FULL PAPER

Sokoto Journal of Veterinary Sciences
(P-ISSN 1595-093X/ E-ISSN 2315-6201)
Sadiq *et al.* /*Sokoto Journal of Veterinary Sciences* (2013) 11(1): 7-12.
http://dx.doi.org/10.4314/sokjvs.v11i1.2

Prevalence of *Brucella* antibodies in donkeys (*Equus asinus*) in Borno and Yobe states, Nigeria

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Abstract

A cross-sectional study was designed to study the serological prevalence of antibodies against *Brucella spp* by using Rose Bengal Plate Test (RBPT) and Microtitre Serum Agglutination Test (MSAT). A total of six hundred (600) adult donkeys comprising of 393 males and 207 females were sampled from three local government areas each, of Konduga, Monguno and Ngala in Borno state and Bursari, Geidam and Machina in Yobe state. Overall prevalence of brucellosis was 33 (5.50%), out of which 14 (2.33%) male and 19 (3.17%) female donkeys were positive by both RBPT and MSAT. There was statistically significant association between female sex of donkeys and positive serological reaction ($p<0.05$). Out of the 300 sera sampled from Borno state, comprising of 193 male and 107 female donkeys, 18 (6.0%) tested positive. Whereas, 15 (5.0%) of the 300 (200 males and 100 females) sera sampled from Yobe state were positive. It can be concluded that the overall prevalence of brucellosis among donkeys in Borno and Yobe states in north eastern Nigeria in this study was 5.5%. The prevalence rate was higher among female donkeys than in males.

Keywords: Borno, Brucellosis, Donkey, Nigeria, Yobe.
Received 16-01-2012 Accepted 09-11-2012

Introduction

Donkeys are related to horses and zebras; they are all members of the family Equidae i.e. they are equines. Donkeys originate from the semi-arid parts of the world, but are now kept in a variety of different environments, although they are rarely found in the humid tropics (Pearson and Ouassat, 2000). The domestic donkey (*Equus asinus*) is a descendant of the African wild ass, *Equus africanus*, which is indigenous to the African continent and is usually divided into a chain of races of subspecies spreading from the Atlas Mountains eastwards to Nubia, down the Red Sea and probably as far as the border of present-day northern Kenya (Haltenorth & Diller 1980; Groves, 1986; Blench, 2004). The great majority of donkeys in the world (probably over 95%) are kept specifically for work. Their most common role is for transport; whether riding, pack transport or pulling carts, they may also be used for farm tillage (Starkey & Starkey, 2004). In certain countries they may assist in threshing, raising water,

milling or other operations (Starkey & Starkey, 2004). Donkeys are not conventional sources of meat; the primary function of donkeys in Nigeria has traditionally been as pack animals (Blench, 2004). Borno and Yobe states in north eastern Nigeria have a large donkey population and these donkeys have significant socioeconomic benefits to the rural communities. However, despite prominent role in rural agricultural system, donkeys have been subjected to poor management, lack of knowledge, lack of health care and negative attitudes from the community (Gutema *et al*., 2009). Despite these factors, donkeys have continued to serve as draught animals (packing, carting, threshing, pulling water from well and riding) (Blench, 2004). Knowledge of diseases of donkeys is scanty, and is often extrapolated from knowledge of diseases of horses (Pearson *et al.,* 1997). Serological surveys of *Brucella* antibodies in horses in Northern Nigeria indicate a prevalence of 4.8% (Bale & Kwanashie, 1984), 14.7% (Ehizibolo *et al.,* 2011). Ocholi *et al*. (2004a) have reported the isolation of *Brucella abortus* biotype 1 from a foal. In another report *Brucella abortus* was isolated from hygroma fluid in a horse with Carpal bursitis (Ocholi *et al*., 2004b). Donkeys are subjected to variety of health problems including brucellosis (Abdalla *et al*., 2010). 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 (Godfroid *et al.,* 2005; WHO, 2006). Transmission from infected livestock to man can either be direct through contact with infected material, or indirect through consumption of animal products (Smits & Cuttler, 2004). The overall seroprevalence of *Brucella* antibodies in the donkeys in Gaderef state in Sudan was 2.12% based on RBPT (Abdalla *et al*., 2010). Serological prevalence rate of between 0.20% and 79.70% have been reported in livestock in various parts of Nigeria to date (Cadmus *et al*., 2006). Equines may be a reservoir of brucellosis and my also play an important role in the epidemiologic patterns of this disease (Abdalla *et al*., 2010). This study was designed to determine the prevalence and distribution of antibodies against *Brucella spp* in donkeys in two north-eastern states of Borno and Yobe, Nigeria. The present study was conducted to provide base line information on the serological prevalence of brucellosis in donkeys with a view to assisting veterinary authorities in disease control policies and planning research priorities.

