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Original Article

Seroprevalence and risk factors of anti-brucella antibodies in cattle in Khartoum State, the Sudan

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ABSTRACT

Objective: This cross-sectional study was conducted from April to July 2012 to estimate the prevalence of brucellosis and investigate the risk factors that enhance its occurrence in cattle in Khartoum state, the Sudan.
Material and methods: A total of 300 serum samples were taken from jugular veins of cattle and screened by Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT). The RBPT-positive samples were all tested using c-ELISA.
Results: Antibodies were detected with an overall seroprevalence of 25.7% using RBPT and 22.7% using SAT while slightly less than two thirds of the RBPT-positive samples were cELISA-positive. The herd and within-herd seroprevalences were 76.7% (n=23) and from 10.0-80.0%. Moreover, significant statistical dissimilarities were not observed between the seroprevalence of the different categories of the investigated risk factors by RBPT. Only milking method (χ^2=3.976; *P*=0.046) was found to have an influence on the RBPT-positive status for brucella infection in the univariate analysis. Additionally, natural breeding (OR=3.61; 95% CI 1.192–10.96; *P*=0.023) was the only observed risk factor with an increased odd of being RBPT positive. The Kappa analysis showed an almost perfect agreement between the results of the RBPT and the SAT tests.
Conclusion: The prevalence of anti-brucella antibodies in Khartoum state was relatively higher; therefore, brucellosis in cattle is, perhaps, a significant public health problem. It is recommended to raise awareness of cattle owners and/or herders on the routes of transmission of brucellosis.

KEYWORDS

Brucella, Cattle, Khartoum, Risk factors, Seroprevalence

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INTRODUCTION

Brucellosis is an infectious rapidly transmitted bacterial disease of ruminants primarily, but it can infect other animals like equines, canines and felines. It has also a zoonotic demission and is one of the important diseases in humans (Radostits et al, 2007). In cattle, brucellosis is typically caused by *Brucella abortus*, less often by *B. melitensis*, and sporadically or rarely by *B. suis*. Infection is widespread in the world and generally characterized by inflammation of the genital organs and fetal membrane, abortion, sterility, and formation of localized lesions in the lymphatic system and joints (Radostits et al, 2007; OIE, 2009; Cadmus et al., 2010). In pregnant cows, retained placenta or failure to expel fetal membranes, inflammation of the uterine endometrium (endometritis), abortion, birth of dead or weak calves (dummy calves or fading calves), repeat breeding, infertility (failure to conceive) as well as reduction of milk yield or agalactia (complete loss of milk yield) are characteristic to brucellosis (Radostits et al, 2007; Aparicio, 2013). Additionally, brucella species are localized in the udder in cows and are excreted in milk in high amounts (Gwida et al., 2010). Naïve cattle often become infected by ingestion of brucella-contaminated feed or water. Besides, infected semen has been incriminated to be one of the transmission routes of the infection to recipient cows. Human beings become infected as result of ingestion of raw or unpasteurized infected milk or dairy products, inhalation of contaminated dust, and contact with infected uterine contents, discharges and infected carcasses (Omer et al., 2000; Radostits et al, 2007). Swine, horses, and dogs acquire or contract brucellosis by natural breeding, through contact with infected cattle or pigs, and by feasting on contaminated fetuses and placentas or drinking milk (Omer et al., 2000; Radostits et al, 2007). Furthermore, new brucella strains have recently been detected and identified from aquatic creatures, indicating an increase in the modes of transmission of the organism as this could be a risk factor to consumers, hunters and researchers (Bishop et al., 1994; Cloeckaert et al., 2001).

In developing countries, where there is no national brucellosis control and eradication programme in place, the disease is of paramount importance from economic point of view (Radostits et al, 2007). Serological investigations have demonstrated that brucellosis is occurring in the Sudan and evidence of infection has been found in large and small ruminants (cattle, sheep, goats, and camels), wildlife and human beings. *B. abortus* biovars 1, 3, 6 and 7 and *B. melitensis* biovars 2 and 3 were found to be associated with the disease (Musa et al., 2008).

