



## Prevalence and Risk Factors of Ruminants Brucellosis in Jabel Aolia Locality, Sudan

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### ABSTRACT

This cross-sectional study was carried out to determine the prevalence and risk factors of brucellosis in ruminants' population in Jebel Aolia locality, Khartoum State. A total of 393 serum samples were collected from 53 herds, out of which 207 were bovine, 84 ovine, 82 caprine and 20 camel serum samples. The serum samples were screened for presence of brucella antibodies using RBPT and the positive samples were confirmed by c-ELISA test. The results pointed out that, prevalence of brucellosis among herds/flocks of cattle, camel, sheep and goats were 76 % (22/29), 20% (1/5), 13% (1/8) and 18% (2/11) respectively. The individual animal prevalence were 19% (39/207), 5% (1/20), 1 % (1/84) and 4%(3/82) respectively. A survey using a questionnaire was conducted to collect the required epidemiological data. The risk factors were investigated using logistic regression analysis. The test revealed that only abortion cases (OR.001, CI.00-.247,  $p$ -value.014) and source of water (OR1.51, CI 2.949-7.745E5,  $p$ -value.021) were significant ( $P<0.05$ ) risk factors. This study provides necessary information about prevalence and risk factors of the disease in the study area which help the decision makers in setting the priority of disease control.

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### INTRODUCTION

Brucellosis is a highly contagious zoonotic, and economically important bacterial disease of animals worldwide (OIE, 2000). The disease causes a decrease in reproductive efficacy and an increased

abortion rates in animals (Rijpens, *et al*, 1996). The disease is transmitted from animals to humans by ingestion of infected food products, direct contact with an infected animal, or inhalation of aerosols

(Gerald and Maloney, 2001). It is widely distributed in developing countries (Ozekicit *et al.*, 2003). The disease is caused by different species of the genus *Brucella*. The old classification of the genus included six species namely *Brucella abortus*, *Br. melitensis*, *Br. suis*, *Br. neotomae*, *Br. ovis* and *Br. canis* (Moreno and Moriyon, 2001). Latter a marine species has been noted as *Br. maris* (Sohn *et al.*, 2003). The epidemiology of brucellosis in animals is influenced by factors associated with the transmission of the disease among herds and the factors influencing the maintenance and survival of the bacteria and spread of infection within herds (Crawford *et al.*, 1990). In Iran prevalence of 3.66% was reported by Akbarmehr and Ghiyamirad (2011). In Africa individual animal prevalence rate in bovine of 5.0%, 0.3%, 8.2% and 2.77% was reported in Kampala by (Makita *et al.*, 2011), in Libya by El Sanousi and Omer (1985), in Eritrea by Omer *et al.* (2000) and Scacchia *et al.*, (2013) respectively. A unit (herd) seroprevalence of 35.9% was reported in Eritrea by Omer *et al.* (2000). The prevalence of camel brucellosis was studied by many researchers; in Ethiopia (1.5%) prevalence rate was reported by Warsame *et al.*, (2012). In Libya Gameel *et al.*, (1993) reported individual animal prevalence of 4.1%. Whereas, in Egypt El-Boshy *et al.*, (2009) reported a prevalence rate of (7.35%). In small ruminant; Ashenafi *et al.*, (2007), Yesuf *et al.*, (2010), Ferede *et al.*, (2011) and Tesfaye *et al.*, (2012) reported 3.2%, 2.5%, 1.2% and (3.8%) prevalence rate respectively. In Eritrea according to Omer *et al.* (2000) the individual prevalence of 3.8% was reported in goats and 1.4% in sheep and unit (herd) prevalence of 33.3% (goats) and 16.7% (sheep) were found in the eastern part of Eritrea, while in western Eritrea the individual prevalence rate in goats was

14.3% and the units prevalence was 56.3%. In Nigeria Cadmus *et al.*, (2006) reported 0% prevalence in sheep while Bertu *et al.* (2010) reported 14.5% prevalence rate in small ruminants. In Asia a high prevalence of small ruminant brucellosis (72.9%) was reported in the Palestine by Shuaibi (1999), while in Yemen according to (Hosie *et al.*, 1985) the prevalence among goats was 0.4% and among sheep was 0.6%. Akbarmehr and Ghiyamirad (2011) reported 4.2% prevalence rate in small ruminants in Iran. According to Mohammed *et al.*, (2013) small ruminants brucellosis in different regions of Abu Dhabi Emirate, the United Arab Emirates was 8.0%.

