

Serological survey for *Brucella* antibodies in donkeys of north-eastern Nigeria

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Abstract A cross-sectional epidemiological study was conducted to determine seroprevalence and risk factors influencing the presence of *Brucella* antibodies in donkeys of Borno State, north-eastern Nigeria. The study aimed at providing baseline information that may be used in planning a control policy against equine brucellosis. Blood samples were collected from 601 donkeys, comprised of 374 males and 227 females from the six agricultural zones of the state between March 2013 and September 2014. The sera obtained were tested for *Brucella* antibodies using Rose Bengal plate test (RBPT) and competitive enzyme-linked immunosorbent assay (cELISA). Of the 601 donkeys tested, 43 (7.2%) and 40 (6.7%) were seropositive by RBPT and cELISA, respectively. A seroprevalence of 8.6% was obtained in male and 3.5% in female donkeys. According to age, the highest seroprevalence of 9.6% was obtained from donkeys of age group 4–6 years. With respect to pregnancy status, a higher seroprevalence (6.8%) was obtained from pregnant donkeys compared to 3.8% obtained from the non-pregnant ones. There were statistically significant associations between the presence of antibodies and sex ($p < 0.05$) and the presence of antibodies and age ($p < 0.05$) of the studied donkeys. However, no statistically

significant association ($p > 0.05$) was observed between the pregnancy status and presence of antibodies. The study concludes that *Brucella* infection is present in donkeys in all the agricultural zones of the state. The relatively high seroprevalence (7.2%) obtained is of public health concern because of the close interaction between donkeys, ruminants, and humans in the study area.

Keywords Equine · Donkeys · Brucellosis · Borno · Seroprevalence

Introduction

Brucellosis is an infectious disease caused by various gram-negative bacteria of the genus *Brucella* (Acha and Szyfres, 2001). This disease is the cause of significant economic losses in livestock production due to reproductive disorders and reduced production of affected animals (Santellano-Estrada et al., 2004). Brucellosis is a zoonotic disease affecting humans and various species of domesticated and feral animals. High-risk groups include those exposed through occupation in contexts where animal infection occurs, such as slaughterhouse workers, hunters, farmers, and veterinarians (FAO, 2003; FAO et al., 2006). The presence of reservoir animals contributes to the perpetuation of brucellosis. In the extreme northern parts of Nigeria especially the rural areas of the Sahel savannah, donkeys play significant roles in transportation of farm produce, persons, and water and are used to shepherd small ruminants. Cross transmission of brucellosis can occur between cattle, swine, sheep and goats, and other species including dogs, donkeys, horses, feral swine, bison, reindeer, and camels (Than, 2007). In Nigeria, studies on animal brucellosis have been carried out in cattle, sheep, and goats (Tijjani et al., 2012). However, a small number of

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surveys have been carried out to determine the epidemiologic role of species other than domesticated ruminants and such studies have involved camels (Adamu et al., 2014b) poultry (Gugong et al., 2012; Adamu et al., 2014a) and dogs (Cadmus et al., 2011). Some reports on equines have concerned clinical cases (Ocholi et al., 2004). To the best of our knowledge, only three studies available in the literature that were carried out to determine epidemiologic patterns of brucellosis in equines in some isolated parts of northern Nigeria. These include study on some horse stables in Kaduna and Jos (Ehizibolo et al., 2011), some stables in Maiduguri metropolis (Tijjani et al., 2012) and a study on donkeys in three Local government areas each of Borno and Yobe States (Sadiq et al., 2013). Therefore, the objective of this study was to determine the seroprevalence and likely risk factors influencing the presence of *Brucella* antibodies in donkeys in all the agricultural zones of Borno State of the north-eastern region of Nigeria. This may provide baseline information that may be used in planning a control policy against equine brucellosis in the study area.

Materials and methods

Study area

The study was conducted in Borno State, in the north-eastern part of Nigeria between the months March 2013 and September 2014. The state lies between latitudes 100 02' N and 130 04' N and longitude 110 04' E and 140 04' E and covers an area of 69,436 km². The state has 27 local government areas (LGAs), divided into six agricultural zones (Fig. 1). The zones were distributed between the two vegetational zones of Sahel savannah in the northern and Sudan savannah in the southern parts of the state.

Study design

A cross-sectional epidemiological study was designed to investigate the epidemiological patterns of *Brucella* infection and possible risk factors influencing the presence of *Brucella* antibodies in the donkey population in the north-eastern region of Nigeria. The study was carried out between March 2013 and September 2014 following anecdotal reports of increased frequencies of brucellosis infection among donkeys.