Risk factors associated with brucella-infections in animals have comprehensively been assessed by Omer et al. (2000). Omer et al. (2000) indicated that any risk factor that enhances the spread of brucellosis among animals belongs to one of the following three categories: (1) characteristics of animal populations, (2) management practices and (3) the biology of the disease (Omer et al., 2000). Nevertheless, Crawford et al. (1990) sorted out the factors and classified them into (1) factors associated with the transmission of the disease between herds and (2) factors influencing the maintenance and spread of infection within herds. Even so, the main risk for a herd to be infected with brucellosis is introducing undiagnosed infected animals into the herd, aborting cows are the most important source of risk for the spread of brucella-infection (Schelling et al., 2003; Aparicio, 2013). Other risk factors of brucella-infection in cattle include density of animal populations and herd size, type and breed of animal (dairy or beef), husbandry systems and environmental factors (Omer et al., 2000). The objectives of this study were to determine the prevalence of brucellosis in cattle and to investigate the risk potential in Khartoum State.

MATERIALS AND METHODS

Study area: Khartoum state is located in central Sudan, in the semi- arid zone between the latitude 15.08ºN to 16.39ºN and longitude 31.36ºE to 34.25ºE **(Figure 1)**. The resident cattle in Khartoum including indigenous ecotypes and exotic breeds as well as their crosses are about 236,909 heads (MARF, 2009). The system of animal breeding or animal production in Khartoum state is in general semi-intensive depending on the natural range in the vicinity of the villages and the town outskirts as well as individual houses (MARF, 2009).

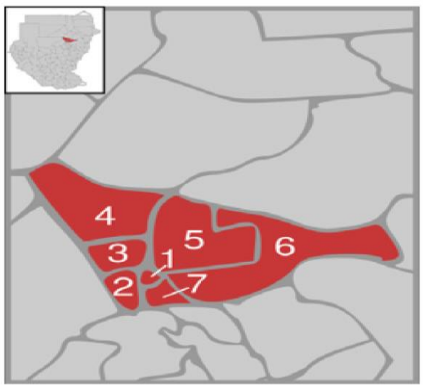

Figure 1: Map of study area. Selected localities were 1=Khartoum, 2=Ombada, 3=Omdurman, 5=Bahry and 6=East Nile or Sharg Al-Neeyl. Adopted from Nurelhuda et al. (2009)

Study design and sampling strategy: This cross sectional study was conducted for 3 months, from April to July/2012, employing a multistage sampling strategy as described by Martin et al. (1988) and Thrusfield (2007). Out of the 7 localities of Khartoum state, 5 were randomly or conveniently selected, namely; Khartoum, Ombada, Omdurman, Bahry, and Sharg Al-Neeyl **(Figure 1)**. Within the selected localities, 13 peasant association or farm collections or villages and 30 farms or cattle herds and individual animals were randomly and/or conveniently sampled (Thrusfield, 2007). The number of selected animals per farm/herd was from 2 to 49.

Sample size: The sample size (n) for estimating the prevalence of anti-brucella antibodies among cattle in Khartoum state was calculated according to Thrusfield (2007) and relying on some parameters including level of confidence (95.0%), desired level of precision (±5%) and the expected prevalence of brucellosis in cattle in the study area of 25.3% as indicated Ali (2011). The required sample size was found to be 300 individual animals by using the following formulaa:

$$n = \frac{(1.96)^2 P_{exp.} (1-P_{exp.})}{d^2}$$

Where, $(1.96)^2$=constant, n=required sample size, P_{exp}=expected prevalence, d=desired absolute precision.

Collection of samples: Whole blood samples for serum were collected aseptically from the milk or jugular vein of animals according to OIE (2009).

Laboratory procedures: All of the three laboratory tests were conducted at the department of Brucellosis, Research Veterinary Institute, Soba, the Sudan.