In Sudan Animal brucellosis was discovered early in 1904 and was first reported by Bennett (1943) in Khartoum. Later many researchers surveyed the disease in different animal species and different locations in Sudan. El-Ansary *et al.*, (2001) reported 5% prevalence rate in cattle in Kassala State. Whereas Ebrahim (2013) reported 25.7% prevalence rate in cattle in Khartoum State. In Kuku Dairy Scheme, Khartoum North, the herd prevalence rate was 90% and the individual animal prevalence was 24.9% (Angara, 2005). In Kassala State, El Ansary *et al.*, (2001) reported zero prevalence of camel brucellosis; while in Darfur States Raga (2000) reported 5.3%. In Khartoum State (Saad, 2013) found 5.8% individual prevalence. In small ruminant according to El-Ansary *et al.*, (2001) the positive reactors were 4% of goat's and 1% of sheep in Kassala State. Omer *et al.*, (1989 to 1990) reported prevalence rate of sheep Brucellosis of 0.01%. Omer *et al.*, (2007) studied the prevalence of brucellosis in Kassala, Eastern Sudan during 2004, 2005 and 2006 the result was 0.1%, 0.4%, 2.1% prevalence rate in sheep and 0.2%, 0.6%, 5.6% in goats. Musa (2005) investigated sheep brucellosis in Darfur

States and reported a prevalence rate of 3.3%. Omran (2011) investigated the disease in Sinnar State and reported 4.1% prevalence rate in sheep. According to Ali (2013) the seroprevalence was 2.5% in sheep in North Kordofan State. A prevalence of 2.0% in goat was reported by Magzub (2001) in Khartoum.

The main objective of this research was to estimate the prevalence of brucellosis among ruminants population in Jebel Aolia locality, Khartoum State.

## **MATERIALS and METHODS**

### **The study area**

Jebel Aolia locality is one of the seven localities of Khartoum State, it located in southern part of the State. Like the other parts of the state, the climate is semi-desert, dry and hot in summer. The average rainfall is 150 mm per year. The breeds of cattle found in Jebel Aolia are local breed mainly Butana and crossbred of mostly less than 75% foreign blood. The main breeds of goats are Saneen, Nubian and Shami goats and crossbred between them, while desert sheep are the main sheep breeds found in the locality. The main breeds of camels are Bushari and Kabashi mainly raised for milk curative purposes.

According to the Ministry of Agriculture and Animal Resources (Anon, 2009) the numbers of livestock holdings in Jebel Aolia locality were 410 for cattle, 16 for camel, 71 for sheep and 783 for goat and the number of animal are 20,360(h) of cattle , 45 (h) of camel , 9,317(h) of sheep and 17,819(h) of goats. Most of the goats are raised in the residential areas.

### **The sample size and design**

The study was carried out during the period from (April, 2012 to April 2014). The survey covered the farmed animals only. The need to use the herd as the basic statistical unit for the economic study, beside the lack of an appropriate sampling frame necessitated multi stage cluster

sample to be used in this study (Otte and Gumm, 1997). The number of clusters (herds) for each animal species was calculated using the following formula according to Bennett *et al.*, (1991).

$$C = P (1-P) D/SE^2n.$$

Where

c: the number of clusters to be sampled,

P: the expected prevalence,

D: the design effect of using cluster sample instead of simple random sample,

SE: the standard error of the estimate and

n: the average cluster size.

With the exception of camel where the whole herds were covered.

Accordingly in the first stage a total of 53 herds/flocks were included in the study, 29 herds of cattle, 5 herds of camel, 11 herds of goats and 8 herds of sheep. In the second stage the individual animals were selected randomly.

