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Seroprevalence of Brucella antibodies in Donkeys (*Equus asinus*) in Yobe south senatorial zone, Northeastern Nigeria

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A cross-sectional survey was conducted to determine the seroprevalence and risk factors influencing the presence of Brucella spp. antibodies in donkeys in Yobe south senatorial zone, Nigeria. The study was aimed at determining the importance of Brucella spp. infection in donkeys (Equus asinus). A total of 200 sera samples from of 105 males and 95 female donkeys were collected and screened for brucellosis using the rose bengal plate test (RBPT) and the indirect enzyme-linked immunosorbent assay (iELISA). Data obtained were analyzed to determine associations and risk factors. The analysis revealed that 21.5% and 18.5% were seropositive by RBPT and iELISA respectively, with 22.0% and 20.0% of the male and female donkeys being seropositive by RBPT, and 19.0% and 17.9% of the male and female donkeys being seropositive by iELISA, respectively. There was a statistically significant association between donkey age and positive rate of iELISA for detecting Brucella infection. Though the positive rate was higher for males than females, there was no statistically significant association between sex and location of donkeys and the sensitivities of RBPT and iELISA for detecting Brucella infection. In conclusion, this study indicates that brucellosis exists with high seroprevalence particularly among male and adult donkeys and is of public health significance and economic importance because it can lead to infertility and abortion in the stock.

Key words: *Brucella antibodies, iELISA, rose bengal plat test, seroprevalence*

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Brucellosis is a global bacterial zoonotic disease recognized as a major cause of significant economic losses in livestock due to its primary effect on the reproductive system in affected animals with a concomitant reduction in production and also poses a serious threat to human health [10]. The disease is highly contagious zoonotic disease of ruminants, canines, swine and equines [16]. It is caused by *Brucella* species, with *B. abortus* and *B. melitensis* causing infection in donkeys [23]. This disease causes significant economic losses for farmers and livestock industries due to reproductive disorders and decreased production of affected animals [24]. The disease affects high-risk groups including those exposed through occupation in contexts where animal infection occurs, such as abattoir workers, hunters, veterinarians and livestock farmers [12, 13]. Brucellosis is characterized by frequent abortion and infertility in a variety of animal species and undulating fever in humans [16]. It is an endemic disease in Nigeria, with serological studies in various parts of the country indicating the existence of the disease virtually in all domestic animals and humans [17]. Northeastern Nigeria has a large population of donkeys and these donkeys provide significant socioeconomic benefits to pastoral communities. However, despite their prominent role in rural agricultural systems, donkeys have been subjected to poor management, lack of knowledge, lack of health care and negative attitudes

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from the community [15]. Despite these factors, they have continued to be used as pack animals and to pull water from wells [7]. The majority of donkeys in the world (perhaps over 95%) are kept specifically for labor. Their greatest role is for transport that is, for riding, pack transport or pulling carts, and they may also be used for farm tillage. In certain countries they may assist in threshing, raising water milling or other operations. Donkeys are not conventional sources of meat; although they may be used for meat in the eastern part of the country, the primary function of donkeys in Nigeria has traditionally been as pack animals [7]. In spite of the endemic nature of brucellosis, the occurrence and prevalence are difficult to determine due to uncoordinated reporting and undedicated government effort with respect to consistent epidemiological investigation of the disease in livestock population in Nigerian [17]. In the northern parts of Nigeria particularly the rural areas of the Sahel savannah, donkeys play important roles in carrying farm produce, people, and water and are used to shepherd small ruminants. Transmission of brucellosis can occur between ruminants, swine, equines, canines and camels. In Nigeria, several serological studies have shown that brucellosis is widespread in livestock, equines and humans [3, 4, 6, 25]. Equines may be a reservoir of brucellosis and also play an important role in the epidemiologic patterns of this disease [2]. The objective of this study was to determine the prevalence of brucellosis and the risk factors influencing the presence of *Brucella* spp. antibodies in donkey in Yobe south senatorial zone, Yobe State, Nigeria. This may perhaps provide baseline information that may be used in designing control and prevention policies for brucellosis in Yobe State and the country at large.

Materials and Methods

Study area

The study was carried out in four local government areas of the Yobe State south senatorial zone namely Fika, Fune, Nangere and Potiskum (Fig. 1). Yobe State is located in the arid-zone of the north-eastern part of Nigeria, within latitude 10° 30' N and longitude 13° 10' E with a total area of 47,153 km^2. The state has a population of 2,321,591 [21]. The arid zone has rather austere climatic conditions with a dry season from late November to early May and an average daily peak temperature especially in April and May of 34.4–37.8°C. Yobe State shares an international border with the Republic of Niger, which enhances transborder movement of livestock between the two countries. The state is one of the leading livestock producers in Nigeria [8].

