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Serological Prevalence of Brucellosis among Donkeys (*Equus asinus*) in Some Local Government Areas of Yobe State, Nigeria

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ABSTRACT

A cross-sectional study of prevalence of antibody against *Brucella* organisms was conducted among donkeys in three local government areas of Yobe State, northeastern Nigeria. Three hundred adult donkeys of both sexes were sampled, with 100 samples each from local government areas Bursari, Gaidam, and Machina. The overall serological prevalence of brucellosis among donkeys in the three local government areas by both rose bengal plate test (RBPT) and microtiter serum agglutination test (MSAT) was 15 (5.0%), of which five (1.7%) were male and 10 (3.3%) were female donkeys. The overall prevalence by competitive enzyme-linked immunosorbent assay (cELISA) was 10 (3.3%), of which four (1.33%) were male and six (2.0%) were female donkeys. There was a significant association between the female sex of the donkeys and the serological reaction to RBPT, MSAT, and cELISA ($P < .05$). Of the 100 sera sampled from Bursari, five (5%) were positive by RBPT/MSAT, which comprised two (2%) male and three (3.0%) female donkeys. There was no significant association between the sex of the donkeys and the serological reaction ($P > .05$). Of the 100 samples from Gaidam, four (4.0%) tested positive by RBPT/MSAT, of which one (1.0%) was male and three (3.0%) were female donkeys ($P < .05$). Six (6.0%) of the 100 donkey sera samples from Machina tested positive by both RBPT and MSAT, which comprised two (2.0%) male and four (4.0%) female donkeys. There was no significant association between the male sex of the donkeys and the serological reaction ($P > .05$); however, there was a significant association between the female sex of the donkeys and the serological reaction to both RBPT and MSAT ($P < .05$). Of the 100 samples from Bursari and the 100 samples from Gaidam, three (3%) from each were positive by cELISA, which comprised one (1%) male and two (2%) female donkeys. There was no statistically significant association between the sex of donkeys and the serological reaction ($P > .05$). Of the 100 samples tested from Machina, two (2%) male and two (2%) female donkeys were positive for *Brucella* antibodies by cELISA. No statistically significant association was demonstrated between the sex of the donkeys and the serological reaction to cELISA ($P > .05$). Because of the importance of donkeys to humans as a source of draft power, infected donkeys could be a source of *Brucella* infection to humans through close contact, through the respiratory system via contaminated dust or droplets, and through aborted fetuses and discharges from the genitalia.

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1. Introduction

Donkeys originate from the semiarid parts of the world, but are now kept in a variety of different environments, although they are rarely found in the humid tropics [1]. The wild ass, *Equus africanus*, is indigenous to the African continent and is usually divided into a chain of races of subspecies spreading from the Atlas Mountains eastward to Nubia, down the Red Sea, and probably as far as the border of present-day northern Kenya [2-4] (Groves 1966). Despite having a prominent role in the rural agricultural system, there is a lack of knowledge regarding donkeys and they have been subjected to poor management, lack of health care, and negative attitudes from the community [5]. Despite these factors, donkeys continue to serve as draft animals (packing, carting, threshing, pulling water from a well, and riding) [4]. Donkeys are prone to variety of health problems, including brucellosis [6]. Brucellosis, also known as "undulant fever," "Mediterranean fever," "Gibraltar fever," or "Malta fever," is a major zoonotic disease, widely distributed in both humans and animals, especially in the developing world [7,8]. Transmission from infected livestock to man can either be direct through contact with infected material or indirect through consumption of animal produce [9]. The epidemiology of brucellosis is complex. Important factors that contribute to the prevalence and spread in livestock include farming system and practices, farm sanitation, livestock movement, mixing and trading of animals, and sharing of grazing grounds [10-12]. The overall seroprevalence of *Brucella* antibodies in the donkeys in Gedaref State in Sudan was 2.12%, based on RBPT [6]. Detailed studies confirming the problem of brucellosis in the livestock in Nigeria have been documented by several authors [13-22], with evidence of the spread of the disease in all parts of the country, which is usually accompanied by severe economic losses. To date, serological prevalence rates of between 0.20% and 79.70% have been reported in various parts of the country [21]. Equines may be a reservoir of brucellosis and may also play an important role in the epidemiologic patterns of this disease [6]. This study was designed to study the prevalence of *Brucella* antibodies in donkeys in three local government areas of Yobe State, northeastern Nigeria. The study aimed to provide documented information on the prevalence of the disease, with a view to assisting veterinary authorities in disease control policies and planning research priorities in the State.