### **The sample collection and laboratory analysis**

The blood samples were collected using disposable syringes. For cattle 5-10ml venous blood was withdrawn from the milk vein whereas, for the other animal species the blood was withdrawn from the jugular vein. The blood samples were transferred to the college of Veterinary Medicine, Sudan University of Science and Technology in thermo flasks with minimal possible shaking, where serum was separated and preserved in cryo tubes at -20 °C. Later the serum was transferred to Veterinary Research Institute (VRI) in Soba for serological testing.

All serum samples were subjected to Rose Bengal plate test (RBPT) as described by Alton *et al.*, (1988) as screening test. The positive results were confirmed by c-ELISA. The kits were obtained from the Veterinary Laboratory Agency, New Haw, Addlestone and Surrey KT 15 3NB United Kingdom. The test was run according to the manufacturer instructions.

For collection of data regarding the risk factors associated with brucellosis, a questionnaire was designed containing different items required to collect the necessary data. Each farm owner was personally interviewed by the researchers.

#### Data analysis

Serological analysis data were stored in the Microsoft excel spread sheet and the herd prevalence and individual prevalence were calculated.

Data on risk factors were first analyzed using frequency analysis to know the distribution of potential risk factors, then univariable analysis cross tabulation was performed to test the association between each brucellosis seropositive status and potential risk factors and finally multivariable logistic regression was used to analyze associations of the various risk factors with the seroprevalence of the disease using Wald test. Only variables with *P*-values <0.25 in univariable analysis were tested in the logistic regression model. Only

risk factors with *P*-values <0.05 in the logistic regression were considered to be significant.

#### RESULTS

This study investigated the prevalence of animal brucellosis in Jebel Aolia locality in Khartoum State and revealed that the overall herd/flock reactivity by RBPT was 64% (90%for cattle, 20%for camels, and 36%for goats and38%for sheep). The confirmatory test by competitive enzyme linked immunosorbent assay (c- ELISA) revealed overall herd/flock reactivity of 49% (76%for cattle,20% for camels, 18% for goats and13 %for sheep herd) (Table 1). The positive sample of serum by RBPT was 21 %(35% for cattle,5% for camels,9% for goats and5%for sheep sample). The confirmatory test (c-ELISA) indicated overall herd/flock reactivity of 11% (19% for cattle, 5% for camels,4% for goats and1% for sheep sample) (Table 2).

**Table 1: The herd/flock prevalence rates of brucellosis in ruminants In Jebel Aolia locality**

Diagnostic tests Animal species	RBPT		c-ELISA	
	No.	+ve herd	No.	+ve herd
Cattle	29	26(90%)	29	22(76%)
Camels	05	01(20%)	05	01(20%)
Goats	11	04(36%)	11	02(18%)
Sheep	08	03(38%)	08	01(13%)
Total	53	34(64%)	53	26(49%)

**Table 2: The Individual animal prevalence rate of brucellosis in ruminants in Jebel Aolia locality**

Diagnostic tests Animal species	RBPT		c-ELISA	
	No.	+ve	No.	+ve
Cattle	207	72 (35%)	207	39(19%)
Camels	020	01(05%)	020	01(05%)
Goats	082	07(09%)	082	03(04%)
Sheep	084	04(05%)	084	01(01%)
Total	393	84(21%)	393	43(11%)

