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RESEARCH ARTICLE

Epidemiological Study of Brucellosis in Camels (*Camelus dromedarius*) in Khartoum State, Sudan

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ABSTRACT

A study was conducted from April to September 2012, to determine the seroprevalence and risk factors for *brucellosis* infection in camels (*Camelus dromedarius*) in Khartoum State, Sudan. A total of 415 camels in 39 herds were included in the study from four localities and blood samples were collected and screened by RBPT. Twenty four samples tested positive giving an individual animal prevalence rate of 5.8%. All RBPT positive reactors were further tested by c-ELISA which confirmed 21 seropositive cases out of 24 RBPT reactors (87.5%). Eighteen herds were found seropositive among the 39 herds included in the study giving a herd prevalence of 46%. In the univariate analysis there was a significant increase in seropositivity of *brucellosis* in camel with respect to age and herd size ($P \leq 0.05$). Conversely, governorate, locality, sex, feeding, type of management, type of production, contact with other camels, source of new camels, source of water, housing, contact with other ruminants and contact with dogs were not found significantly associated with *brucellosis* ($P \geq 0.05$). Multivariate analysis showed that large herd size comprising more than 20 camels was significantly associated with seroprevalence of camel brucellosis (Exp B=5.660; 95% CI: 1.258 –25.463; $P \leq 0.05$). The results of the present study indicate that *brucella* exists within the camel herds in Khartoum State. The disease is widely distributed among large camel herds in the State. Further studies need to be done on *brucella* infection in the other ruminants to determine which measures should be followed for control of brucellosis.

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INTRODUCTION

Camel farming is an important economic income for pastoralist populations in Africa and Asia due to their characteristics to adapt to adverse environmental conditions. Actually, the main production of camels is aimed to both milk and meat with an increased demand of the populations for these products among the North Africa and the Middle-East populations. In addition, camel racing is popular in the Arabian Gulf countries and Northern Africa (Abbas and Agab, 2002). *Brucellosis* is one most important zoonotic disease. Although it has been eradicated in many developed countries in New Zealand Europe, Australia, Canada, Israel, Japan (Gul and Khan, 2007), It remains endemic in regions of Africa, Mediterranean, Middle East, parts of Asia or Latin America (Refai, 2002).Previous studies showed that *B. abortus and B. melitensis* are the most frequent bacteria isolates from dromedary (Abou-Eisha, 2000; Hamdy and Amin, 2002).

Brucellosis is the responsible for important economic losses resulting from clinical disease, abortion, and reduced or decreased milk production. In addition, the disease constrains free animal movement and trade (Wernery and Kaaden, 2002).Control of *brucellosis* in animal is the first step to control the infections to humans and the application of eradication plans are fundamental. Sanitary measures carried out must be according to the epidemiological situation of the area. Due to the scarce information about *brucellosis* in camels and epidemiological data, the aim of the study was the determination of the seroprevalence of *brucellosis* in camels and the identification of the risk factors that influence the presence of the disease.

MATERIALS AND METHODS

Study area

The study was carried out in Khartoum State, Sudan covering an area of about 20971 Km² and located between latitudes 15° 8`- 16° 39` N and longitudes 31° 36 - 34° 25` E in the semi desert tropics. The State is dominated by the semi desert climate which is characterized by very hot /dry summer and cold in winter. The average temperature ranges from 21.6σº to 37.7σº. (The mean annual evaporation rate is 7.7 mm/day), and the average relative humidity ranges from 21% to -38%. According to the official agricultural census report (2009), the camel population in the study area is 6585 camels.

Study design

Data were collected as part of a study on the seroepidemiology of *brucella* infection in camels herding in Khartoum State. A cross–sectional study was carried out during April to September 2012 to estimate the seroprevalence of *brucellosis* in camels and its risk factors. Multistage random sampling was designed based on state, governorate, locality, herds and individual animals. Selection among the fragments of the state was based on simple random sampling. Four localities were included in the study. The sample size of the animals was calculated as described by Thrusfield (2005) determined by using the formula given for simple random sampling method. The expected prevalence of brucellosis in our study was estimated at 45% based on previous work carried out by Musa and Shigidi(2001).Accordingly, the calculated sample size was 380 however, 415 camels were screened from the study area.

Blood samples were aseptically collected through jugular puncture and allowed to clot. Samples were transported in refrigerator to the Soba Veterinary Research Institute and processed in the following 12 hours. Serum was obtained by centrifugation and stored at -20σº until laboratory test was performed by rose bengal plate test (RBPT) and enzyme- linked immunosorbent assay (ELISA).

Questionnaire design

Information about location, age, gender, breed, body condition and contact with other farms was collected for each camel sampled. Thirty nine camel owners were interviewed by using questions. By doing so risk factors that had possible association with *brucellosis* among herds were investigated to support serological result, such as herd size, type of management, type of production, source of water, feeding, housing, contact with other ruminant, contact with other camel herds, contact with dogs, source of new camels, milking hygiene, herd man education, awareness of *brucellosis*, awareness of fetus and fetal membrane disposal and veterinary supervision.

