



## Brucellosis in Sinnar State, Sudan: A One Health Approach

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DOI: [10.5281/zenodo.10041822](https://doi.org/10.5281/zenodo.10041822)

Submission Date: 22 Sept. 2023 | Published Date: 26 Oct. 2023

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### Abstract

Stratified random samples consisting of 400 bovines, 200 ovine, 200 caprine and 120 human serum samples were collected from dairy farms and slaughterhouses in the seven localities of Sinnar State to detect the seroprevalence of Brucellosis using the One Health approach. The seroprevalence of brucellosis was 6.5% in bovine, 3.5%, in caprine, and 1.5%, in ovine. This difference in prevalence was statistically significant with  $p \leq 0.05$ . The overall prevalence of human brucellosis was 10%. The prevalence of human brucellosis was 11.25% (n= 80) with the Rose Bengal Plate test in individuals working with cattle. In dairy cows the prevalence in dairy cattle (6.7%) was significantly higher than bulls (5%) prepared for slaughtering, with  $p \leq 0.05$ , while the prevalence in dairy farmers was slightly higher (11.3%), compared to (11.1%) in slaughterhouses' workers. The difference in the prevalence between the seven localities was of high statistical significance with  $p \leq 0.05$ . There was significant correlation (0.968) between the prevalence of brucellosis in bovine and human, with  $p \leq 0.05$ . According to sex and age of cattle (cows, heifers, and bulls), the prevalence in Sinnar State was highest (10.4%) in cows, compared to 3.4% in heifers and 2.5% in bulls when Rose Bengal Test was used. This difference was statistically significant with  $p \leq 0.05$ , using one way analysis of variance. All samples had been tested by Rose Bengal Plate test (RBPT) and Competitive ELISA (cELISA) was used as a confirmatory test for the positive sera and only 46.2% of these positive sera had been confirmed positive with ELISA. It could be concluded that the high prevalence of brucellosis in animals and humans is a real threat to public health.

**Keywords:** Brucellosis; Human Brucellosis; RBPT; cELISA

## INTRODUCTION

The concept of one health is defined as a common approach between several sectors and disciplines, as it works at the global and even local levels to achieve optimal health results in the interdependence between humans, animals, plants, and the environment that they share (CDC and NCEZID, 2022). The one health approach helps support global health security by improving cooperation and communication between humans, animals, and the environment, address common health threats such as zoonotic diseases, antimicrobial resistance, and food safety and security (CDC and NCEZID, 2019).

The hazards of Brucella lie in its association with multiple species of animals and their dairy products and humans (humans in contact with affected animals and their product). Its presence in the environment in a significant way had importance in many sciences, preventive, zoonosis, public health and one health approach. The infection in humans is primarily caused by direct contact with infected cattle, sheep and goats, pigs, dogs, or by ingesting unpasteurized and contaminated animal products. Also, infection by inhaling airborne agents were also reported (Pappas et al., 2006; HCL, 2020).

Brucellosis is one of the most common zoonotic bacterial diseases and is recognized as a re-emerging and neglected zoonotic disease (Corbel, 2020). This disease affects mainly during daily activities of livestock as well as from animal produce (Pal et al., 2017). Although brucellosis was being almost eradicated from most of the developed countries, it is still a major public and human and animal health problem in many developing countries, where livestock are a major source of food and income (FAO, 2003).

While, Brucellosis in Sudan was first reported from human cases as early as 1904, (Hasseb, 1905), *Brucella abortus* was first isolated from a dairy farm in Khartoum (Bennett, 1943). In Sinnar State few studies were conducted to detect the prevalence of brucellosis, but it was restricted to few areas (Omran, 2011). Therefore, a comprehensive study of the prevalence of brucellosis in different localities of Sinnar State is needed. This study aims to detect the prevalence of Brucellosis using one health approach.

## MATERIALS AND METHODS

### Study Area

The study was 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 latitudes 12:5 and 14:7 and longitudes 32:58 and 35:42.

### Area description

The dairy sheds in these farms were in a very poor hygienic conditions, usually cleaned every 3-7 days, and animals were milked in a different area from the rest of the herd, but the pregnant animals usually gave birth inside the barn and there was no separate area for the new borne.

As for the slaughterhouses, samples were taken from the two slaughterhouses located in Sinnar locality. Cleanliness and caring for the carcasses were poor, and all the workers were found not to observe personal hygiene or the cleanliness of the slaughterhouse.