Rose Bengal Plate Test (RBPT): The RBPT was carried out as described by Ferede et al. (2011) and OIE (2009).

Serum Agglutination Test (SAT): The SAT was carried out as described by OIE (2009).

Competitive enzyme-linked Immunosorbent assay (cELISA): The cELISA kit was obtained from the Central Veterinary Laboratory, Weybridge, UK, and was conducted according to the instructions of the manufacturer.

Questionnaire survey: A well developed and pretested structured questionnaire was administered, when collecting blood samples, to livestock owners or farms workers to gather information on potential risk factors such as number of parity and body condition, as well as information related to herd management like production type, breeding type and share males for breeding, water source, herd size, veterinary service, type of floor, waste disposal, disposal of placenta, separate pen for calving, milking method and history of previous abortions and retained placenta.

Data management and analyses: The generated tests' and questionnaire-gathered data were transferred into Microsoft Excel spreadsheet database, then imported to the Statistical Package for Social Sciences (SPSS) for Windows® version 22.0 (SPSS Inc., Chicago, Illinois) for conducting appropriate statistical analyses. Descriptive statistics, frequencies and cross-tabbing were obtained for each variable (potential risk factors). Univariate and multivariate analyses by means of the 2-tailed chi-square test and logistic regression model were conducted to test the hypothesized variation of the potential risk factors between test-positive and test-negative animals. Associations in the chi-square test and logistic regression model were deemed significant when $p \leq 0.05$ but factors that are biologically known to be associated with brucella-infection in cattle with a $p \leq 0.25$ in the Univariate analysis were entered into the final logistic regression model.

RESULTS

Overall seroprevalence of brucellosis: The overall seroprevalences were 25.7% (n=77) with a 95% CI from 20.76 to 30.64 using RBPT and 22.7% (n=68) with a 95% CI from 17.96 to 27.44 using SAT. Out of the 77 RBPT-positive sera, 66.2% (n=51) were confirmed to be positive by c-ELISA (95% CI from 55.63 to 76.77). Furthermore, the herd seroprevalence was 76.7% (n=23) with a 95% CI from 61.57 to 91.83 and the within-herd prevalence ranged from 10.0% to 80.0%.

RBPT-estimated seroprevalences by risk factors: With exception of the risk factors that contain category(ies) with expected count less than 5 or with constant category(ies) (no samples tested), statistical significant differences at *p-value* ≤0.05 were not observed between the seroprevalences of the categories of the investigated risk factors by RBPT **(Table 1, 2,** and **3)**.

Sharg Alneeyl locality and Eidbabekir showed prevalences of 30.3% (95% CI from 22.15 to 38.45) and 45.5% (95% CI from 24.69 to 66.31) which were the highest compared to other localities and villages. Moreover, the seroprevalence was higher in female animals (25.8%), >6 years old cattle (26.6%), cattle that

Table 1: Seroprevalences and univariate analysis of brucellosis in cattle by individual animal risk factors in Khartoum state (from April to July 2012)