Sample collection

A 10 m*l* of blood sample was collected aseptically from the jugular vein of each donkey into plain vacutainer tubes. Each sample was labeled with an identification number, and information about sex, age, and location of the donkeys was documented for data analysis. The samples were transported on ice packed in coolers to the Teaching and Research Laboratory of the Department of Veterinary Medicine at the University of Maiduguri, and centrifuged (at 3,000 G for 5 min) to obtain clear sera. The harvested sera were stored at −20°C until tested for evidence of *Brucella* antibodies.

Animal studies ethic

The experiment was performed in accordance with a published protocol for the care and use of experimental animals [22] and was approved by the Faculty of Veterinary Medicine Ethics and Research Committee at the University of Maiduguri.

Serological tests

Serological tests were conducted in the Teaching and Research Laboratory of the Department of Veterinary Medicine at the University of Maiduguri; the rose bengal plate test (RBPT) was carried out in accordance with the method described by Alton *et al*. [5], and the results were recorded. The RBPT antigen was obtained from the Animal and Plant Health Agency (Addlestone, Surrey, U.K.). Briefly, 30 μl of serum were dispensed onto a white glossy ceramic tile and mixed with an equal volume of RBPT antigen using a sterilized applicator stick. The mixture was then shaken gently at room temperature for about 4 min and any visible agglutination or the appearance of a typical rim was taken as a positive result; the result was then taken as negative if there was no agglutination. An indirect enzyme-linked immunosorbent assay (iELISA) kit was obtained from IDvet, (Innovative Diagnostics, Montpellier, France), and the iELISA test was conducted in accordance with the manufacturer's instructions in the Microbiology, Laboratory Department of Veterinary Microbiology, University of Maiduguri.

Data analysis

Prevalence was calculated using the number of positive samples divided by the total number of samples tested and expressed as a percentage. Data were analyzed using IBM SPSS Statistics version 21.0. The statistical methods used include descriptive statistics to determine percentages. Relationship between disease positivity and factors were determined using the χ^2 test and Fisher's Exact Test to test for association. Strength of association was calculated using the odds ratio (OR) and 95%, confidence interval (CI).

Results

The overall seroprevalence rates of brucellosis in were 21.5% and 18.5% in the 200 samples from donkeys tested using RBPT and iELISA, respectively. Out of the 105 male donkeys tested, 24 (22.0%) and 20 (19.0%) were seropositive using RBPT and iELISA, respectively, while of the 95 female donkeys tested, 19 (20.0%) and 17 (17.9%) were seropositive using RBPT and iELISA respectively. There was no statistically significant association between prevalence of brucellosis and sex of the donkeys tested in the study area (P>0.05; Table 1). Based on age distribution, seroprevalence was higher among adult (over 3 years old) donkeys (40, 24.1%; 35, 21.1%) than in young (1–3 years old) donkeys (3, 8.8%; 2, 5.9%) using RBPT and iELISA, respectively. There was no statistically significant associa-