### Samples size:

Sample size from animals were determined statistically according to the formula given for stratified random method and cross section sample for human samples. The relevant formula for 95% confidence and 5% precision according to Singh and Masuku (2014):

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

Where:

n= required sample size.

P<sub>exp</sub>= expected prevalence.

d= desired absolute precision.

Accordingly, 24 farms were selected to collect data and 400 serum samples to detect the prevalence of bovine brucellosis, of these 360 samples were from dairy farms and 40 from bulls in the slaughterhouses. Two hundred sheep (Fifty milking and 150 from slaughterhouses) and two hundred goats (Fifty milking and 150 from slaughterhouses) were also tested for brucellosis antibodies. One hundred and twenty human blood samples were also collected twenty human blood samples of which 92 were from dairy farmers and 28 were from workers in the slaughterhouses (Table 1).

### Sampling methods

After shaving and swabbing with 70% Alcohol and drying, 5 ml of blood was collected from jugular vein using disposable plain Vacutainer, which then 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 Eppendorf tube, labeled and frozen at -20°C until used.

### Human samples

Five ml of blood were collected from the superficial veins on the dorsum aspect of the hand after cleaning and disinfection with 70% Alcohol using a disposable syringe, the blood was collected under the supervision of Sinnar hospital officials. The blood was collected in a plain vacutainer, labeled, saved in icebox and sent to Sinnar Veterinary Researches Laboratory for serum separation. The serum was frozen in labeled labeled Eppendorf tubes at -20°C until used.

**Table 1: Sample size**

Locality	Cattle sera	Dairy farmers sera	Sheep sera	Goat sera	Small ruminants' farmers sera
Sinnar	150	30	60	60	15
Singa	50	25	20	20	10
E. Sinnar	60	5	40	40	3
Al Suki	30	5	20	20	3
Al Dindir	50	5	20	20	3
Abu Hejar	30	5	20	20	3
Al Dali	30	5	20	20	3
Total	400	80	200	200	40

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

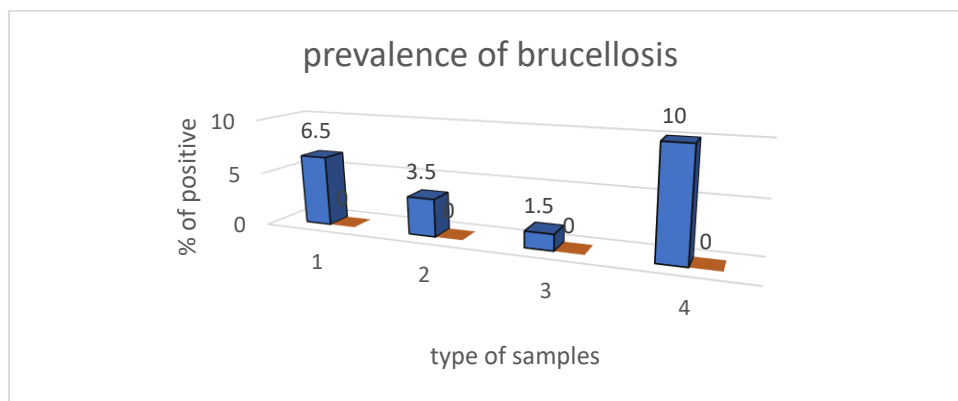
Rose Bengal antigen was supplied by the Department of Brucella- Central Veterinary Laboratory Soba. The results were documented according to presence or absence of agglutination or ring formation (Singh and Masuku, 2014).

**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**

The prevalence of bovine brucellosis was 6.5% (n= 400), 3.5% (n=200) in goats and 1.5% (n=200) in sheep and the overall prevalence of human brucellosis was (10%) (n=120) (Fig. 1).



\*1: Bovine. 2: Ovine. 3: Caprine. 4: Human.

**Fig. 1: Prevalence of Bovine and Human Brucellosis in Sinnar State using Rose Bengal Test (RBPT)**

The overall 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 2-1). The prevalence of human brucellosis was 11.25% (n= 80) in individuals working with cattle. The difference in the prevalence between the localities was of high statistical significance with  $p \leq 0.05$  (Table:2-1).

There was highly significant correlation (0.968) between the prevalence of brucellosis in cattle and human with  $p \leq 0.05$ . (Table: 2-2).