2. Materials and Methods

2.1. Sample Collection

Sampling was done by cluster sampling method [23], in which the villages where donkeys were kept were identified and considered as clusters in each local government area. In each cluster, both male and female donkeys were selected, according to proportion of each sex, by simple random sampling using balloting. Three hundred donkeys were used in this research, with 100 donkeys each selected from local government areas Bursari, Machina, and Gaidam. Ten clusters were selected in each of the three local governments of Bursari, Machina, and Gaidam. Of the 100 samples from Bursari, 62 were male and 38 were female donkeys. Sixty-six male and 34 female donkeys were sampled from Gaidam, whereas 72 male and 28 female donkeys were sampled from Machina. After proper restraint of the donkey, 5 mL of blood was aseptically collected from the jugular vein, using a hypodermic syringe and a needle. Blood in the syringe was gently transferred into a sterile plain Bijou bottle, labelled, and placed in a slanting position for 1 hour to get the serum separated from the clotted blood. Serum samples were placed in a cool box containing ice packs and taken to the laboratory where they were stored in a refrigerator before serological analysis.

2.2. Serological Tests

Serological tests were conducted in the laboratory using the rose bengal plate test (RBPT) and the microtiter serum agglutination test (MSAT) as screening and standard tests for brucellosis, respectively, according to Alton et al. [24] and the Office International des Epizooties manual [25]. All sera samples were subjected to both RBPT and MSAT to quantify the antibody titers and results recorded.

2.3. Rose Bengal Plate Test

The serum samples and antigen were brought to room temperature after removing from refrigerator; only sufficient antigen for the day's tests was removed from the refrigerator. Three drops of the serum sample were placed on a glass slide, the antigen bottle was gently shaken, and an equal volume of antigen was placed near each serum spot. Immediately after the last drop of antigen was added, the serum and antigen were mixed thoroughly using a sterile wire loop to produce a circular or oval zone approximately 2 cm in diameter. The mixture was agitated gently for 4 minutes at ambient temperature by gently rocking the slide. Agglutination reactor was immediately read after the 4-minute period; any visible reaction was considered positive. A control serum that gives a minimum positive reaction was tested before each day's tests to verify the sensitivity of test conditions. The degrees of agglutination were recorded as very strong (++++), strong (+++), moderate (++), low (+), and negative (−), according to the World Health Organization [8].

2.4. Microtiter Serum Agglutination Test

Rigid U-bottomed microtitration plates were marked off with one row of 12 wells assigned to each specimen, and eight specimens were assigned to each plate. High, low, and negative reference sera of known titers were included in each day's run, as well as an antigen control. Phosphate buffered saline (PBS, pH 7.2) diluent was added to wells 2 through 12 in each row in 0.05-mL (50-mL) amounts with a calibrated pipette dropper. A 1:10 dilution of each serum specimen was made by adding 0.1 mL of serum to 0.9 mL of PBS with 5 m*M* ethylenediaminetetraacetic acid (EDTA), pH 7.2, in a small beaker. The chelating agent EDTA was added to reduce nonspecific reactions. After mixing, 0.1 mL (100 μL) of the 1:10 dilution of serum was added to the first well

of each row of the microtitration plate, and doubling dilutions were made with a 0.05-μL automatic microdiluter (Dynatech Corp, Dynatec Labs, TX). A 1:10 dilution of antigen was made by adding 0.1 mL of Serum Agglutination Test (SAT) antigen to 0.9 mL PBS in a small beaker. An equal amount (0.05 mL [50 μL]) of the 1:10 dilution of the antigen was added to each well. The serum dilutions were doubled by the addition of antigen, so that the dilution in the first well of each specimen was 1:20 instead of 1:10. The plates were sealed with aluminium foil plate sealers to prevent evaporation. The contents of the plates were mixed manually by tapping the edges of the plate for 20 seconds. After mixing, the plates were incubated for 24 hours in a 37°C incubator. They were read against a black background. A thin piece of translucent paper or facial tissue was placed over the top of the plate to facilitate reading. The tests were read against opacity standards prepared by diluting the working strength antigen 1 in 4, 2 in 4, and 3 in 4 to correspond to 25%, 50%, and 75% agglutination. Phenol saline was used as the 100% control, and the undiluted working strength antigen was used as the 0% control. The results are scored as the degree of clearance (1+ = 25%, 2+ = 50%, 3+ = 75%, 4+ = 100%) over the serum dilution, as described by the World Health Organization [8]. A serum sample having a titer of 30 or more IU/mL is considered to be positive [25].