**Table 3: The potential risk factors examined for animal brucellosis in Jebel Aolia, Khartoum State**

Risk factor	Distribution & (%)	$\chi^2$	No. +ve	Exp(B)(OR)	95% C.I For Exp(B)	P-value
Unit						
Jebel Aolia	41 (77.4)	4.178	17	.793	.012-51.372	.913
Elazhry	12 ( 22.6)		9			
Owner education						
Illiterate	23 (43.4)	4.247	15			.098
Rather good	11 (20.8)		4	-		
Well educated	19 (35.8)		7			
Type of the herd						
One species	24 (45.3)	5.44	16	.386	.023-6.343	.505
Multi-species	29 (54.7)		10			
Veterinary care						
Have veterinarian	43 (81.1)	1.791	23	.601	.032- 11.290	.734
No veterinary care	10 (18.9)		3			
Abortion cases						
No abortion case	33 (62.3)	5.638	12	.001	.00-.247	.014
Have abortion case	20 (37.7)		14			
Knowing the cause of abortion						
Well knowledge	15 (28.3)	4.197	4	.472	.033-6.792	.581
Bad knowledge	38 (71.7)		22			
Breeding						
Have a bull	48 (90.6)	1.865	25	2.463	.059- 102.415	.636
Borrow bull	5 (9.4)		1			
Feeding and watering animals						
Separate	33 (62.3)	7.483	21	20.741	.586- 734.237	.096
Together with other species	20 (37.7)		5			
Source of water						
Have source	47 (88.7)	.016	25	1.511E3	2.949- 7.745E5	.021
Common canal	6 (11.3)		1			

The result of frequency (Table 3) revealed that more than 77% of the farms are located in Jebel Aolia unit. More than half (55%) animals raised were multi species and about 60% of the animals were purchased from other sources. In breeding their animals owners depend on natural breeding that 91% have their own bulls. To reduce the cost of

feeding 45% of the farmers depend on grazing land. About 89% of the farms have their own source of water and 62% of the owners fed their animals separately. 38% of the herds have abortion cases and 49% of them were positive to brucellosis. The knowledge about the causes of the abortion was very poor among the owners; most of

them (72%) have no knowledge. Although most of the owners (43%) were illiterate, 62% of them vaccinate their animal with the officially recommended vaccines except for brucella vaccine where only 4% of the herds were vaccinated. The chi-square univariable analysis was performed to test the relationship between prevalence and potential risk factor. The test revealed 9 variables were statistically significant ( $p$ -value  $\leq 0.25$ ). The test revealed there was significant association between the rate of infection and the unit ( $p$ -value=0.041), owner education ( $p$ -value = 0.120), type of herd ( $p$ -value = 0.020), dealing with sick animal ( $p$ -value = 0.181), have abortion case ( $p$ -value = 0.018), knowledge of the owner about the cause of abortion ( $p$ -value = 0.041), have their own bull for breeding ( $p$ -value = 0.172), feeding and watering animals ( $p$ -value = 0.006) and the source of water ( $p$ -value = 0.092). The multi variable analysis revealed that only abortion case (OR.001, CI.00-.247,  $p$ -value .014) and source of water (OR1.51, CI 2.949-7.745E5,  $p$ -value .021) were significant ( $P < 0.05$ ) risk factors.

## DISCUSSION

The RBPT is widely used in Sudan for brucellosis screening for regulatory control and for export requirements. Although the test is very sensitive and is suitable for screening herds, it can give false positive results due to vaccination with *B. abortus* strain 19 vaccine or for cross reactions with other bacteria (OIE, 2004). This fact justifies the use of c-ELISA as a confirmatory test. Among the other animals tested cattle were found to be mostly affected by the disease, despite climatic conditions of the State (persistence of the sun light at the most hours of the day, dry desert weather and low humidity) which may not favor survival of brucella organisms for long periods. The high prevalence rate in cattle in this study (76%) is attributed to the management

practices where animals are kept overcrowded and reared in open system in which animals of different ages and sex aborted and pregnant ones are housed together in high stocking density. More over in the traditional sector, infected animals are usually kept for breeding despite the fact that congenital infection is a major epidemiological means of spread of the disease, as it is well known that as high as 20% of calves born by infected heifers could be found persistently infected with brucella (Nielsen and Duncan, 1990). Camels came after cattle in harboring the infection. All camel herds in the locality were tested because they were few and there were no specialized dairy camel farms. On the other hand, the low prevalence rate of brucellosis in sheep was attributed to the fact that sheep kept are mainly males brought from range lands (where they usually raised in extensive system) for marketing purposes, the levels of brucella infections tend to be relatively high in intensive farms (Anonymous, 1986). In the current study only goats raised in farms were tested, although there were a great number of goats raised inside the domestic houses because of the difficulty in collecting samples from individual animals in houses.

