



Seroprevalence of Brucellosis Among Sheep and Goats in Sinnar State, Sudan

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Abstract

This study was conducted in Sinnar State to estimate the prevalence of brucellosis in sheep and goats in year 2015 between February-May a four months period. All seven localities of the State were visited with the objectives to study the epidemiology of brucellosis, estimate its prevalence, and identify the endemic area in the State. four hundred serum samples were collected divided as follows: 200 from sheep and 200 from goats. All samples were screened by the Rose Bengal Plate test (RBPT). Competitive ELISA (cELISA) was used as a confirmatory test for only the positive sera. The prevalence was 1.5% and 33.3% in sheep, 3.5% and 42.9% in goats, when tested by RBPT and cELISA respectively. The highest prevalence, in sheep was 5% and 10% in goats in Abu Hejar Locality when tested by RBPT, all these animals were positive with cELISA. Post Hoc test was used and there was a significant difference between Sinnar and Singa also between Abu Hejar and Al Dali at $p \geq 0.05$. Between sheep and goats Post Hoc test showed a significant difference with significance level 0.004 at $p \geq 0.05$.

Keywords: Seep; goat; Brucellosis; Rose Bengal Plate test; cELISA

INTRODUCTION

Brucellosis is an important zoonosis that causes abortion in naturally infected small ruminants and is of great public health concern in many countries (Benkirane *et al.*, 2015). Brucellosis is an economically important disease of livestock causing reproductive losses through infertility, delayed heat, and economic losses from international trade bans (McDermott and Arimi, 2002).

Sir David Bruce isolated *B. melitensis* from a British soldier who died from Malta fever in Malta in 1887. In 1905, Zammit, a Maltese physician, identified goats as the source of infection (Ariza and Bosilkovski, 2007). *Brucella Ovis* is the causative agent of ovine brucellosis, primary manifestations are lesions of the epididymis, tunica, and testis in rams; placentitis and abortion in ewes; and occasionally prenatal death in lambs (Merck, 2013). The signs of brucellosis in goats are similar to those in cattle, characterized by abortion and mastitis. Abortion usually occurs in the final 2 months of pregnancy, and in a susceptible flock it may reach epidemic proportions. Arthritis and orchitis may occur (Merck, 2013).

There are no previous studies on brucellosis in small ruminants in the State, as the state is rich in livestock so many studies must be conducted to provide more information about the disease in the area.

MATERIALS AND METHODS

- **Study Area:**

A cross-sectional analytical study had been conducted in the year 2015 during the period between February- May in Sinnar State localities (Sinnar, Singa, Eastern Sinnar, Al Suki, Al Dindir, Abu Hejar and Al Dali).

Sinnar State is located in the southeastern part of Sudan (250 km from Khartoum) between latitudes 12:5 and 14:7 and longitudes 32:58 and 35:42.

- **Samples size:**

Sample size of the studied animals was determined statistically according to the formula given for simple random method. The relevant formula according to Singh and Masuku (2014) for 95% confidence and 5% precision is:

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

Where:

n= required sample size.

P_{exp}= expected prevalence.

d= desired absolute precision.

Brucella growth was confirmed by bacteriological and biochemical tests as described by Maymona et al. (2014).

- **Sampling procedures:**

After shaving and swabbing with 70% alcohol and drying, five ml of blood collected from jugular vein using disposable plain vacutainer. Vacutainers were labeled and saved in icebox and sent to Sinnar Veterinary Researches Laboratory. After centrifugation at 300 rpm for 5 minutes, the serum was separated from the clot with a pipette with a disposable tip to an Epindorph tubes, labeled and frozen at -20°C until used.

Methods:

- **Rose Bengal Plate test (RBPT):**

Rose Bengal antigen was supplied by the Department of Brucella in the Central Veterinary Laboratory at Soba, Khartoum. The results were documented according to the presence or absence of agglutination or ring formation (Singh and Masuku, 2014).

- **Enzyme-linked immune sorbent assay (c-ELISA):**

Just positive samples of RBPT were tested by cELISA. The test was carried out as described by Animal Health Veterinary laboratory Agency, U. K (Singh and Masuku, 2014).