Risk factors	No. tested	No. positive	%	95% CI Lower - Upper	df	χ^2	*P-value*
Locality					4	3.963	0.411
Sharg Alneeyl	122	37	30.3	22.15 - 38.45[a]			
Bahry	66	13	19.7	10.10- 29.30[a]			
Khartoum	37	11	29.7	14.98 - 44.42[a]			
Omdurman	28	5	17.9	3.700- 32.10[a]			
Ombada	47	11	23.4	11.30- 35.50[a]			
Villages					12	19.780	0.071*
Waddafeaa	9	3	33.3	2.510- 64.09[a]			
Elsheglah	48	15	31.2	18.09 - 44.3 [a]			
Omdawban	11	0	00.0	00.00- 00.00			
Falasteen	22	4	18.2	2.080- 34.32[a]			
Darelsalam	25	7	28.0	10.40- 45.60[a]			
Elfetiahab	28	5	17.9	3.700- 32.10[a]			
Soba	37	11	29.7	14.98 - 44.42[a]			
Elkadaro	49	7	14.3	4.500- 24.10[a]			
Kaforri	18	6	33.3	11.53 - 55.07[a]			
Garwalah	17	7	41.2	17.80- 64.60[a]			
Kuku	7	2	28.6	-4.88 - 62.08[a]			
Eidbabekir	22	10	45.5	24.69 - 66.31[a]			
Eleisialat	7	0	00.0	00.00- 00.00			
Sex					1	0.695	0.404*
Male	2	0	0.0	00.00- 00.00			
Female	298	77	25.8	20.83 - 30.77[a]			
Age (yrs)					1	1.653	0.438
≤6	172	43	25.0	18.53 - 31.47[a]			
>6	128	34	26.6	18.95 - 34.25[a]			
Body condition					1	0.361	0.629
Good	280	73	26.2	21.05 - 31.35[a]			
Poor	20	4	22.7	4.340- 41.06[a]			
Breed					2	1.046	0.593*
Local	11	2	18.2	-4.60- 41.00[a]			
Cross	287	75	26.2	21.11 - 31.29[a]			
Exotic	2	0	0.0	00.00- 00.00			
Number of parity					2	0.918	0.821
≤4	184	47	25.5	19.20- 31.80[a]			
>4	114	30	26.3	18.22 - 34.38[a]			

*Different superscripts indicate significant difference at P≤0.05, *=risk factor with category that has expected count less than 5, or with constant category*

have good body condition (26.2%), cross breed cattle (26.2%) and cattle that have given birth > 4 times (26.3%) when compared to other categories of the same risk factor.

The highest seroprevalences among the categories of management factors were found in animals of small size (≤30) and dairy herds (30.5%, CI 23.16-37.84 and 26.3%, CI 21.26-31.34), in addition, 31.8% (CI 22.98-40.62) in cattle where vet services were inaccessible, 27.9% (CI 22.35-33.45) in cattle where milking was manual, 40.9% (CI 20.36-61.44) in cattle of the same farm in which breeding was mainly artificial, and 26.6% (CI 18.30-34.90) where watering of animals was by using underground water.

Extensive animal production system and concrete floor were constant categories, *i.e.*, no samples were tested; therefore a seroprevalence of 25.7% (95% CI from 20.76 to 30.64) for the other categories of the same risk factor was estimated.

Table 2: Seroprevalences and univariate analysis of brucellosis in cattle by herd management risk factors in Khartoum state (from April to July 2012)

Risk factors	No. tested	No. positive	%	95% CI Lower - Upper	df	χ^2	*P-value*
Herd size					1	0.108	0.122
Small	151	36	30.5	23.16 - 37.84[a]			
Large	149	41	22.5	15.80- 29.20[a]			
Production type					1	2.475	0.116*
Dairy	293	77	26.3	21.26 - 31.34[a]			
Beef	0	0	0.0	00.00- 00.00			
Dual	7	0	0.0	00.00- 00.00			
Veterinary service					1	3.253	0.071
Accessible	193	43	22.3	16.43 - 28.17[a]			
Inaccessible	107	34	31.8	22.98 - 40.62[a]			
Milking method					1	3.976	0.046
Machine	49	7	14.3	4.500- 24.10[a]			
Manual	251	70	27.9	22.35 - 33.45[a]			
Breeding					2	5.478	0.065
Artificial	22	9	40.9	20.36 - 61.44[a]			
Natural	222	59	26.6	20.79 - 32.41[a]			
Both	56	9	16.1	6.470 - 25.70[a]			
Water source					1	0.079	0.779
Tap water	191	48	25.1	18.95 - 31.25[a]			
Underground	109	29	26.6	18.30 - 34.90[a]			
Production system							
Semi-intensive	300	77	25.7	20.76 - 30.64[a]	-	-	-
Extensive	0	0	0.00	00.00 - 00.00			
Floor							
Concrete	0	0	0.00	00.00 - 00.00	-	-	-
Normal ground	300	77	25.7	20.76 - 30.64[a]			