Materials and methods

Sample Collection

Sampling was done by cluster sampling method (Thrusfield, 2008) in which sixty (60) villages where donkeys were kept were identified in three local government areas each, of Konduga, Monguno and Ngala in Borno state and Bursari, Geidam and Machina in Yobe state, were considered as clusters. In each cluster both male and female donkeys were selected, according to proportion of each sex, by simple random sampling using balloting. Six hundred (600) donkeys were used in this research, comprising of 300 donkeys in each state; one hundred (100) donkeys were selected from each, Konduga, Monguno and Ngala (from Borno state) and Bursari, Machina and Geidam (from Yobe state), local government areas. Ten (10) clusters were selected in each of the six (6) local government areas of Konduga, Monguno and Ngala (from Borno state) and Bursari, Machina and Geidam (from Yobe state). Ten (10) donkeys were selected in each of the clusters. Following proper restraint of the donkey, 5ml of blood was aseptically collected from jugular vein, using hypodermic syringe and needle. Blood in the syringe was gently transferred into sterile plain bijou bottle, labelled, and placed in a slanting position for one hour to get the serum separated from the clotted blood. Serum samples were placed in cool box containing ice packs and taken to the laboratory where they were stored in a refrigerator before serological analysis.

Serological Tests

Serological tests were conducted in the laboratory using Rose Bengal Plate Test (RBPT) and Microtitre Serum Agglutination Test (MSAT) [both from Veterinary Laboratories Agency U.K] as screening and standard test for brucellosis respectively according to Alton *et al* (1988) and OIE manual, (2004). All sera samples were subjected to both RBPT and MSAT in order to quantify the antibody titres and results recorded. In RBPT the degrees of agglutination were recorded as very strong (++++), strong (+++), moderate (++) low (+) agglutination and negative (−) according to WHO (2006). The results for MSAT are scored as the degree of clearance (1+68 = 25%, 2+ = 50%, 3+ = 75%, 4+ =100%) over the serum dilution as described by WHO (2006). A serum titre greater than 1:40 (containing >30 IU per ml) is considered to be positive (OIE Manual, 2004).

SPSS-17® 144 statistical software was used to analyze data collected. The prevalence rate and odds ratio (OR) and 95% confidence interval on OR were calculated using two by two (2 x 2) contingency table to test association between occurrence of antibodies against *Brucella* spp and sex of donkeys and local government areas.