Diagnostic techniques

(A) Rose Bengal Plate Test (RBPT)

All serum samples collected were initially screened by RBPT using RBPT antigen (Soba Veterinary Research Institute). Sera samples were stored at 4°C and left at room temperature before the run of the test. RBPT was carried out as described by (Alton *et al*., 1975). Thirty µl of RBPT antigen was added to each well and 30µl of test serum was placed alongside the antigen. The antigen and test serum were mixed thoroughly by wooden applicator. The plate was shacked for 4 minutes and the degree of agglutination reactions were read and recorded as + + ++ (coarse clumping and clearing), + + + (clumping and some clearing), + + (visible fine agglutination), + (weak fine agglutinations using magnifying glass) and in case of positive reactions, and 0 (no agglutinations) in negative reactions.

(B) Competitive Elisa (cELISA):

The competitive enzyme-linked immunosorbent assay kit was obtained from Veterinary Laboratories Agency, Department of Environment, Food and Rural Affairs, Surrey, United Kingdom. Initially the diluting buffer, washing solution, stopping solution, conjugate solution and controls were reconstituted as directed by the manufacturer. Test serum was added per each well of the micro titer plate which had sixty columns (wells). 100 µl of the prepared conjugate solution was then dispensed in all wells. The plate was then shaken for 2 minutes. In order to mix the serum with the conjugate solution. The plate was then covered with the lid and incubated at room temperature for 3 minutes. The content of the plate was then discarded and rinsed 5 times with washing solutions and then dried. 100 µl of the substrate chromogen solution was added to all wells. The plate was kept at room temperature for 10 minutes. The reaction was slowed by adding 100 µl of the stopping solution to each well.

Results: The lack of color development indicated that the sample tested was positive. A positive / negative cut-off can be calculated as 60% of the mean of optical density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to below this value should be regarded as being positive.

Statistical analysis

Statistical analysis was performed on SPSS 16.0 (SPSS, IBM, and New York, USA). The animal and herd seroprevalence was based on RBPT positivity. Questionnaire survey was performed and analysed by using Chi-square. Potential risk factors with $P \leq 0.05$ were considered significant at this level. Significant risk factors in the univariate analysis were subjected to multivariate analysis using logistic regression. Exp B was used to indicate the strength of association with risk factors involved in the occurrence of the disease. All risk factors with $P \leq 0.05$ were considered significant.

RESULTS

Prevalence of brucellosis

A total of 415 camels were screened from 39 herds. Twenty four camels were positive to the RBPT giving an individual prevalence of 5.8%. The positive reactors with RBPT (24) were further confirmed using c-ELISA. Accordingly, 21 seropositive camels were observed (87.5%). Out of 39 examined herds 18 camel herds were positive for brucellosis (46%).

Table 1: Univariate analysis for the association between the prevalence and risk factors of brucellosis diagnosed by RBPT in 415 camels in Khartoum State using the Chi-square (χ^2) test

Risk factors	No. tested	No. positive	Percentage %	Degree of freedom	χ^2	P-value
Governorate				1	.728	0.393
Khartoum North	208	10	4.8%	-	-	-
Omdurman	207	14	6.8%	-	-	-
Locality				3	5.084	0.166
Karare	60	1	1.7%	-	-	-
East Nile	208	10	4.8%	-	-	-
Ombeda	77	6	7.8%	-	-	-
Omdurman	70	7	10%	-	-	-
Herd size				1	10.503	0.001
Small	165	2	1.2%	-	-	-
Large	250	22	8.8%	-		-
Management				1	.626	0.429
Semi-intensive	188	9	4.8%	-	-	-
intensive	227	15	6.6%	-	-	-
Type of Production				2	.744	0.689
Racing	188	9	4.8%	-	-	-
Meat	145	9	6.2%	-	-	-
Milk	82	6	7.3%	-	-	-
Feeding				1	.073	0.786
Equipment	270	15	5.6%	-	-	-
Ground	145	9	6.2%	-	-	-
Water source				2	2.181	0.140
Underground	60	1	1.7%	-	-	-
Surface	355	23	6.5%	-		-
New camel				1	.441	0.506
Herd	333	18	5.4%	-	-	-
Purchase	82	6	7.3%	-	-	-
Contact ruminants				1	2.239	0.130
No	368	19	5.2%	-	-	-
Yes	47	5	10.6%	-	-	-
Contact camels				1	.73	0.786
No	270	15	5.6%	-	-	-
Yes	145	9	6.2%	-	-	-
Sex				1	.163	0.686
Male	172	9	5.2%	-	-	-
Female	243	15	6.2%	-	-	-
Age				1	4.553	0.033
Immature	258	10	3.9%	-	-	-
Mature	157	14	8.9%	-	-	-
Contact dogs				1	.728	0.393
No	208	10	4.8%	-	-	-
Yes	207	14	6.8%	-	-	-
Housing				1	.073	0.786
Closed	270	15	5.6%	-	-	-
Open	145	10	6.9%	-	-	-

The significant level ≤0.05

Univariate analysis

The Univariate analysis by Chi-square on camel risk factors revealed two variables with P≤0.05 (herd size and age). Large herd size ≥20 recorded highly significant association with seropositivity of *brucellosis* (P=0.001). Moreover, mature camels ≥4 years showed also significant effect on the seroprevalence of *brucellosis* (P=0.033) (Table 1) . Significances are usually expressed as P≤0.05: significant, P>0.05: non-significant, P≤0.01: more significant and P≤0.001: highly significant.