*Different superscripts indicate significant difference at P≤0.05, *=risk factor with category that has expected count less than 5, or with constant category*

The relationship between risk factors and brucellosis: The proportions of sero-positive differed between the individual and management risk factors. As **Table 3** depicted, only one risk factor (milking method; χ^2=3.976, df=1, *P*=0.046) was significantly associated with RBPT-positive status for brucella infection. However, none of the individual or other management risk factors were statistically correlated to brucellosis in the univariate analysis

Results of the logistic regression analysis assessing the combined relationship between risk factors that were correlated to brucella infection in the univariate analysis with RBPT-positive status for brucella are shown in **Table 4**. The regression coefficients (Exp(B)) express 'odds ratios' (OR) (=the increased or decreased probability (OR≠1)) of sero-positivity occurrence in comparison to the reference (OR=1). Natural breeding type (OR=3.61; 95% CI 1.192–10.96; *P*=0.023) was the only risk factor that was associated with increased odds of being RBPT positive. Conversely, the rest of the factors were not associated with increased odds of being RBPT positive. Furthermore, village and production type were not in the equation (excluded) as residual chi-square was not computed because of redundancies.

RBPT and SAT agreement: A 91% measure of agreement with a *P-value* of 0.027 was observed between RBPT and SAT and this agreement is almost perfect **(Table 5)**.

DISCUSSION

Brucellosis is one of the disease that have drawn attention and concern at it causes economic losses in cattle, besides to its zoonotic dimension (Radostits et al, 2007). The disease can be diagnosed using several serological tests including rose Bengal test (RBT), SAT, complement fixation test (CFT), radial immunodiffusion (RID), ELISA and others (Mwelwa, 2012). The seroprevalences reported in this study using RBPT and SAT were not statistically different and almost perfect agreement was

Table 3: Seroprevalences and univariate analysis of brucellosis in cattle by management risk factors in Khartoum state (from April to July 2012)

Risk factors	No. tested	No. positive	%	95% CI Lower - Upper	df	χ^2	*P-value*
Shared bull					1	1.892	0.169
Yes	128	38	29.7	21.78 - 37.62[a]			
No	172	39	22.7	16.44 - 28.96[a]			
Retained placenta					1	1.374	0.241
Yes	162	46	28.4	21.46 - 35.34[a]			
No	138	31	22.5	15.53 - 29.47[a]			
Abortion history					1	0.148	0.701
Yes	123	33	26.8	18.97 - 34.63[a]			
No	177	44	24.9	18.53 - 31.2 [a]			
Calving pen					1	0.002	0.965
Yes	8	2	25.0	-5.01 - 55.01[a]			
No	292	75	25.7	20.69 - 30.71[a]			
Placenta disposal					1	0.897	0.421
Yes	115	20	28.7	20.43 - 36.97[a]			
No	185	57	23.8	17.66 - 29.94[a]			
Presence of dogs					1	0.225	0.636
Yes	186	46	24.7	18.50 - 30.90[a]			
No	114	31	27.2	19.03 - 35.37[a]			
Waste disposal					1	0.458	0.499
Yes	223	55	24.7	19.04 - 30.36[a]			
No	77	22	28.6	18.51 - 38.69[a]			

*Different superscripts indicate significant difference at P≤0.05, *=risk factor with category that has expected count less than 5, or with constant category*