Sample size estimation and sampling

An individual animal was considered to be the unit for analysis in this study. The sampling frame for the study comprised all the donkey population in the six agricultural zones in the state. The sample size of the cross-sectional study was calculated using OpenEpi© version 2.3 (OpenEpi, Atlanta, GA,

USA). The sample size was determined using of hypothetical proportion of 50% since an earlier study on this specie in the state or in any other state in northern Nigeria was not available. Power of 80% was assumed and alpha (α) level was considered to be 0.05. The hypothetical proportions of exposure among the donkey population was deduced from unpublished reports and interactions with the staff of the state veterinary service involved in animal disease monitoring in the study area over the years. A minimum sample size of 348 was calculated using Fleiss Method with continuity correction. All animals that were 1 year and above were sampled. The samples were drawn from all zones based on the distribution and population of donkeys available in the Local Government Areas in the zone. To increase precision of the estimate of the seroprevalence and power of the study, a total 601 donkey blood samples comprising of 374 males and 227 females was randomly collected from all the agricultural zones. Approximately 10 ml of blood samples was collected aseptically from the jugular vein of each animal in all selected LGAs using syringe and needles following proper restraint. Each sample was labeled with unique identification number and information about, sex, age, breeds, and location were recorded for data analysis. The samples were transported in ice packed coolers to the laboratory. The tubes containing blood samples were set tilted on a table over night at a room temperature to allow clotting. Next morning, the clotted blood samples in the tubes were centrifuged (at 3000g for 5 min) to obtain clear sera. The sera were stored at -20 °C until tested.

Tests for brucellosis

The sera obtained from the collected blood samples were subjected to Rose Bengal plate test (RBPT) and competitive enzyme-linked immunosorbent assay (cELISA) tests. The RBPT antigen and COMPLISA kit were procured from Animal Health and Veterinary Laboratory agency (AHVLA), UK. The tests were performed according to the manufacturer's instructions.

Data analysis

The specific seroprevalence was calculated as the number of animals tested positive by the RBPT/cELISA divided by the number of animals tested in each categorical variables (sex, age, and pregnancy status). Data generated were analyzed using GraphPad InStat 3 Statistical Package (Graphpad Software Inc., California, USA). Chi-squared test was employed to test for association between the presence of *Brucella* antibodies and the variables sex, age, and pregnancy status. For all the analyses, a *p* value less than 0.05 was considered to be significant.

