Research Article

SEROPREVALENCE OF BRUCELLOSIS AMONG CATTLE IN SINNAR STATE, SUDAN

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Abstract

This study was conducted in Sinnar State. The objectives were to study the epidemiology of brucellosis, estimate its prevalence, and identify the endemic area in the seven localities of the State. Four hundred serum samples collected from cattle were divided as follows: 40 from adult males, 178 from heifers and 182 from cows. All samples were subjected to serological investigation using the Rose Bengal Plate test (RBPT). Competitive ELISA (cELISA) was used as a confirmatory test for the positive sera. The prevalence of *brucella* antibodies in the State was 6.5% with RBPT and 46.2% of these positive sera were confirmed as positive with cELISA. The highest prevalence was 39.1% in Singa locality when tested by RBPT, all these samples were confirmed positive with cELISA. It could be concluded that despite of its low prevalence, brucellosis constitutes a big problem in the State, which threats both animal and public health. Control measures should promptly commence and the spread of bovine brucellosis should be taken seriously.

Keywords: Cattle; Brucellosis; Rose Bengal Plate test; cELISA

INTRODUCTION

Brucellosis in cattle is usually caused by biovars of *Brucella abortus* (Bankole *et al.*, 2010). Clinically, the disease usually characterized by one or more of the following signs: abortion, retained placenta, still or weak birth, orchitis, epididymitis and rarely arthritis, with excretion of the organisms in uterine discharges and in milk (OIE, 2012). The Centre of Disease Control and Prevention (CDC) has labeled Brucella species as highly weaponizable (Atluri *et al.*, 2011). The duration of the disease and its long convalescence means that brucellosis is an important economic as well as a medical problem for the patient because of time lost from exhibiting activities (Corbel, 2006).

In Sinnar State few studies were conducted to detect the prevalence of brucellosis, but it was restricted to Sinnar area (Omran, 2011). Therefore, a comprehensive study of the prevalence of brucellosis in different localities of Sinnar is needed.

MATERIALS AND METHODS

• Study Area:

A cross-sectional analytical study had been carried out during the period between February- May 2015 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 latitudes12:5 and 14:7 and longitudes 32:58 and 35:42. Total cattle population in the state was estimated at 6,383,134 heads, dominated by Kenana and Butana breeds (Ministry of Agriculture, Sinnar, 2011) state-website - http://www.sennarstate.gov.sd). Other cattle breeds, which are foreign, are exemplified by Kory (from Chad) and Red Ambararro (from Ethiopia).

- **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 = (1.96) 2Pexp (1-Pexp)
    d2

Where:
n= required sample size.
Pexp= expected prevalence.
d= desired absolute precision.

- **Sampling procedures:**

Five ml of blood collected from jugular vein using disposable plain vacutainer after shaving and swabbing with 70% alcohol and drying, 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):**

The prevalence of bovine brucellosis in Sinnar State was 6.5% (n= 400) using the Rose Bengal Plate test. The prevalence of brucella antibodies in cattle was 7.3% in Sinnar locality, 20% in Singa, 3.3% in East Sinnar, 30% in Abu Hejar, 0% in Al Suki, Al Dindir and Al Dali (Table 1).

Table 1: Prevalence of Brucella antibodies in cattle in Sinnar State by RBPT.

Locality	Cattle serum	
	+ve	% Of positive
Sinnar	11	7.3
Singa	10	20
E. Sinnar	2	3.3
Al Suki	0	0
Al Dindir	0	0
Abu Hejar	3	10
Al Dali	0	0
Total	26	6.5

Traditional cattle farms in Singa recorded the highest prevalence based on zoning in each locality which was 30%, 20% in Algalaa dairy farm in Sinnar locality, 20% in Wad Al Nayyal in Abu Hejar and 3.6% in Rowena village in East Sinnar locality with RBPT (Table 2).

Table 2: Prevalence of Brucella antibodies in cattle according to area in each locality by RBPT

Locality	Area	Cattle serum	
		+ve	% of positive
Sinnar locality	Algalaa dairy farm	4	20
Singa	Traditional farm	3	30
East Sinnar	Rowena	2	3.6
Abu Hejar	Wad Al Nayyal	2	20

When sample were compared according to sex and age, the prevalence in the State was 10.4% in cows, 2.5% in bulls and 3.4% in heifers. The prevalence in Sinnar was 10.3%, Singa 39.1%, Abu Hejar 21.2% and 0% in E. Sinnar, Al Suki, Al Dindir and Al Dali in cows. The prevalence in males was 6.7% in Sinnar and 0% in other localities. Prevalence in heifers was 4.4% in Sinnar, 4.5% in Singa and 7.4% in E. Sinnar. It was also 0% in other localities (Table 3).