observed between the results obtained by the two tests. This did confirm the findings of Genc et al. (2005) who found no variation between the results of the two tests in Ardahan Province, Turkey. Genc et al. (2005) investigated 163 serum samples collected from aborted cows from 19 settlements. The samples were positive for *B. abortus* as shown by cELISA (68.1%), CFT (65.6%), RBPT (58.9%) and SAT (55.2%). However, moderate (Kappa=0.55) and good agreements between RBPT and SAT have previously been observed (Senein and Abdelgadir, 2012). Furthermore, in dogs, Talukder et al. (2011) indicated that the sensitivity of RBT was 100% and that of SAT was 66.7% in comparison to ELISA, while the specificity of the RBT was 96.3% and of the SAT was 100%. In general, RBT is considered less sensitive than other tests like CFT and ELISA, consequently, using a second confirmatory test for the RBT-positives was recommended. In average, RBT has a sensitivity of around 81.2% and a specificity of around 86.3% when put side-by-side to cELISA (Angara et al., 2004; OIE, 2009; Salih et al., 2014). For SAT, a sensitivity of 95.6% and a specificity of 100% were noted when the test was used for bacteremic human patients (Memish et al., 2002). In this regard, many factors could probably lead to incorrect classification of a sample as positive or negative (false-positive or false-negative) using RBPT or SAT, including contaminated or expired rose Bengal antigen, inappropriate antigen and/or sera temperature when conducting the test, and overestimation of the agglutination reaction and misinterpretation of the actual result (Unger et al., 2003). The perfect agreement noticed between the two tests herein could possibly be explained by their sensitivity and specificity. The SAT could perhaps not accurately discriminate between S19 vaccinated and naturally infected animals as does by the RBT (Mwelwa, 2012). The results of the confirmatory testing of the RBPT-positive samples by cELISA, typified the findings of Mwelwa (2012) who indicated that the ELISA in general detects antibodies that could have been missed by the RBT, SAT or CFT.

The RBPT-estimated seroprevalence in this study was compatible with the one reported by Ali (2011), who found a seroprevalence of 25.3%, while it was lower than the prevalences reported by Omer et al. (2000), Angara et al. (2004), and Solafa et al. (2014) from different parts of Khartoum state, which were 31.0, 35.0, 40.8, and 29.4%, respectively. On the other hand, it was higher than the 8.4% that was observed in Eldein locality, Darfur, by Senein and Abdelgadir (2012). Prevalences from other countries like Ethiopia, Uganda, Rwanda, Egypt, Jordan, Bangladesh, Iran, and Brazil ranged from

Table 4: Multivariate associations of risk factors with brucellosis seropositivity in cattle in Khartoum state (from April to July 2012)

Risk factors	No. tested	No. positive	%	Exp(B)	95% CI Lower-Upper	*P-value*
Villages				-	-	-
Eleisialat	7	0	00.0			
Umdawban	9	0	00.0			
Waddafeaa	48	3	33.3			
Elsheglah	11	15	31.2			
Falasteen	22	4	18.2			
Darelsalam	25	7	28.0			
Elfetiahab	28	5	17.9			
Soba	37	11	29.7			
Elkadaro	49	7	14.3			
Kaforri	18	6	33.3			
Garwalah	17	7	41.2			
Kuku	7	2	28.6			
Eidbabekir	22	10	45.5			
Production type				-	-	-
Beef	0	0	0.0			
Dairy	293	77	26.3			
Dual	7	0	0.0			
Herd size						
Large	149	41	22.5	ref		0.228
Small	151	36	30.5	1.53	0.767–3.043	
Veterinary service						
Accessible	193	43	22.3	ref		0.699
Inaccessible	107	34	31.8	1.12	0.622–2.083	
Milking method						
Machine	49	7	14.3	ref		0.446
Manual	251	70	27.9	2.17	0.296–15.94	
Breeding type						
Both	56	9	16.1	ref		0.072
Artificial	22	9	40.9	1.90	0.837–4.094	
Natural	222	59	26.6	3.61	1.192–10.96	
Shared bull						
No	172	39	22.7	ref		0.335
Yes	128	38	29.7	1.43	0.689–2.981	
Retained placenta						
No	138	31	22.5	ref		0.390
Yes	162	46	28.4	1.38	0.660–2.894	

-=risk factor not in equation

Table 5: Kappa analysis between RBPT and SAT outcomes

Test	SAT-Negative	SAT-Positive	Total
RBPT-Negative	223	0	223
RBPT-Positive	9	68	77
Total	**232**	**68**	**300**

2.13% to 15% (Aguiar et al., 2007; Berhe et al., 2007; Dinka and Chala, 2009; Al-Majali et al., 2009; Kaoud et al., 2010; Mohammed et al., 2011; Akbarmehr and Ghiyamirad, 2011; Megersa et al., 2011; Rahman et al., 2011; Jönsson, 2013).