As a comparison with other relative studies, a lower bovine prevalence (3.66%) was reported by Akbarmehr and Ghiyamirad (2011) in Iran. Makita *et al.*, (2011) reported 5.0% individual animal prevalence of bovine brucellosis in Kampala using (c-ELISA). In Libya El Sanousi and Omer (1985) reported overall reactivity of 0.3%. In Sudan lower cattle prevalence (25.7%) was reported in Khartoum State by Ebrahim (2013) by RBPT, Also a lower rate (5%) was reported in Kassala State by El-Ansary *et al.*, (2001). However, based on c-Elisa, a higher result of 24.9% was obtained by Angara *et al.*, (2009) in Kuku Dairy Scheme Khartoum North, Sudan. They also reported higher

herd prevalence rate (90%) using the same test. The higher rates in Kuku Scheme were due to high foreign blood breed kept beside the presence of the animals in close contact in collections.

Almost the same rate (5%) of camel brucellosis was reported by El-Taweel (1999) in Egypt. However, higher rates of 7.0%, 7.02%, 8% , and 15.8%, were also reported by Radwan *et al.*, (1995), El-Boshy *et al.*, (2009), Mohammed *et al.*, (2013) and Dawood (2008) in Saudi Arabia, Nobaria city (Egypt), Abu Dhabi and Jordan respectively. In Sudan Raga (2000) and Saad (2013) reported slightly higher rates of camel brucellosis of 5.3% and 5.8% in Darfur and Khartoum States respectively. Lower/negative rates of camel brucellosis were reported in Kassala State, Sudan (0%) United Arab Emirates (1.5%), Ethiopia(1.5%) and Libya (4.1%), by El-Ansary *et al.*, (2001), Afzal and Sakkir (1994), Warsame *et al.*,(2012) and Gameel *et al.*, (1993) respectively.

Regarding small ruminant brucellosis, the infection rate reported in the current study is far lower than that reported by Shuaibi (1999) in the Palestine (72.9%). The very high rate of infection in small ruminant reported by Shuaibi (1999) was due to the fact the herds tested were selected purposely for being suspected to the infection due to abortions or the presence of human cases, whereas herd tested in the current study were selected randomly. The result of goats brucellosis obtained in the current study was almost similar to that obtained by Omer *et al.*, (2000) in Eritrea in case of herd prevalence (36% versus 35.9%) and individual animal prevalence (9% versus 8.2%). On the other hand the herd prevalence rate in case of sheep brucellosis was higher in this study than the rate of (38% versus 16.7%) obtained by Omer *et al.*, (2000) in Eritrea, this also true for the individual animal prevalence. All

comparisons were based on RBPT. Lower sheep prevalence (0.6%) was reported in Yemen by Hosie *et al.*, (1985). The current result was higher than (0%) rate in sheep reported by Cadmus *et al.*, (2006) in Nigeria.

In Sudan almost similar result (1% prevalence rate) was reported in sheep by El-Ansary *et al.*, (2001) in Kassala State, Sudan. Ali (2013) reported lower prevalence in North Kordofan (2.5%) by RBPT in sheep. Omer *et al.*, (1989 to 1990) reported lower prevalence in (Alkdr) quarantine, Khartoum State and (Portsudan) quarantine, Red sea State where the prevalence rate of sheep brucellosis was 0.01%. Also Omer *et al.* (2007) reported low prevalence in Kassala Eastern Sudan during 2004- 2006; the result were 0.1%, 0.4% in 2004, 2005 however, in 2006 the prevalence was 2.1% in sheep. Musa (2005) reported higher rate (3.3%) of sheep brucellosis in Darfur States. Also higher result was reported by Omran (2011) in Sinnar (4.1%) in sheep. According to Omer *et al.*, (2000) almost similar result of the individual prevalence of 3.8% in goats was obtained in Eritrea, but a higher (33.3%) unit (herd) prevalence was reported. In Yemen (Hosie *et al.*, 1985) reported lower (0.4%) prevalence in goats. El-Ansary *et al.*, (2001) reported similar individual prevalence (4%) in goats in Kassala State, Sudan. A lower prevalence of 2.0% in goats was reported by Magzub (2001) in Khartoum. Omer *et al.*, (2007) reported lower prevalence in goats in Kassala eastern Sudan during 2004-2006, in where the prevalence rate was 0.2%, 0.6 % in 2004, 2005 while in 2006 it was 5.6%.