RESULTS

- **Rose Bengal Plate Test (RBPT):**

Prevalence of brucellosis in sheep, goats in Sinnar state by RBPT was 1.5% in sheep when n= 200, in goats was 3.5% when n= 200. In Sinnar locality it was 1.7% in sheep and goat, and Al Suki recorded 5% in both species. Abu Hejar recorded 10% and 10% in sheep and goat respectively. Other localities recorded 0% in sheep, but the prevalence was 5%, 10% and 0% in East Sinnar, Al Dindir and Al Dali respectively (Table: 1). Oneway ANOVA was used to determine the differences between species when Rose Bengal test was used which showed significant variances (.008) at (P≤0.05) (Table 1).

Table 1: Prevalence of brucellosis in sheep, goats and humans, in Sinnar state by RBPT:

Locality	Sheep sera		Goat sera		Sig. .008
	+ve	%	+ve	%	
Sinnar	1	1.7	1	1.7	
Singa	0	0	0	0	
E. Sinnar	0	0	1	5	
Al Suki	1	5	1	5	
Al Dindir	0	0	2	10	
Abu Hejar	1	5	2	10	
Al Dali	0	0	0	0	
Total	13	1.5	7	3.5	

Wad Al Nayyal area recorded the highest prevalence in the state based on zoning in each locality which was 20% in goats when in sheep it was 10%. There was an equality in the prevalence between sheep and goats in the abattoir of Al Suki locality and Al Galaa area in Sinnar locality, as they recorded 10% and 6.7%, respectively. On the other hand, the village of Wad Al Abbas in East Sinnar locality recorded 0% for sheep and 5% for goats. with RBPT (Table 2).

Table 2: Prevalence of Brucella antibodies in sheep and goat according to area in each locality by RBPT

Locality	Area	Sheep		Goat	
		+ve	%	+ve	%
Sinnar	Al Galaa	1	6.7	1	6.7
E. Sinnar	Wad Al Abbas Village	0	0	1	5
Al Suki	Al Suki Abattoir	1	10	1	10
Abu Hejar	Wad Al Nayyal	1	10	2	20

Percentage of antibodies of brucella in sheep, goats in Sinnar state by ELISA was 33.3% when n= 3 in sheep, 42.9% when n= 7 in goats. In Sheep the percentage was 100% when n= 1 in Al Suki and 0% in the others. Prevalence of brucella antibodies in goats was 100% when n=1 in Sinnar (Algalaa area) and Al Suki Abattoir, in Abu Hejar (Wad Al Nayyal) when n= 2 the prevalence was 50% and in the others was 0%. (n=number of RBPT positive samples) (Table 3).

Table .3: Percentage of brucellosis in sheep and goats in Sinnar locality by ELISA:

Locality	Sheep		Goat	
	+ve	%	+ve	%
Sinnar	0	0	1	100
East Sinnar	0	0	0	0
Al Suki	1	100	1	100
Al Dindir	0	0	0	0
Abu Hejar	0	0	1	50
Total	1	33.3	3	42.9

Post Hoc Tests had showed no significant differences between overall samples of sheep and goats (.369) (Table 4).

Table 4: Post Hoc Test between species using RBPT:

Species	Species	Sig.
Sheep	Goat	.369

Post Hoc test when used to complain the significant variance between sheep and goats within localities showed no significance differences in all localities except in Al-Dindir locality there were significant difference between sheep and goat (.007) at ($p \leq 0.05$) (Table 5).

Table 5: Post Hoc tests between species within localities:

Locality	Sig.
Sinnar	1.000
Singa	1.000
E. Sinnar	.383
Al Suki	1.000
Al Dindir	.007
Abu Hejar	.587

DISSCUSION

Brucellosis occurs in all farm and some wildlife animal species and humans in Sudan (Wegdan *et al.*, 2016). The current study is the first one, which investigated brucellosis in small ruminants in the seven localities of the State of Sinnar, covering a large number of large farms, small breeders and some slaughterhouses. The study was conducted during using cELISA as a confirmatory test for RBPT positive samples.