The herd seroprevalence in this study was lesser than the findings of Solafa et al. (2014) and Angara et al. (2004) who found a herd seroprevalences of 90.3% and 93.3%. Opposite to that, Berhe et al. (2007) and Megersa et al. (2011) in Ethiopia, Jönsson (2013) in Uganda, and Aguiar et al. (2007) in Brazil found 42.31%, 26.1%, 10.7% and

63.0% of the surveyed herd as brucellosis seropositive, correspondingly.

The within-herd prevalence was similar to the findings of Angara et al. (2004) who reported within herd prevalences of 60.0% and 55.6%. Nonetheless, it was higher than the one reported in Ethiopia by Berhe et al. (2007) who found a within-herd prevalence that ranged between 0 to 15.15%.

Variations in individual animal, herd, and within-herd prevalences could doubtless be ascribed to dissimilarities in the size of tested samples in each study. The different investigated ecosystems or areas could be another reason behind these dissimilarities, considering the fact that each area has its specific and unique indigenous components and risk factors. Furthermore, divergent prevalences of brucella in cattle might possibly be explained by differences in the investigated animal production systems and husbandry (intensive or extensive). Accuracy measurements of diagnostic tests (sensitivity, specificity, positive predictive value, and negative predictive value) are not always the same, therefore, variations are expected as different tests were used in each study. A good example is that cELISA can eliminate some, but not all, false-positive serological reactions caused by *Enterobacteriaceae* and also eliminate most of false-positive reactions from vaccination with S19 that cannot be detected by RBT and SAT (OIE, 2009).

There are certain inter-related factors that shape up the clinical picture or appearance of brucella-infection in cattle, affect the degree and duration of the immune response and the concentration of anti-brucella antibodies (Jönsson, 2013). These factors include species of the infected animal, age, sex, pregnancy and stage of pregnancy in female animals and virulence of the involve strain of brucella (Jönsson, 2013). Detection of anti-brucella antibodies in a samples obviously evinces previous exposure to brucella and does not essentially denote that the animal is having an on-going or an active infection at the time when the sample was taken (Godfroid et al., 2002). Furthermore, it has also been reported that transmission of brucella species from natural reservoirs to susceptible animals relies on some factors such as herd size, density of animal populations in a given farm or locality, presence of host animals, management style, animal movement and dynamics, husbandry systems, type and/or breed of animal and ecological conditions (Cloeckaert et al., 2001).