2.5. Competitive Enzyme-Linked Immunosorbent Assay

The freeze-dried smooth lipopolysaccharide is reconstituted to 1 mL with distilled water and further diluted 1:1000 with 0.05 *M* carbonate buffer (pH 9.6). To coat the microplates, 100-μL volumes of LPS solution are added to all wells, and the plates are covered and incubated for 18 hours at 4°C. Unbound antigen is removed by washing all microplate wells four times with phosphate buffered saline with Tween (PBST). Volumes (50 μL) of monoclonal antibody (MAB) (M84 in this example) diluted appropriately in PBST/EDTA are added to each well, followed immediately by 50-μL volumes of serum diluted 1:10 in PBST/EDTA. Plates are incubated for 30 minutes at ambient temperature, with shaking for at least the initial 3 minutes. Test sera are added to the plates and may be tested singly or in duplicate. The controls, calibrated against the Office International des Epizooties ELISA standard sera, are set up in duplicate wells and include a strong positive, a weak positive, a negative control serum, and a buffer control. Unbound serum and MAb are removed by washing the microplate four times with PBST. Volumes (100 μL) of commercial goat anti-mouse immunoglobulin G (heavy and light chain) horseradish peroxidase conjugate diluted in PBST (predetermined by titration) are added to each well, and the plates are incubated at ambient temperature for 30 minutes. Unbound conjugate is removed by four washing steps. Volumes (100 μL) of substrate/chromogen (1.0 m*M* H_2O_2 and 4 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) [ABTS]) are added to each well, the plates are shaken for 10 minutes, and color development is assessed in a spectrophotometer at 414 or 405 nm. If required, 100-μL volumes of 4% sodium dodecyl sulfate may be added directly as a stopping reagent [25]. The control wells containing MAb and buffer (no serum) are considered to give 0% inhibition, and all data are calculated from these absorbance readings (between 1.000 and 1.800) using the following equation:

Percent inhibition (%I) = 100 − (absorbance [test sample]/absorbance [buffer control] × 100)

SPSS 17 statistical software (IBM SPSS Inc) was used to analyze data collected. The prevalence rate and odds ratio (OR) were calculated using two by two (2 × 2) contingency table to test association between occurrence of brucellosis and sex of donkeys, as well as local government areas.

3. Results

All the samples were serologically tested by RBPT, MSAT, and competitive enzyme-linked immunosorbent assay (cELISA). Of the 300 sera samples, only 15 (5.0%) were serologically positive by both RBPT and MSAT, of which five (1.67%) were male and 10 (1.33%) were female donkeys, and only 10 (3.3%) tested positive by cELISA, which comprised four (1.33%) male and six (2.0%) female donkeys. There was a significant association between the female sex of the donkeys and the serological reaction to RBPT, MSAT, and cELISA ($P < .05$; 95% confidence interval [CI] OR = 1.087-3.153) (Table 1).

Of the 100 sera sampled from Bursari, five (5%) samples tested positive by RBPT/MSAT, which included two (2.0%) male and three (3.0%) female donkeys (Table 2). There was no statistically significant association between the sex of the donkeys and the serological reaction ($P > .05$). Of the 100 samples from Gaidam, four (4.0%) tested positive, of which one (1.0%) was male and three (3.0%) were female donkeys (Table 2). There was no statistically significant association between the male sex of the donkeys in Gaidam and the serological reaction ($P > .05$); however, there was a statistically significant association between the female sex of the donkeys and the serological reaction to both RBPT and MSAT, with 95% CI OR of 1.230-4.386 ($P < .05$). Six (6.0%) of the 100 donkey sera samples from Machina tested positive by both RBPT and MSAT, of which

Table 1
Distribution of sex-specific prevalence of *Brucella* antibodies among donkeys

Sex	Number Sampled	RBPT (%)		MSAT (%)		cELISA (%)		χ^2	OR	95% CI on OR	
		Positive	Negative	Positive	Negative	Positive	Negative			Upper	Lower
Male	200	5 (1.67)	195	5 (1.67)	195	4 (1.33)	196	3.310	0.320	0.088	1.160
Female	100	10 (1.33)	90	10 (1.33)	90	6 (2.0)	94	7.895	1.851	1.087	3.153
Total	300	15 (5.0)	285	15 (5.0)	285	10 (3.3)	290				

cELISA, competitive enzyme-linked immunosorbent assay; MSAT, microtiter serum agglutination test; OR, odds ratio; RBPT, rose bengal plate test, 95% CI, confidence interval.