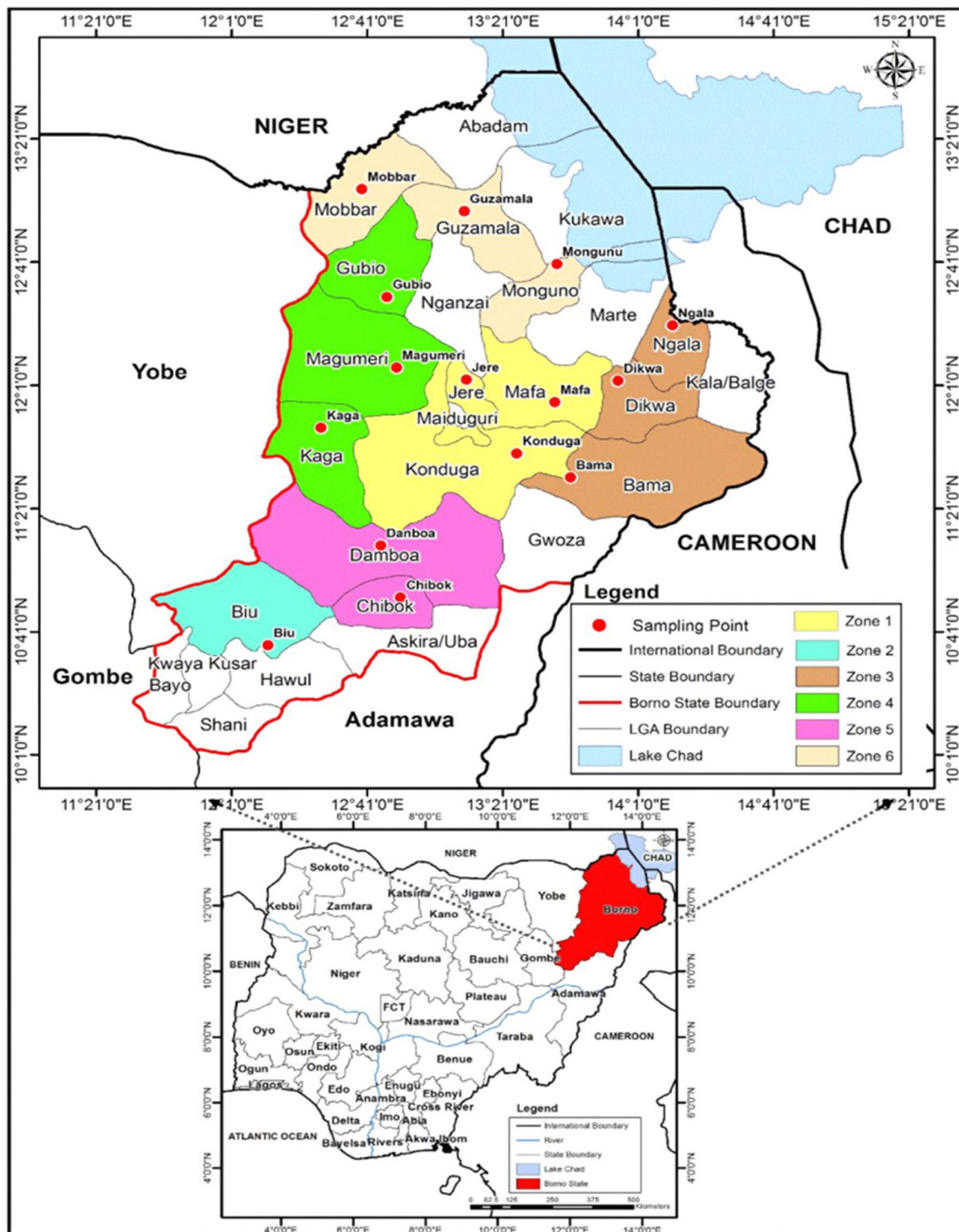


Fig. 1 Map of Borno State showing local government areas and sampling sites

Results

Of the 601 donkeys tested in Borno State, 43 (7.2%) and 40 (6.7%) were seropositive to *Brucella* infection by RBPT and cELISA, respectively. Out of the tested 374 males, 33 (8.8%) and 32 (8.6%) were seropositive by RBPT and cELISA, respectively. Also of the 227 females, 10 (4.4) and 8 (3.5%)

were seropositive by RBPT and cELISA, respectively. Using the cELISA results, there was a significant statistical association between the sex of donkeys studied and the presence of *Brucella* antibodies ($p < 0.05$) as shown in Table 1.

The age group 4–6 years showed the highest age-specific seroprevalence by both RBPT (9.6%) and cELISA (8.8%). This was followed by age group 1–3 years (7.4 and 6.9%)

Table 1 Sex-specific seroprevalence of brucellosis in donkeys in Borno State using on RBPT and cELISA

Sex	No. tested	RBPT + ve (%)	cELISA + ve (%)	95% CI	<i>p</i> value
Male	374	33 (8.8)	32 (8.6)	5.8–11.4	0.02
Female	227	10 (4.4)	8 (3.5)	1.1–5.9	
Total	601	43 (7.2)	40 (6.7)	4.7–8.7	

No. number of animals, +ve positive, CI confidence intervals on cELISA

and the least (2.1%) by both tests was observed in the age group 7–9 years. There was significant statistical association between the age group of the donkeys and positive serological reaction ($p > 0.05$) as shown in Table 2.

The distribution of *Brucella* antibodies based on pregnancy status in donkeys in the state is presented in Table 3. Out of the 227 female donkeys tested, 7 (3.8%) of the 183 non-pregnant and 3 (6.8%) of the 44 pregnant donkeys were seropositive by RBPT. Also 5 (2.7%) of the non-pregnant and 3 (6.8%) of the pregnant donkeys were seropositive by cELISA. There was no statistically significant association between the pregnancy status and presence of *Brucella* antibodies ($p > 0.05$).

Discussions

Despite the extensive studies of brucellosis in cattle and small ruminants in different parts of Nigeria, only few studies have been conducted on prevalence of brucellosis in equine in the country. The previous studies of seroprevalence of brucellosis using RBPT and SAT in horses have been reported to be between 6.7 and 22.7% in some parts of northern Nigeria (Ehizibolo et al., 2011) and in donkeys was 5.0 and 6.0% in three local government areas each of Borno and Yobe States, respectively (Sadiq et al., 2013).

