa and Bomo tested positive. Using the LFA test, 2 (0.4%) samples from Hanwa tested positive while 1 (0.2%) each from Bomo and Basawa districts tested positive.

Based on the RBPT, cattle sampled from Saulawa and Furana Districts in Ikara LGA had the highest seroprevalence of 0.8%. However, with respect to the LFA test, the highest prevalence of 0.4% occurred in animals from Hanwa District in Sabon-Gari LGA.

Table 1: Detection rate of *Brucella* antibodies in cattle using RBPT and LFA in 4 Local Government Areas in the North Senatorial District of Kaduna State, Nigeria

LGA	No. of samples tested	No. +ve According to Test Type (%)	
		RBPT	LFA
Ikara	104	9 (1.8)	6 (1.2)
Kubau	130	2 (0.4)	2 (0.4)
Makarfi	177	2 (0.4)	2 (0.4)
Sabon-Gari	89	1 (0.2)	4 (0.8)
Total	500	14 (2.8)	14 (2.8)

KEY: RBPT = Rose Bengal Plate Test; LFA = Lateral Flow Assay.

Table 2: Distribution of sampled cattle according to sex in 4 Local Government Areas in the North Senatorial District of Kaduna State

LGA	No. of sampled animals	Sex	
		Male (%)	Female (%)
Ikara	104	33 (6.6)	71 (14.2)
Kubau	130	40 (8.0)	90 (18.0)
Makarfi	177	53 (10.6)	124 (24.8)
Sabon-Gari	89	15 (3.0)	74 (14.8)
Total	500	141 (28.2)	359 (71.8)

Table 3: Seroprevalence of bovine brucellosis from sampled cattle in 4 Local Government Areas from the North Senatorial District of Kaduna State according to sex

LGA	No. of Males +ve			No. of Females +ve		
	NS	RBPT (%)	LFA (%)	NS	RBPT (%)	LFA (%)
Ikara	33	1 (0.2)	2 (0.4)	71	8 (1.6)	4 (0.8)
Kubau	40	0 (0)	0 (0)	90	2 (0.4)	2 (0.4)
Makarfi	53	0 (0)	0 (0)	124	2 (0.4)	2 (0.4)
Sabon-Gari	15	0 (0)	0 (0)	74	1 (0.2)	4 (0.8)
Total	141	1 (0.2)[a]	2 (0.4)	359	13 (2.6)[b]	12 (2.4)

KEY: RBPT = Rose Bengal Plate Test; LFA = Lateral Flow Assay; LGA = Local Government Area; +ve = Positive test response; NS= Number sampled; a, b shows significant difference.

Using the RBPT, none of the animals from Kurmin-Kogi District in Ikara LGA, or Dutsen-Wai District in Kubau LGA, or Makarfi District in Makarfi LGA, as well as in Basawa and Bomo Districts in Sabon-Gari LGA was positive. Likewise, with respect to the LFA, none of the animals from Dutsen-Wai District in Kubau LGA and Makarfi District in Makarfi LGA was positive.

Contrarily, all the 4 districts sampled in Ikara LGA tested positive using the LFA with 0.2% seroprevalence in cattle from Ikara District and 0.4% in Saulawa District. Only cattle sampled from Dutsen-Wai and Makarfi Districts in Kubau and Makarfi LGAs, respectively, had negative seroprevalence serological responses using both the RBPT and LFA tests.

Seroprevalence of brucellosis in cattle based on sex

The distribution of cattle sampled according to sex and their associated seroprevalence rates for brucellosis using the RBPT and LFA tests are presented in Tables 2 and 3. The results showed that a total of 141 male and 359 female cattle were sampled.

Of the 141 males sampled, only 1 (0.2%) was seropositive using the RBPT while 2 (0.4%) were positive using the LFA (Table 3). Furthermore, of the 359 females sampled, 13 (2.6%) and 12 (2.4%) were seropositive using the RBPT and LFA, respectively.

Table 4: Seroprevalence of bovine brucellosis by age in 4 Local Government Areas of the North Senatorial District of Kaduna State

	Age < 1 year			Age 1-5 years			Age > 5 years		
LGA	NS	RBPT (%)	LFA (%)	NS	RBPT (%)	LFA (%)	NS	RBPT (%)	LFA
Ikara	26	0 (0)	2 (0.4)	21	4(0.8)	1 (0.2)	57	5 (1.0)	3 (0.6)
Kubau	29	0 (0)	0 (0)	37	1 (0.2)	0 (0)	64	1 (0.2)	2 (0.4)
Makarfi	23	0 (0)	0 (0)	99	0(0)	1 (0.2)	55	2 (0.4)	1 (0.2)
Sabon-Gari	15	0 (0)	0 (0)	46	1 (0.2)	2 (0.4)	28	0 (0)	2 (0.4)
Total	93	0 (0)	2 (0.4)	203	6 (1.2)	4 (0.8)	204	8 (1.6)	8 (1.6)