Results

A total of six hundred (600) adult donkeys comprising of 393 males and 207 females were sampled. Thirty three (5.50%) samples tested positive for antibodies against *Brucella* spp, out of which 14 (2.33%) male and 19 (3.16%) female donkeys were positive by both RBPT and MSAT (Table 1). There was no statistically significant association between male sex of donkeys and positive serological reaction (p>0.05), this was however significant among the females OR=1.735 (1.267-2.238; p<0.05). Out of the 300 serum sampled from Borno state 193 were from male while 107 were from female donkeys. Eighteen (6.0%) of the sera samples tested positive for antibodies against *Brucella* organisms out of which 9 (3.0%) each of the serum samples were from male and female donkeys. There was no significant association between sex of donkeys in Borno state and positive serological reaction (p>0.05) (Table 2). Fifteen (5.0%) of the 300 sera sampled from Yobe state gave serologically positive test by both RBPT and MSAT (Table 2). This comprised of 5 (1.66%) male and 10 (3.33%) female donkeys, there was no significant association between male sex of donkeys and positive serological reaction (p>0.05). However this was significant for female sex of donkeys in Yobe state OR= 2.111 (1.420-3.139; p<0.05) (Table 2). Five (5%) of the 100 donkey sera from Konduga local government tested positive for antibodies against *Brucella* spp, comprising of 1(1%) male and 4(4%) females. There was no significant association between the male sex of donkeys with positive serological reaction (p>0.05); however, this was significant among the female sex of donkeys OR=1.900 (1.155- 3.125; p<0.05). Out of the 100 samples from Monguno local government area of Borno state, five (5%) tested positive by both RBPT and MSAT, comprising of 2(2%) male and 3(3%) female donkeys. There was significant association between the female sexes of donkeys from Monguno with positive serological reaction OR=2.228 (1.034-5.028; p<0.05). A total of eight (8%) donkeys from the 100 sampled in Ngala local government area were positive for antibodies against *Brucella* organisms by both RBPT and MSAT. Out of which 6(6%) were male while 2(2%) were female donkeys. There was no significant association between both sexes of donkeys sampled from Ngala local government area of Borno state with positive serological reaction (p>0.05) (Table 3). Five (5%) samples tested positive from 100 donkey sera samples collected from Bursari local government area of Yobe state. This comprised of 2 (2%) and 3(3%) male and female donkeys respectively. There was no significant association between both sexes of donkeys sampled from Bursari local government with positive serological reaction (p>0.05). Four (4%) sera samples comprising of 1 (1%) male and 3(3%) females tested positive from 100 donkeys sampled from Geidam local government area of Yobe state. There was no significant association between male sexes of donkeys sampled from Geidam local government with positive serological reaction (p>0.05). However, this was significant for female sexes of donkeys from Geidam local government of Yobe state OR=2.323 (1.230-4.386; p<0.05). Six (6%) sera samples collected from Machina local government area of Yobe state tested positive for antibodies against *Brucella* spp. This comprised of 2(2%) male and 4(4%) female donkeys. There was significant association between the female sexes of donkeys from Machina local government area of Yobe state with positive serological reaction OR=2.611 (1.346-5.066; p<0.05) (Table 3).

Table 1: Sex specific prevalence of antibodies against *Brucella* spp among donkeys

	Serological reaction (MSAT/RBPT)				Odds Ratio	95% CI on OR	
Sex	Positive (%)	Negative	Total	X^2	(OR)	Lower	Upper
Male	14(2.33)	379	393	8.229	0.366	0.179	0.745
Female	19(3.16)	188	207		1.735	1.267	2.238
Total	33(5.50)	567	600				

Table 2: State specific prevalence of antibodies against *Brucella* spp among donkeys by sex

State	Sex	Serological Reaction (RBPT/MSAT)		Total	X^2	Odds Ratio (OR)	95% CI on OR	
		Positive (%)	Negative				Lower	Upper
Borno	Male	9(3.0)	184	193	1.715	0.533	0.205	1.385
	Female	9(3.0)	98	107		1.439	0.882	2.346
	Total	18(6.0)	282	300				
Yobe	Male	5(1.66)	195	200	7.895	0.231	0.077	0.695
	Female	10(3.33)	90	100		2.111	1.420	3.139
	Total	15(5.0)	285	300				

Table 3: Local government specific prevalence of antibodies against *Brucella* spp among donkeys by sex