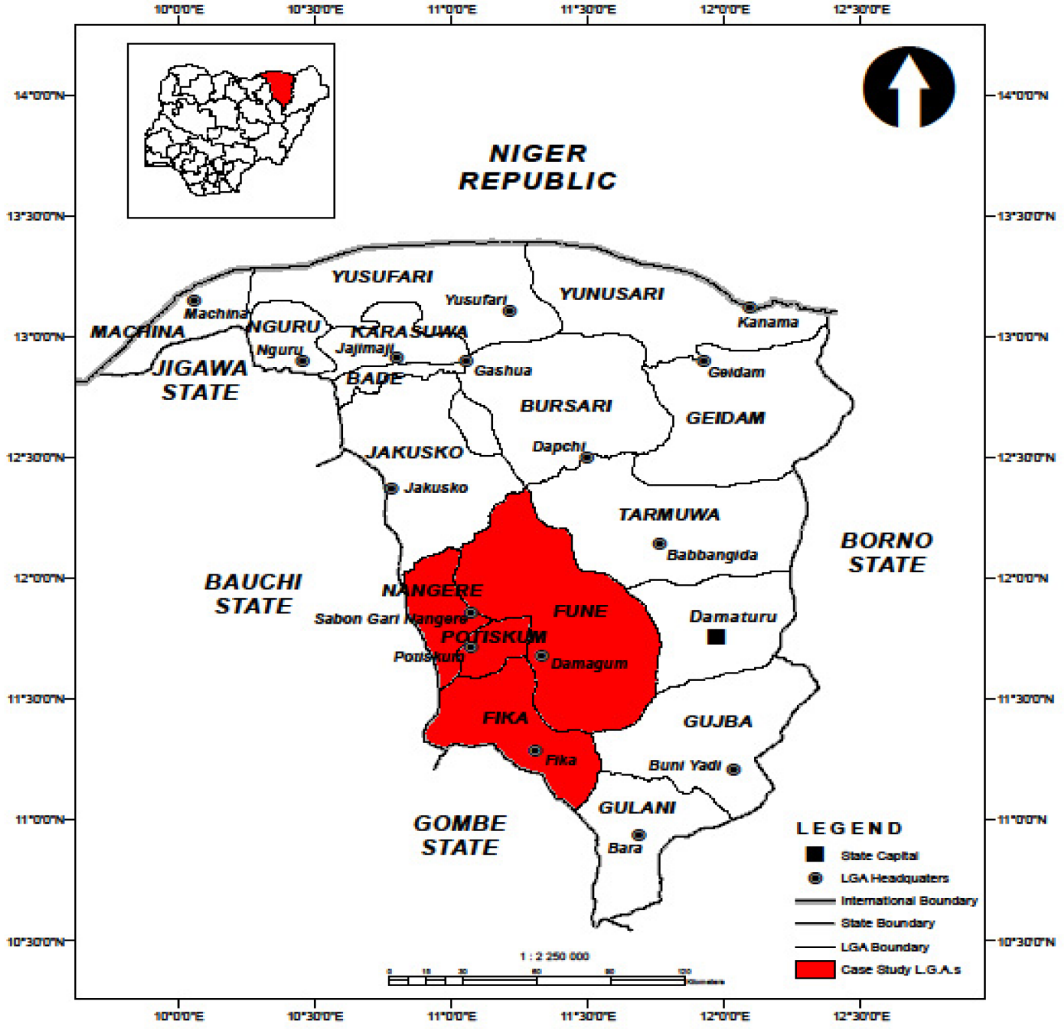

Fig. 1. Map of Yobe State showing the sampling area. Source: Adapted and modified from the administrative map of Yobe State.

Table 1. Prevalence of brucellosis in donkey based on sex using rose bengal plat test (RBPT) and iELISA

Sex	No. tested	RBPT		OR	*P* value	iELISA		OR	*P* value
		+ve no. (%)	−ve no. (%)			+ve no (%)	−ve no (%)		
Male	105	24 (22.9)	81 (77.1)	1.185	0.749	20 (19.0)	85 (81.0)	1.080	0.857
Female	95	19 (20.0)	76 (80.0)	1*		17 (17.9)	78 (82.1)	1*	
Total	200	43 (21.5)	157 (78.5)			37 (18.5)	163 (81.5)		

1*=reference. +ve indicates positive samples; −ve indicates negative samples. OR, odds ratio.

Table 2. Prevalence of brucellosis in donkeys based on age using rose bengal plat test (RBPT) and iELISA

Age Years	No. tested	RBPT		OR	*P* value	iELISA		OR	*P* value
		+ve no. (%)	−ve no. (%)			+ve no (%)	−ve no (%)		
Young	34	3 (8.8)	81 (77.1)	0.305	0.065	2 (5.9)	32 (94.1)	0.234	0.049
Adult	166	40 (24.1)	76 (80.0)	1*		35 (21.1)	131 (78.9)	1*	
Total	200	43 (21.5)	157 (78.5)			37 (18.5)	163 (81.5)		

1*=reference. +ve indicates positive samples; −ve indicates negative samples. OR, odds ratio.

Table 3. Prevalence of brucellosis in donkeys based on location using rose bengal plat test (RBPT) and iELISA

Location	No. tested	RBPT		OR	*P* value	iELISA		OR	*P* value
		+ve no. (%)	−ve no. (%)			+ve no (%)	−ve no (%)		
Fika	50	10 (20.0)	40 (80.0)	0.255	0.069	9 (18.0)	41 (82.0)	0.506	0.089
Fune	50	16 (32.0)	34 (68.0)	0.136		14 (28.0)	36 (72.0)	0.394	
Nangere	50	14 (28.0)	36 (72.0)	0.164		12 (24.0)	38 (76.0)	0.352	
Potiskum	50	3 (6.0)	47 (94.0)	1*		2 (4.0)	48 (96.0)	1*	
Total	200	43 (21.5)	157 (78.5)			37 (18.5)	163 (81.5)		

1*=reference. +ve indicates positive samples; −ve indicates negative samples. OR, odds ratio.

tion between the seroprevalence of brucellosis and age of the donkeys tested using RBPT ($P>0.05$). There was, however, a statistically significant association between seroprevalence of brucellosis and age of the donkeys tested using iELISA ($P<0.05$; Table 2). The prevalence of brucellosis based on location was highest in the Fune local government area (LGA; 16, 32.0%; 14, 28.0%), followed by the Nangere LGA (14, 28.0%; 12, 24.0%), using RBPT and iELISA, respectively and the lowest prevalence was recorded in the Potiskum LGA 3, 6.0%; 2, 4.0%). There was no statistically significant association between prevalence of brucellosis and location of the donkeys tested using RBPT and iELISA ($P>0.05$; Table 3).