KEY: RBPT = Rose Bengal Plate Test; LFA = Lateral Flow Assay; NS = Number sampled within the age group

All the seropositive males, using either tests, were from Ikara LGA. Of the seropositive females using RBPT and LFA, 8 (1.6%) and 4 (0.8%) were respectively from Ikara LGA, 2 (0.4%) and 2 (0.4%) were respectively from Kubau LGA, and also 2 (0.4%) and 2 (0.4%) were respectively from Makarfi LGA. However, of the seropositive females using the two tests, 1 (0.2%) and 4 (0.8%) were respectively from Sabon-Gari LGA.

There was a statistically significant difference (P= 0.0154) in the prevalence rates of brucellosis between the male and female animals using the LFA. There was, however, no statistically significant difference (P=0.1134) in the prevalence rates of brucellosis between the male and female animals using the RBPT.

Seroprevalence of bovine brucellosis by age

Of the 500 cattle sampled from all the four LGAs, 93 were aged less than 1 year old and none of them tested positive for brucellosis using RBPT. However, 2 (0.4%) cattle within this age group from Ikara LGA were positive using LFA (Table 4). Similarly, 203 cattle were within the age group of 1 to 5 years old out of which 6 (1.2%) and 4 (0.8%) were seropositive using RBPT and LFA**,** respectively. Out of the six positive by RBPT, 4 (0.8%) were from Ikara and 1 (0.2%) each from Kubau and Sabon-Gari LGAs**,** respectively. Of the four animals within this age group that were positive using LFA, 1 (0.2%) each was from Ikara and Kubau while 2 (0.4%) were from Sabon-Gari LGA. Of the 204 cattle that were over 5 years old, 8 (1.6%) were positive using RBPT with 5 (1.0%), 1 (0.2%), and 2 (0.4%) coming from Ikara, Kubau, and Makarfi LGAs, respectively**.** Also, 8 (1.6%) were positive using LFA with 3 (0.6%), 2 (0.4%), 1 (0.1%) and 2 (0.4%) coming from Ikara, Kubau, Makarfi and Sabon-Gari LGAs, respectively (Table 4).

There was no statistically significant difference (P= 0.1004) in prevalence rates of brucellosis between the three different age groups evaluated.

DISCUSSION

This study showed an overall sero-prevalence of 2.8% for bovine brucellosis was obtained by both RBPT and LFA. This is lower than a previous report by Mbuk *et al.* (2011) who recorded a prevalence of 7.1% in a study on brucellosis in migratory Fulani cattle in Kaduna State. The difference in prevalence between the one obtained in this study and that by Mbuk *et al.* in 2011 could perhaps be due to the fact that the animals in this study were more sedentary while those by Mbuk and co-workers were migratory and had higher risk of coming down with the disease by way of contact with contaminated pastures and water and other infected animals.

The occurrence of bovine brucellosis in the study area can be a source of worry as the animals are under extensive and sedentary system and the fact that in pastoralist settings, cattle are usually herded with sheep and goats (Shehu *et al.,* 1999a). As reported in another study on brucellosis in small ruminants much earlier by Shehu *et al.* (1999b) and of recent by Kaltungo (2012) whereby a variety of domestic animals were kept together. It is also noteworthy of the fact that the Fulani pastoralists have a disease reducing management practice which includes their rapid disposal of aborting animals and those with poor fertility and reduced milk yields (Racloz *et al*., 2013). This attitude may be the reason for a reduced seroprevalence in this study suggesting that a higher seroprevalence may be obtained under other management practices. The occurrence of brucellosis in these animals could have a lot of public health significance in view of the social and cultural settings of the pastoralists along with those of the communities they live closely with as reported by Shehu *et al.* (1999b). Furthermore, chickens have been reported by Gugong *et al.* (2012) to be infected with *Brucella* spp while Baek *et al*. (2003) reported such infections in dogs.

This study has also shown that female cattle had higher brucellosis prevalence which could probably be attributed to the fact that more female animals live longer as they are kept for production while the males are usually sold for family sustenance and also as a means of curtailing infighting among bulls particularly when cows are on heat. This agrees with the reports of Kaltungo *et al.,* (2013) and Zubairu *et al.* (2014) who both reported a higher seroprevalence in female animals than in males. The presence of a sugar alcohol, Erythritol in the uterus of female animals is a contributing factor to the high prevalence in females than in males as Brucella has a high affinity for this sugar (Radostits *et al*., 2006).

Though not statistically significant, higher seroprevalence was obtained in older cattle (> 5yrs) than younger ones. This agrees with the reports of Zubairu (2014) who reported higher prevalence in adult animals compared to younger ones.

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