LGA	Sex	serological reaction (RBPT/MSAT)		Total	X^2	Odd Ratio (OR)	95% CI on OR	
		Positive (%)	Negative				Lower	Upper
Konduga	Male	1(1.0)	55	56	2.768	0.345	0.059	2.011
	Female	4(4.0)	40	44		1.900	1.155	3.125
	Total	5(5.0)	95	100				
Monguno	Male	2(2.0)	70	72	2.673	0.543	0.184	1.599
	Female	3(3.0)	25	28		2.280	1.034	5.028
	Total	5(5.0)	95	100				
Ngala	Male	6(6.0)	59	65	0.382	1.169	0.762	1.795
	Female	2(2.0)	33	35		0.697	0.204	2.387
	Total	8(8.0)	92	100				
Bursari	Male	2(2.0)	60	62	1.081	0.389	0.062	2.442
	Female	3(3.0)	35	38		1.629	0.760	3.491
	Total	5(5.0)	95	100				
Geidam	Male	1(1.0)	61	66	3.121	0.159	0.016	1.591
	Female	3(3.0)	35	34		2.323	1.230	4.386
	Total	4(4.0)	96	100				
Machina	Male	2(2.0)	70	72	4.734	0.171	0.030	0.996
	Female	4(4.0)	24	28		2.611	1.346	5.066
	Total	6(6.0)	94	100				

Discussion

The current study provides the first information on the prevalence of *Brucella* antibodies among donkeys in north eastern Nigeria. The overall prevalence of brucellosis among donkeys in Borno and Yobe states in north eastern Nigeria in this study was 5.5%. The result of the present study is lower than the 14.7% reported for horses by Ehizibolo *et al.* (2011). The difference in prevalence could be attributed to differences in sample size and equine species; 75 horses compared with 600 donkeys in the present study. The prevalence of brucellosis among donkeys in this study was slightly higher than the 4.8% obtained by serum agglutination test (SAT) in horses by Bale and Kwanashie (1984). The slight difference could be due to the specificity of SAT used in that study. The prevalence rate was higher among female donkeys than in males. The association between sexes of donkeys and positive serological reaction was significant among the overall sample population of both states and among female donkeys of Yobe state. The higher prevalence amongst female donkeys obtained in this study was in consonant with similar finding reported by Goz *et al*. (2007). These findings are also in agreement with the works of Kudi *et al*. (1997), Egbe-Nwiyi *et al*. (1999), Junaidu *et al.* (2006) and Sadiq *et al*. (2008) in separate studies on the distribution of seroprevalence of brucellosis by sex of camels who reported higher prevalence in males than in females. Agab (1993); Yagoub *et al*. (1990) also reported that the seroprevalence of brucellosis was three to four-folds higher among adult animals than young ones

and two-folds higher in females compared to males. The prevalence obtained in this study was lower than 54.2% prevalence of brucellosis among 35 donkeys with fistulous withers tested by RBPT in Egypt reported by Esmat and El- Mezyen (1983). This variation could be due to the difference in sample size, that study was conducted on only 35 donkeys with fistulous withers while the present study involved 600 donkeys. The prevalence in the present study was higher than 2.12% of *Brucella* antibodies in 412 donkeys in Gaderef state in Sudan based on RBPT reported by Abdalla *et al*. (2010). The higher prevalence could be attributed to the fact that donkeys in these areas are kept in the same house with other domestic livestock such as goats, sheep and cattle. The donkeys might have contracted the infection through close contact between infected animals through cross transmission between species, through the alimentary tract from contaminated feed or water, through the respiratory system via contaminated dust or droplets, or through the genital system from infected semen while mating. Cross-transmission of *Brucella* organisms across other domestic species could be the most likely source of the infection since donkeys traditionally share the same watering point and graze together with other species on the same pasture. It can be concluded that the overall prevalence of brucellosis among donkeys in Borno and Yobe states in north eastern Nigeria in this study was 5.5%. The prevalence rate was higher among female donkeys than in males. There was no statistically significant association between male sex of donkeys and positive serological reaction ($p>0.05$). Because of the importance of donkeys to human as a source of draught infected donkeys could be a source of *Brucella* infection to human through close contact, through respiratory system via contaminated dust or droplets and aborted foetuses and discharges from the genitalia. There should be officially coordinated system of brucellosis surveillance and reporting in Nigeria to enhance epidemiological trace-back, enforcement and monitoring of control measures. Government coordinated public awareness on the presence of brucellosis among donkeys with emphasis on its economic impact and public health implications are hereby strongly recommended. There is need for improved sanitary measures with proper handling and disposal of vaginal discharges, retained placenta and aborted foetuses by burning and deep burying in the ground.

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