In this study there was significant association between the rate of infection and the owner education ( $p$ - value = 0.120) was reflected on the management of the farms, although 62% of the owners used to vaccinate their animals with the officially

recommended vaccine ,yet only 4% of the herds were vaccinated against brucella. The situation resemble that in Afar region in which the high prevalence of brucella antibodies (9.7%) and wide spread nature of brucella infection were attributed to the absence of brucella vaccination (Teshale *et al.*, 2006). Keeping multi species in 55%of the farms ( $p$ - value = 0.020) is also associated with brucella infection in univariate analysis this result similar to that obtained by (Al-Majali *et al.*, 2009; Muma *et al.*, 2007) in which they found the practice of mixing of cattle, either through grazing or sharing of watering points is an important risk factor for brucellosis .Megersa *et al.*, (2011) also reported that keeping more than two animal species at household level was found to be risk factor for cattle and camels brucella infection when compared to those animals from households that keep only two animal species. This may suggest a possibility of cross species transmission of brucella infection under such mixed herding. The association between the infection and the occurrence of abortion cases in the herd ( $p$ - value = 0.018) and the owner knowledge about the causes of abortion ( $p$ - value = 0.041) is similar to the result obtained by Berhe *et al.* ( 2007), Islam *et al.*,(2010), Matope *et al.*,( 2011),Tesfaye *et al.*,(2011) and Rahman *et al.*,(2011).In individual cattle, the associated of the history of abortion with brucellosis seroprevalence comes in agreement with Aulakh *et al.*,( 2008) who found that there was significant association between the disease and abortion.

Based on the logistic regression model the presence of abortion case (OR.001, CI.00-.247,  $p$ -value.014) and source of water were the only significant ( $P<0.05$ ) risk factors. This result consolidate the findings of Rijpens, *et al.*, (1996) that brucellosis causing a decrease in reproductive efficacy, an increase abortion rate and the bad

sanitary measures in farms plays major role in spreading the infection. Also this result comes in agreement with Tesfaye *et al.*, (2011) who revealed that percentage of 4.4% abortion was associated with brucella antibodies ( $P<0.05$ ) in Addis Ababa. In Bangladesh Islam *et al.*, (2010) also found that increased odd of seropositivity of brucellosis was observed in aborted animal and Rahman *et al.*, (2011) also found a significant association between abortion and occurrence of brucellosis ( $P < 0.01$ ). Similar result also observed by Berhe *et al.* (2007) when investigated 26 herds in Tigray Region of Ethiopia by Fisher's Exact Test revealed that seropositivity to brucellosis had statistically significant association with history of previous abortions and stillbirths. Similar studies on risk factor of brucellosis in Khartoum State by (Ishag, 2013) and (Saad, 2013) in which they found education level, abortion cases, source of feed and water source were significant in sheep and herd size, age of animal and mixed breed were significant in camel respectively.

## CONCLUSION

Prevalence of cattle brucellosis is higher than other species, numbers of camel in the locality are very few so the prevalence of camel in this study cannot give a real idea however, it gives estimation about presence of brucellosis. There is no increase in prevalence in sheep and goat brucellosis comparing with other localities. Abortion case and source of water were significant ( $P<0.05$ ). Finally the risk factors that contribute to the spread of brucellosis in animals in Jebel Aolia may include mixing of infected animals with healthy animals, mixed herding of different animal species, lack of brucellosis control measures and lack of knowledge of brucellosis.

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