Table 3: Prevalence of brucellosis in cattle according to sex and age when tested by RBPT

Locality	Cows		Bulls		Heifers	
	+	% of positive	+	% Of positive	+	% Of positive
Sinnar	7	10.3	1	6.7	3	4.4
Singa	9	39.1	0	0	1	4.5
E. Sinnar	0	0	0	0	2	7.4
Al Suki	0	0	0	0	0	0
Al Dindir	0	0	0	0	0	0
Abu Hejar	3	21.4	0	0	0	0
Al Dali	0	0	0	0	0	0
Total	19	10.4	1	2.5	6	3.4

- **Confirmation of the positive sera using Enzyme-linked immune sorbent assay (c-ELISA):**

The percentage of *brucella* antibodies in the 26 sample which were positive with RBPT in Sinnar State was 46.2%. Of this, 54.5% was in Sinnar locality, Singa recorded prevalence of 50% and 33.3% in Abu Hejar locality (Table 4).

Table 4: Percentage of Bovine Brucellosis in Sinnar State by ELISA

Locality	Cattle serum	
Sinnar	+ve	% Of positive
	6	54.5
Singa	5	50
E. Sinnar	0	0
Al Suki	0	0
Al Dindir	0	0
Abu Hejar	1	33.3
Al Dali	0	0
Total	12	46.2

When samples tested by cELISA, three areas in Sinnar locality showed prevalence of 100%. These were Sinnar Sugar Factory Farm, Sinnar Abattoir and Western Countryside. Singa traditional farms reported the highest prevalence in Singa locality which was 66.7%. In Abu Hejar the highest prevalence was 50% in Wad Al Nayyal area (Table 5).

Table 5: Prevalence of Brucella antibodies in cattle according to area in each locality by ELISA

Locality	Area	Cattle serum	
		+ve	% Of positive
Sinnar locality	Sinnar Sugar factory farm	1	100
	Sinnar Abattoir	1	100
	Western Countryside	2	100
Singa	Traditional farm	2	66.7
Abu Hejar	Wad Al Nayyal	1	50

When results ordered according to animal's sex and age the percentage was 42.1% (n=19) in cows, 100% (n= 1) in bulls and 50% (n= 6) in heifers in Sinnar State.

Percentage of Brucellosis in cows was 57.1% in Sinnar locality, 33.3% in Singa, 33.3% in Abu Hejar and 0% in other localities. Percentage in bulls was 100% in Sinnar and 0% in others. Percentage in heifers was 33.3% in Sinnar, 100% in Singa and 0% in the other localities (n= positive samples for RBPT) (Table 6).

Table 6: Percentage of Bovine Brucellosis in Sinnar state according to animal's sex and age using ELISA:

Locality	Cows		Bulls		Heifers	
	+	% Of positive	+	% Of positive	+	%Of positive
Sinnar	4	57.1	1	100	2	33.3
Singa	3	33.3	0	0	1	100
East Sinnar	0	0	0	0	0	0
Suki	0	0	0	0	0	0
Dindir	0	0	0	0	0	0
Abu Hejar	1	33.3	0	0	0	0
Al Dali	0	0	0	0	0	0
Total	8	42.1	1	100	3	50

- **Analysis of variances (ANOVA):**

One-way ANOVA was used to determine the differences between localities when Rose Bengal test was used. It showed significant differences at *P (0.05)* (Table 7). ANOVA between cows, ox and heifers shows significant variances (Table 8).

Table 7: One-way ANOVA-between Localities using RBPT:

	Df	Mean Square	Sig.
Between Groups	6	313.	000.
Within Groups	1313	051.	
Total	1319		

Table 8: One-way ANOVA-between cows, bulls and heifers using RBPT:

	Df	Mean Square	Sig.
Between Groups	2	.453	.001
Within Groups	797	.060	
Total	799		

DISSCUSION

According to my knowledge, this study was the first one which studied brucellosis in all the seven localities of Sinnar State.

This research aimed to study the epidemiology of brucellosis, estimate its prevalence, and identify the endemic area in Sinnar State using cELISA as a confirmatory test for RBPT positive samples.

The RBPT is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction (WHO, 2006).

In Sinnar State there were few records about brucellosis. This study revealed that Sinnar and Singa localities had high prevalence of bovine brucellosis. This may be attributed to the mixing of the local herds with the large numbers of infected Ethiopian cattle that cross the Sudanese border (Hundum and Regasse, 2009).

The percentage of seropositive cattle in Sinnar State localities by RBPT was 6.5%. In Sinnar locality was 7.3%, Singa was 20%, East Sinnar 3.3%, and in Abu Hejar 10%, while no reactors were identified in Al Suki, Al Dindir and Al Dali localities. The percentage of seropositive cattle in all localities of Sinnar State agreed with those of El -Ansary *et al.* (2001) who reported 5% prevalence rate in cattle in Kassala State and with Minja (2002) in Nigeria (7.6%), but disagreed with that reported by Gasim (2009) in Khartoum State (22.4%). Many researchers in Africa reported high prevalence rates which disagree with this study such as Hundum and Regasse (2009) who reported 15.2% in Ethiopia, Bertu et al. (2010) reported 15% in Nigeria, Magona et al. (2009) reported 18.1% in Uganda.