The Rose Bengal Plate Test (RBPT) is often used as a rapid screening test (RuizMesa *et al.*, 2005) The sensitivity of RBPT is reported to be very high (>99%) but the specificity can be disappointingly low (Barroso *et al.*, 2002). As a

result, the positive predictive value of the test is low and a positive test result thus requires confirmation by a more specific test (Smits and Kadri, 2005).

Prevalence of brucellosis in sheep and goats using RBPT was 1.5% and 3.5%, respectively. This finding was in line with El-Ansary et al. (2001) who found 1% for sheep and 4% for goats in Kassala State, and Magzub, (2001) reported a prevalence of 2.0% in goats in Khartoum State. The results also were in agreement with the findings of Akbarmehr and Ghiyamirad, (2011) who reported a prevalence rate of 4.2% in small ruminants in Iran. Omer et al., (2007) also reported prevalence rate of sheep Brucellosis of 0.01% in Libya. This study also was in agreement with the results of Omran (2011) who reported 4.73% in goats but did not agree with his results obtained in sheep 4.95%. Reviriego et al. (2000) reported lower prevalence rates than those reported in this study 0.7% in sheep and 0.1% in goats. Abu Hejar and Al Dindir localities showed very high prevalence in goats, which was the highest one in the State 10% and 10% in goats and low in sheep 5%, 0%, respectively. El-Ansary et al. (2001) in Kassala State, Sudan 1% in sheep reported a similar result. Abdallah et al. (2015) found the overall seroprevalences of ovine brucellosis 2.5% (n = 8) with 95% CI from 0.78 to 4.22 and 22.0% (n = 70) with 95% CI from 17.5 to 26.6, by RBPT and SAT, respectively. However, no any; 0.0% (0/8) with 95% CI from 0.00 to 0.00, of the samples which were RBPT-positive was positive by cELISA. Mohamed *et al.* (2018) studied caprine brucellosis and found the overall seroprevalence of 11.4% (n=35), out of these 35 RBPT-positive samples, the positivity of 18 and 17 were confirmed by SAT and cELISA, respectively. Shuaib et al. (2018) found a percentage of 9.3 (n=47, 95% CI 6.80 - 11.9) of the RBPT-screened sheep serum samples were positive reactors, of which the cELISA confirmed the positivity of 55.3% (26/47). El Sanousi (2012) explained the decrease in the spread of *brucella* to several factors, including the climate of Sudan, which is characterized by long daylight hours and high temperatures, in addition to low humidity, which affects the continuity of brucella's survival. This is in addition to the fact that the Bedouin pastoral system is inferior to the system of raising goats and sheep in Sudan. It reduces the gathering of animals together and their stay outside the pens for long periods, which helps to better ventilate the pens, clean them, and expose them to the sun.

Algalaa areas reported the higher prevalence in Sinnar locality 6.7 in both species, while Wad Al-abbas village in East Sinnar reported 5% in goat as the highest rate, Al-Suki abattoir reported 10% in both and Wad Al-Nayyal reported 10% and 20% in Abu-Hejar locality in sheep and goats respectively. This highly prevalence in some areas compared with other areas in same locality which recorded 0% may be attributed to the big number of animals in one farm and bad husbandry practice and This also may be attributed to the system of management applied (Tigist et al., 2011).

These cELISA provide similar or better sensitivity than both RBT, but like classical tests, ELISA are unable to differentiate infected animals from animals recently vaccinated with the Rev-1 vaccine (Ferreira et al., 2003). Thirty three percent of the samples which were positive in the RBPT were confirmed positive when using ELISA as confirmatory test in sheep sera samples. In Al Suki locality all positive samples to RBPT were confirmed positive by ELISA, while other localities were negative with RBT and were not tested with ELISA. The percentage of brucella antibodies in goats 73 when tested by ELISA was 42.9% in the whole State, the highest rate 100% which was reported in Sinnar and Al Suki localities may be attributed to bad housing in these two localities. The rate 50% was reported in Au Hejar locality. Singa, East Sinnar, Al Dindir and Al Dali localities were negative with RBPT.

CONCLUSION

It could be concluded that despite of its low prevalence, brucellosis represents a problem in the State that threatens animal health and public health together and should be taken seriously.

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Competing Interest

The authors declare that they have no competing interests.

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