Among all of the investigated potential risk factors in this study, milking method was the only correlated risk factor to seropositivity in the univariate analysis using chi-square test. Jönsson (2013) made a comparable observation and found no dependencies could be established between brucellosis and the investigated risk factors other than with geographical location. However, Solafa et al. (2014) found a relationship between brucella-seropositivity and many potential risk factors in the univariate analysis including administrative unit (P=0.041), type of herd (P=0.020), have abortion case (P=0.018), knowledge of the owner about the cause of abortion (P=0.041), and feeding and watering (P=0.006). This noticed discrepancy could perhaps be attributed to good management or good farming practices (GFP), general hygiene and application of biosecurity measure in the surveyed farms in the present study. Though almost all risk factors were not related to brucellosis, there were differences in the seroprevalences between the categories of each risk factor. For instance, the seroprevalence was higher in dairy cattle than in cattle raised for dual purposes (milk and meat). This could probably be due to the fact that dairy cattle are kept for long period for production, hence, the longer time at risk and an increased chance of coming in contact with infected fetal membranes and contaminated environment. In addition, diary cattle are subjected to many stress factors like pregnancy, calving and lactation (Langomi et al., 2000). In addition, milking is one of the important modes of transmission as brucella species are presumably to be transmitted from cow-to-cow when milked by the same person (milker) or if the similar teat cup is used for milking of more than one cow (Aparicio, 2013). Negreiros et al. (2009) reported the presence of the infection in mixed (beef and dairy) cattle farming (OR=1.8 [1.2-2.7]) and Ogata et al. (2009) considered dairy farming (OR=0.63) as protective factors. Furthermore, the prevalence of brucellosis was higher among animals of farms where veterinary services were inaccessible and this was contrary to the findings of Samartino (2002) and Luna-Martínez and Mejía (2002) in Argentina and Mexico, where veterinary services, individual animal care or population medicine, played an insignificant role in avoiding the introduction and spreading of brucellosis into cattle farms or herds. Delivering adequate animal health services would, with no doubt, help to the control spreading and manage the incidence of diseases in general and infectious diseases in particular. This has been proved by Al-Majali (2005) and Al-Majali et al., (2009) who found out that applying chemical decontaminants (disinfectants) and delivering of adequate veterinary care for individual animals and herds were protective factors at least against brucella-infection among cattle and camel herds.

Whether a bull was shared for breeding or not, the prevalence of brucellosis was not statistically different and this risk factor did not influence the occurrence of

the disease (χ^2=1.892; $P \leq 0.25$). Yet, only natural breeding type was significant in the multivariate analysis using logistic regression. Kaoud et al. (2010) indicated that using of exogenous fertilizing system (OR=3.2) was one of the imperative factor for the introduction and transmission of brucella-infection amid farm animals. Poor hygienic practices, before and/or after artificial insemination (AI), and the use of inappropriate techniques during AI might affect the prevalence of brucellosis in cattle and boost its transmission. However, bulls do not usually transmit brucellosis by venereal route or mechanically from infected to non-infected cows or even if they are infected. Bulls may discharge semen that contains brucella but unlikely to transmit the infection. The risk of spreading of the infection by an infected bull is much higher when the semen is used for artificial insemination (Radostits et al., 2007; Aparicio, 2013). Jergefa et al. (2009) reported a higher seroprevalence on farms that used artificial insemination and Azevedo et al. (2009), Chate et al. (2009) and Silva et al. (2009) as well.

History of retained placenta or failure to expel fetal membranes after calving was not correlated to increased or decreased prevalence of brucellosis, but contrary, McDermott et al. (2002) and Kubuafor et al. (2000) did note an association. Retention of the placenta and inflammation of the wall of the uterus (metritis) are common sequelae to abortion due to brucellosis (Radostits et al., 2007). Aparicio (2013) reported that brucella-infected cows were expected to abort 3 to 4 times more than unexposed cows. As the study found insignificant association between failure to expel placenta and brucella-positive status, possible occurrence of other abortion-inducing diseases or conditions (abortifacients) should be thought about. These might likely be tick borne diseases such as theileriosis or infectious causes of abortion like leptospirosis or dietary deficiencies.

Virtually investigated potential risk factors were not statistically related to brucellosis in cattle in the present study and this could likely be due to the similar epidemiological status of the disease in the surveyed farms. Another elaboration might be the selection of animals and the number of sampled animals from each farm, as seropositive farms or individual animals could have been missed.

CONCLUSION

The prevalence of anti-brucella antibodies in Khartoum state is relatively high. Therefore, brucellosis in cattle is potentially a significant public health problem. With exception of milking method, none of the individual or the other management risk factors had an effect on the occurrence of brucellosis in cattle in Khartoum state. However, it is recommended to put efforts to raise awareness of cattle owners and/or herders on the routes of transmission of brucellosis and its zoonotic nature. Additionally, magnifying the importance of practicing farm and personal hygiene. Brucellosis should be investigated in other animal species in close contact with cattle and stray animals to understand the role of these animals in the epidemiology of brucellosis in cattle.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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