Multivariate analysis

All potential risk factors with two categorical variables and P≤0.20 on univariate analysis (herd size, source of water, contact with other ruminants and age) were subjected to multivariate analysis by logistic regression (Table 2). Large herd size ≥20 was only identified as risk factor for camel *brucellosis* (P≤0.05; ExpB=5.660; 95% CI: 1.258 – 25.463). Camels in large size herds were six times more likely to suffer from *brucellosis* than those in small herds.

DISCUSSION

Brucellosis is a widespread disease in camels. The infection rate is higher in intensive camel production system (Abbas and Agab, 2002). In production system where mixed herding is practiced, the disease circulates in sheep, goats and cattle, and further spreads to dromedaries (Musa *et al.,* 2008; Al-Majali *et al*., 2008). Despite the advances made in surveillance and control, the prevalence of brucellosis is increasing in many developing countries due to various sanitary, socioeconomic, and political factors (Pappas *et al*., 2006).

Table 2: Multivariate analysis for risk factors associated with camel brucellosis in 415 camels in Khartoum State using logistic regression.

Risk factors	No tested	Positive %	Exp (B)	95% C.I. for Exp (B)	P value
Herd Size	415				
Small	165	1.2%			
Large	250	8.8%	5.660	1.258-25.463	0.024
Water source	415				
Underground	60	1.7%			
Surface	355	6.5%	0.373	0.047-2.985	0.353
Contact ruminants	415				
No	368	5.2%			
Yes	47	10.6%	1.315	0.437-3.985	0.626
Age	415				
Immature	258	3.9%			
Mature	157	8.9%	1.900	0.761-4.743	0.129

The significant level ≤0.05

In our study and based on the results of RBPT, the prevalence of *Brucella* of examined camels was (5.8 %). This result was in accordance with that recorded in Ethiopia (Teshome *et al.*, 2003). However, higher prevalence was recorded in Sudan (Musa and Shigidi 2001; Omer *et al*., 2010), Saudi Arabia (Abbas and Agab 2002), Jordan (Al- Majali *et al*., 2008; Dawood, 2008), and Nigeria (Sadiq *et al*., 2011). The differences in the prevalence of camel *brucellosis* from different countries may be attributed to varying husbandry and management practices.

By the univariate analysis, the presence of seropositive camels was significantly associated (P≤0.05) with the variables: age and herd size. Herds with more than 20 camels were more frequently affected. Seroprevelance was 8.8% in large herds and 1.2% in small herds. This result was in agreement with that previously reported by Abbas and Agab (2002), Bati (2004), Al-Majali*et al*., (2008) and Mohammed *et al.,* (2011). It was suggested that more contact between camels may occur in large herds than smaller ones. The prevalence was lower among the young animals screened in this study compared to the mature ones. In this study seroprevalence of *brucella* was 3.9 % in young and 8.9 % in mature camels. Similar results were recorded by Musa and Shigidi (2001), Bati (2004), Al- Majali *et al*., (2008), Dawood (2008), Omer *et al*., (2010) and Swai *et al*., (2011). Usually young animals are protected by maternal immunity until when the immunity disappears, thus susceptibility seems to be low among them. Also, mature camels are more exposed. The presence of growth factors such as erythritol and hormones favor infection in mature animals. Also the high prevalence seen in mature animals may demonstrate the characteristics of *brucellosis.*

Multivariate analysis showed that herd size comprising more than 20 camels was significantly associated with seroprevalence of camel *brucellosis* in logistic regression (Exp B=5.660; 95% CI: 1.258- 25.463) P<0.05. Similar association was recorded by Bati (2004) (OR=1.5; 95% Cl: 1.03, 2.2, P<0.05), Al- Majali *et al*., (2008) (OR=1.5; 95% Cl: 1.1, 3.7, P<0.05) and Ghanem *et al*., (2009) (OR=5.425; 95% Cl: 2.956, 10.207, P<0.001). The increase in herd size increases the chance of contact between animals leading to more chances of infection. In large herd size and density of animal population together with poor husbandry, management practices, the number of susceptible camels, the virulence of the organisms, presence of reactor animals in the region, absence of veterinary service and lack of awareness about the disease directly increase the infection rate of *brucellosis.*

The results of the present investigation indicate that, *brucella* exist within camel herds in Khartoum State. The large herd size is a major risk factor associated with camel brucellosis.Therefore, frequent screening of the camel herds is recommended to assess the status of the disease and to identify the *brucella* species involved. Moreover, epidemiological studies needed to explore the current status of the disease in other ruminants and to enable the public veterinary authorities to construct concrete program for prevention of the disease within animal herds.

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