Table 2
Distribution of local government-specific prevalence of *Brucella* antibodies using RBPT/MSAT by sex of donkeys

Local Government Areas	Sex	RBPT/MSAT Reaction (%)			χ^2	OR	95% CI on OR	
		Positive	Negative	Total				
Bursari	Male	2 (2%)	60	62	1.081	0.389	0.062	2.442
	Female	3 (3%)	35	38		1.629	0.760	3.491
	Total	5 (5%)	95	100				
Gaidam	Male	1 (1%)	65	66	3.121	0.159	0.016	1.591
	Female	3 (3%)	31	34		2.323	1.230	4.386
	Total	4 (4%)	95	100				
Machina	Male	2 (2%)	70	72	4.734	0.171	0.030	0.996
	Female	4 (4%)	24	28		2.611	1.346	5.066
	Total	6 (6%)	92	100				

two (2.0%) were male and four (4.0%) were female donkeys (Table 2). There was no statistically significant association between the male sex of the donkeys from Machina and the serological reaction ($P > .05$); however, there was a statistically significant association between the female sex of the donkeys and the serological reaction to both RBPT and MSAT, with 95% CI OR of 1.346-5.066 ($P < .05$) (Table 2).

Of the 100 samples from both Bursari and Gaidam, three (3%) samples from each were positive by cELISA, this comprised one (1%) male and two (2%) female donkeys. There was no statistically significant association between the sex of the donkeys and the serological reaction ($P > .05$) (Table 3). Of the 100 samples tested from Machina, two (2%) male and two (2%) female donkeys were positive for *Brucella* antibodies by cELISA. No statistically significant association was demonstrated between the sex of the donkeys and the serological reaction to cELISA ($P > .05$) (Table 3).

4. Discussion

Overall prevalence of *Brucella* antibodies among donkeys in three local government areas of Yobe State was 5% by both RBPT and MSAT and 3.3% by cELISA. The lower overall prevalence (3.3%) by cELISA could be attributed to the fact that cELISA has higher specificity in detecting the *Brucella* antibodies than RBPT and MSAT. This was higher than the 2.12% prevalence of *Brucella* antibodies in 412 donkeys in the Gedaref State in Sudan, based on RBPT reported by Abdalla et al. [6]. The difference could be because of difference in sample size and location; the present study involved 300 donkeys selected from three local government areas in the Yobe State of Nigeria. The prevalence in this study was lower than the 54.2% prevalence of brucellosis among 35 donkeys with fistulous withers tested by RBPT in Egypt [26]. The higher prevalence could be attributed to the fact that Esmat and El-Mezyen's study was based on donkeys with clinical fistulous withers, whereas our study was based on apparently healthy donkeys. The seroprevalence rates found in this work were in consonant with the assertion of Cadmus et al. [21] that seroprevalence rates of brucellosis of between 0.20% and 79.70% have been reported in various parts of the country to date. In this study, there was a statistically significant association between the female sex of the donkeys and the serological reaction to RBPT, MSAT, and cELISA. Although there were more positive female than male donkeys in this study, there was no statistically significant association between the male sex of the donkeys and the serological reaction ($P > .05$); however, there was a statistically significant association between the female sex of the donkeys and the serological reaction to both RBPT and MSAT in Gaidam and Machina local government areas. The higher prevalence among female donkeys obtained in this study was in agreement with that reported by Goz et al. [27]. These findings are also in agreement with the works of Egbe-Nwiyi et al., Kudi et al., Junaidu et al., and Sadiq et al., in separate studies on the distribution of seroprevalence of brucellosis by sex of camels, who reported higher prevalence in males than in females [22,28-30]. Agab and Yagoub et al. also reported that the seroprevalence of brucellosis was three- to fourfold higher among adult animals than young ones and twofold higher in females compared with males [31,32]. Cross-

Table 3
Distribution of local government-specific prevalence of *Brucella* antibodies using cELISA by sex of donkeys

Local Government Area	Sex	cELISA Reaction (%)			χ^2	OR	95% CI on OR	
		Positive	Negative	Total				
Bursari	Male	1 (1.0%)	61	62	1.079	0.295	0.026	3.370
	Female	2 (2.0%)	36	38		1.796	0.775	1.165
	Total	3 (3.0%)	97	100				
Gaidam	Male	1 (1.0%)	65	66	1.471	0.246	0.022	2.817
	Female	2 (2.0%)	32	34		2.021	0.865	4.723
	Total	3 (3.0%)	97	100				
Machina	Male	2 (2.0%)	70	72	1.000	0.371	0.050	2.775
	Female	2 (2.0%)	26	28		1.846	0.657	5.189
	Total	4 (4.0%)	96	100				

transmission of *Brucella* organisms across other domestic species could be the most likely source of the infection, as donkeys traditionally use the same watering point and graze together with other species on the same pasture. Because of the importance of donkeys to humans as a source of draft power, infected donkeys could be a source of *Brucella* infection to humans through close contact, through the respiratory system via contaminated dust or droplets, and through aborted fetuses and discharges from the genitalia.

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