Scacchia et al. (2013) reported prevalence rates of 0.3 % and 2.77% in Libya and Eritrea, respectively which were lower than the current study (6.5%). This also may be attributed to the system of management applied (Tigist et al., 2011).

Samaha et al., (2008) reported a prevalence rate of 7.2% of brucellosis in cattle in Benisuef, Egypt. This disagrees with the current study (6.5%) which also disagrees with Junaidu et al., (2008) who reported a prevalence of 5.1% in Black Bengal. These differences may be attributed to the area of study, breed of animals studied and type of test applied.

In the present study, the prevalence of Brucella antibodies in cattle according to area in each locality by RBPT revealed that the highest rate in Sinnar locality was in Algalaa dairy farm which was 20%, and lowest rate was reported in West countryside villages and in Sinnar abattoir which was 6.7% and 5%, respectively. This variation in the disease incidence may be due to the intensive system of breeding practiced. This result was similar to the findings of Omran (2011) who reported a prevalence of 24% in Sinnar area and to Ebrahim (2013) (25.7%) in Khartoum State. These variations may be attributed to the time and area of the study and management systems adopted.

This study showed that the highest rate (30%) in Singa locality was in traditional farms in Singa town and the lowest rate (10%) was in Singa Abattoir. Poor housing, sharing of water sources and inappropriate husbandry practices may be the main factors that led to the increasing prevalence of the disease in Singa locality. East Sinnar locality reported the lowest rate in the State (3.3%). This may be due to the isolation of animals from each other. This rate was similar to the findings of Sarker et al. (2014) in Sudan, Islam et al. (2013) in Khartoum State and Berhe et al. (2007) in Ethiopia who reported 2.1%, 3.85% and 4.2%, respectively.

In the current study, the prevalence, with RBPT in cattle was 10.4% in cows and 2.5% in bulls. This result was similar to that reported by Hussain et al. (2008) who reported 12% in cows and 1.9% in bulls but disagreed with Kubuafor et al. (2000) who reported 8.5% in cows and 9% in bulls. This may be attributed to the fact that the prevalence of brucellosis in males is lower than that in the females (Kubuafor et al., 2000).

The prevalence of the disease in females, males and heifers in this study was comparable to Mellau *et al.* (2009) who reported that prevalence in cows (14.3%) was higher than heifers (7.54%). This may be due to age factor, as high prevalence depends on multiparous status increases animal susceptibility to infection, and presence of higher concentration of erythritol in the uterus which favors rapid multiplication of the pathogens in adult cows as compared to heifers or bulls (Alton, 1985; Rezaei *et al*., 2010); and this causes the infection to persist, most commonly, in sexually mature animals (Adams, 1998).

The percentage of positive sera to brucellosis in cattle in the State was very high with RBPT samples and only 46.2% of these positive samples were confirmed by ELISA. Akbarmehr and Ghiyamirad (2011) in Iran reported a lower percentage of 3.66% in cattle. Makita *et al.* (2011) reported 5.0% in Kampala by cELISA which disagreed with the percentage reported in this study (46.2%).

This study showed that in Singa locality, traditional farms gave the highest percentage of confirmed positive samples to bovine brucellosis was 66.7%, Singa dairy farms gave 50%. The high rates of Singa locality may be attributed to the very poor housing in the area.

 Statistical analysis showed significant differences between ox (2.5%), cows (10.4%) and heifers (3.4%). There was a significant difference (.001) at (p $\leq$0.05) when ANOVA was used. These variations may be due to the system of breeding and replacement within the herd. Also, there were significant differences between Localities (.000) at (p$\leq$0.05).

In this study, non-significant differences were shown between Singa and Abu Hejar equal to (.628). This may be due to inappropriate housing and sharing of water sources in Singa area (Omer *et al.,* 2007). East Sinnar showed a significant difference with Abu Hejar equal to (.001). Abu Hejar locality and Al Dali locality also showed a significant difference equal to (.000) at (p$\leq$0.05). Non-significant differences were shown between Al Dindir and Abu Hejar (.000) and between Al Dindir and Al Dali localities equal to (.638). Post Hoc Test also showed non-significant differences between Al Suki and Al Dali (.545), Al Dindir and Al Dali (.638) and between East Sinnar and Al Dindir (.407).

CONCLUSION AND RECOMMENDATIONS

It could be concluded that despite of its low prevalence, brucellosis represents a problem in the State that threats animal health and public health together and should be taken seriously. Isolation of pregnant females and increasing in vaccine production are effective for control program. Awareness among humans who have close contact with animal, especially in small villages is an essential preventive tool.

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Competing Interest

The authors declare that they have no competing interests.

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