The seroprevalence of 7.2 and 6.7% of *Brucella* infection obtained in donkeys by RBPT and cELISA respectively in this study is relatively high and of public health concerns because of the close association between donkeys, other livestock and humans. The seroprevalence is higher than the 6.0 and 5.0% obtained in Borno and Yobe States respectively in a study on donkey brucellosis (Sadiq et al., 2013). It is also higher than 0% in Mossoro, Brazil (Dorneles et al., 2013); 3.6% in Darfur, western Sudan (Musa, 2004) and 6.05% in south east Turkey (Tel et al. 2011). The seroprevalence is comparable with 7.3% in Egypt (Gul and Khan, 2007) but lower than 8.5% in Jordan

(Abo-Shehada, 2009) and 36.3% in Peshawar, Pakistan (Safirullah et al., 2014).

The seroprevalence of brucellosis obtained in this study was significantly higher in male than female donkeys. The reason responsible for differences in prevalence of brucellosis infection according to sex is controversial as some researchers have reported that the presence of *Brucella* antibodies were not associated with sex (Wadood et al., 2009; Gul et al., 2013; Badieli et al., 2013) whereas other reports have shown significantly higher prevalence in females than males (Hussein et al., 2005; Sadiq et al., 2008; Junaidu et al., 2011). The significantly higher prevalence obtained among the males compared to females in this study is similar to what was observed by Yohannes et al. (2013) who reported higher proportion of reactors in males (4%) compared to females (1.62%) in an extensive cattle production system in Ethiopia. The basis for this comparison of effect sex on prevalence observed in this study with what was earlier observed in extensive cattle production is the fact that donkeys are often reared together with cattle and are usually exposed to similar risks of infection. Similarly, Bokaie et al. (2009), Chimana et al. (2010), and Mai et al. (2012) reported higher prevalence in bulls compared to cows. However, the earlier few reports on prevalence according to sex of the equine species indicated no significant statistical association between the sex and *Brucella* infection (Sadiq et al., 2013; Safirullah et al., 2014).

In this study, the highest seroprevalence of 8.8% by cELISA was obtained in the age group 4–6 years and the least 2.1% among the age group 7–9 years. This apparent decreased proportion of seropositive animals with increasing age could be due to the fact that some older animals may not exhibit detectable antibody titres possibly due to latency which is common in chronic brucellosis as observed by Matope et al. (2011).

Although apparently higher seroprevalence was obtained among pregnant female donkeys compared to the non-

Table 2 Age-specific seroprevalence of brucellosis in donkeys based on RBPT and cELISA

Age (years)	No. tested	RBPT + ve (%)	cELISA + ve (%)	95% CI	<i>p</i> value
1–3	188	14 (7.4)	13 (6.9)	3.3–10.5	0.04
4–6	272	26 (9.6)	24 (8.8)	5.4–10.2	
7–9	141	3 (2.1)	3 (2.1)	0.3–4.3	
Total	601	43 (7.2)	40 (6.7)	4.7–8.7	

No. number, +ve positive, CI confidence intervals on cELISA

Table 3 Pregnancy status-specific seroprevalence of brucellosis in donkeys in Borno State, based on RBPT and cELISA

Status	No. tested	RBPT +ve (%)	cELISA +ve (%)	95% CI	p value
Non-pregnant	183	7 (3.9)	5 (2.8)	0.5–5.2	0.18
Pregnant	44	3 (6.8)	3 (6.8)	0.6–14.2	
Total	227	10 (4.4)	8 (3.5)	1.1–5.9	

pregnant ones, there was no statistically significant association between pregnancy status and positive serological reactions. This is consistent with observation of Safirullah et al. (2014) who recorded higher seropositivity in female, matured, pluriparous, pregnant, abortive, and lactating mares and female donkeys in a study on epidemiological study of brucellosis in equine species in Pakistan. Walker (1999) and Mekonnen et al. (2011) also reported that sexually matured and pregnant animals are more prone to *Brucella* infection and brucellosis than sexually immature animals of either sex. In contrast to the findings of this study, Ibrahim et al. (2010) and Mai et al. (2012) reported higher prevalence among non-pregnant compared to the pregnant animals. It has been observed that pregnant cattle above 5 months and mares in mid to last 3 months of gestation are more susceptible to *Brucella* infection due to the preferential localization of *Brucella* in the uterus in which allantoic fluid factors such as erythritol stimulate the growth of *Brucella* (Godfroid et al., 2004).

The study concludes that *Brucella* infection is present in donkeys in all the agricultural zones of the state. The relatively high seroprevalence (7.2%) obtained is of public health concern because of the close interaction between donkeys, ruminants, and humans in the study area.

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Compliance with ethical standards

Ethical clearance The experiment was carried out according to the care and use of experimental animals' protocol and was approved by the Faculty of Veterinary Medicine Ethics and Research Committee.

Conflict of interest The authors declare that they have no conflict of interest.

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