Discussion

Brucellosis is a zoonotic disease found in a wide range of animal species, and it can also be spread to humans through contact with infected animals and aborted fetuses. To the best of our knowledge, this study is the first to report information on the prevalence of *Brucella* antibodies among donkeys in Yobe South Senatorial Zone, Yobe State, Nigeria using iELISA. An earlier report on seroprevalence of brucellosis in donkeys in the northeastern part of Nigeria, indicated seroprevalence rates of 5.0% and 6.0% using RBPT and SAT [23]; similarly Tijjani *et al*. [26] reported seroprevalence rates of 7.2% and 6.7% in Borno State using RBPT and cELISA. The overall seroprevalence rates of brucellosis in donkeys in this study were 21.5% and 18.5% by RBPT and iELISA respectively, which were higher than those reported by Sadiq *et al*. [23] in part of Yobe State and by Tijjani *et al.* [26] in Borno State, Nigeria but lower than those by reported by Esmat and El-Mezyen [11] in Egypt and Safirullah *et al*. [24] in Pakistan. The high seroprevalence rates obtained in this study are of public health concern because of the close association between donkeys, cattle and small ruminants kept together in some northern parts of Nigeria and also because donkeys provide significant socioeconomic benefits to pastoral communities. The high prevalence rates could be attributed to the fact that the donkeys were kept together with other domestic livestock, such as cattle, and small ruminants. The donkeys might have gotten infected through close contact with infected animals through grazing, at watering points, or through contaminated dust or droplets. The difference in seroprevalence rates could be the result of an increase in the frequency of the disease due to the lack of vaccination of animals against brucellosis in the country. The seroprevalence rates of brucellosis obtained in this study were higher in male donkeys than in female donkeys using RBPT and iELISA, though there was no statistically significant association between the disease and the sex of the donkeys tested. These results agreed with those reported by Yohannes *et al*. [27] and Tijjani *et al*. [26] who reported higher seroprevalence rates in male donkeys than in female donkeys. On the other hand, they did not agree with those reported by Göz *et al*. [14] in Turkey, Sadiq *et al*. [23] in Nigeria and Junqueira *et al*. [19] in Brazil who reported higher seroprevalence rates in female donkeys than in male donkeys. Similarly, Chimana *et al*. [9] and Mai *et al*. [20] reported higher seroprevalence rates in bulls compared with cows, while Ardo and Abubakar [6] reported higher seroprevalence in stallion than in mares. However, Sadiq *et al*. [23] and Safirullah *et al*. [24] reported that there was no statistically significant association between sex and

Brucella infection in equine species. The high seroprevalence of brucellosis in male donkeys in this study could be the result of relatively few female donkeys being kept. The finding is still in consonance with studies on the distribution of the seroprevalence of *Brucella* infection be according to sex, in which males camels were reported to have higher seroprevalence than female camels [1, 18]. In this study, the seroprevalence rates of *Brucella* infection were higher in adult donkeys than in young donkeys using the RBPT and iELISA tests, and there was a statistically significant association between the age of the donkeys tested and *Brucella* infection using the iELISA test; however, there was no statistically significant association between the age of the donkeys tested and *Brucella* infection using the RBPT test. This is in agreement with the work reported by Safirullah *et al*. [24], who reported higher seroprevalence in adult donkeys than in young donkeys in the Peshawar District of Pakistan. Similarly, Ardo and Abubakar [6] reported a higher seroprevalence in adult horses than in young horses in the Mambilla plateau of Taraba State, Nigeria. Age is one of the fundamental components which can influence the susceptibility of brucellosis, especially in adult animals, as reported in this study. Seroprevalence may rise with age because of a persistent period of response of antibodies among the infested animals due to prolonged exposure. Although highest seroprevalence of brucellosis in donkeys was recorded in the Fune LGA and the lowest seroprevalence was recorded in Potiskum, there was no statistically significant association between the location of the donkeys and the positive serological reactions. In conclusion, the high seroprevalence of *Brucella* infection in the donkeys in the study area demonstrates that brucellosis is still endemic in Yobe State, Nigeria and is of public health concern. Therefore there is a need for improved hygienic measures with appropriate handling of aborted fetuses and retained placentas, and there is a need for advocacy in relation to trans-border animal diseases.

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