**Table 2-1: Prevalence of Bovine and Human Brucellosis in Sinnar State using Rose Bengal Test (RBPT)**

Locality	Cattle Serum	+ve	%	Humans' serum	+ve	%	Significance
Sinnar	150	11	7.3	30	3	10	0.000
Singa	50	10	20	25	3	12	
E. Sinnar	60	2	3.3	5	1	20	
Al Suki	30	0	0	5	0	0	
Al Dindir	50	0	0	5	0	0	
Abu Hejar	30	3	10	5	2	40	
Al Dali	30	0	0	5	0	0	
Total	400	26	6.5	80	9	11.25	

**Table 2-2: The correlation between bovine and human brucellosis**

		Bovine	Human
Bovine	Pearson Correlation	1	.986**
	Sig. (2-tailed)		.000
	N	8	8
Human	Pearson Correlation	.986**	1
	Sig. (2-tailed)	.000	
	N	8	8

\*\* . Correlation is significant at the 0.01 level (2-tailed).

According to sex and age of cattle (cows, heifers, and bulls), the prevalence in Sinnar State was 10.4% (n=183) in Cows, 2.5% (n=40) in bulls and in heifers it was 3.4% (n=177) when Rose Bengal Test was used. This difference was statistically significant with  $p \leq 0.05$ , using one way analysis of variance. The prevalence in Sinnar locality 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. (Table 3).

**Table 3: Prevalence of brucellosis in bulls, cows and heifers tested using RBPT**

Locality	Cows		Bulls		Heifers		significance
	+	%	+	%	+	%	
Sinnar	7	10.3	1	6.7	3	4.4	0.001
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	
<b>Total</b>	<b>19</b>	<b>10.4</b>	<b>1</b>	<b>2.5</b>	<b>6</b>	<b>3.4</b>	

In dairy sector the prevalence in dairy cattle (6.9%) was significantly higher than bulls prepared for slaughtering (2.5%), with  $p \leq 0.05$ , (Table 3), while the prevalence in dairy farmers was 11.3% and 11.1 in slaughterhouse workers (Table 4).

**Table 4: Comparison between +sera collected from cattle in abattoirs, dairy farms, and human**

Source of sample	Cattle sera	+ve	%	Humans' sera	+ve	%	Significance
Abattoirs	40	1	2.5	18	2	11.1	<b>0.03</b>
Dairy farms	360	25	6.9	62	7	11.3	
<b>Total</b>	<b>400</b>	<b>26</b>	<b>6.5</b>	<b>80</b>	<b>9</b>	<b>11.25</b>	

**Serum ELISA Test (cELISA):**

The percentage of Brucella antibodies in cattle's samples positive in RBPT when the positive samples were confirmed using ELISA, in Sinnar State was 46.2% and in human, it was 22.2%, (Table 4). Sinnar locality recorded high prevalence (54.5%) of brucellosis in cattle and 0% in humans. Singa recorded prevalence of 50% in cattle and 0% in humans. In Abu Hejar prevalence of brucellosis was 33.3% and 100% reported in cattle and human respectively, A percentage of 100% in humans was found in Abu Hejar locality (Table 5).

**Table 5: Prevalence of Brucella antibodies in cattle and humans in Sinnar state using (ELISA).**

Locality	Cattle serum		Human serum		Significance
	+	%	+	%	
Sinnar	6	54.5	0	0	<b>0.04</b>
	5	50	0	0	
E. Sinnar	0	0	0	0	
Al Suki	0	0	0	0	
Al Dindir	0	0	0	0	
Abu Hejar	1	33.3	2	100	
Al Dali	0	0	0	0	
<b>Total</b>	<b>12</b>	<b>46.2</b>	<b>2</b>	<b>22.2</b>	

- Percentage of bovine Brucellosis in Sinnar state according to herd composition using ELISA:**

According to animal's sex and age the prevalence was 42.1% (n=19) in cattle, 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 of heifers was 33.3% in Sinnar, 100% in Singa and 0% in the other localities (Table 6).

**Table 6: Percentage of bovine Brucellosis in Sinnar state according to herd composition using ELISA:**

Locality	Cows		Bulls		Heifers	
	+	%	+	%	+	%
Sinnar	4	57.1	1	100	2	33.3
Singa	3	33.3	0	0	1	100
E. 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
<b>Total</b>	<b>8</b>	<b>42.1</b>	<b>1</b>	<b>100</b>	<b>3</b>	<b>50</b>

The prevalence of brucellosis in sheep intended for slaughtering was 0% compared to 4% in goats and 0% in slaughterhouse workers. On the other hand, the prevalence of brucellosis in dairy farms was 2% in sheep, 3.3% in goat and 3.3% in workers of these farms (Table 7). The prevalence of human brucellosis was 2.5% (n= 40) with the Rose Bengal Plate test in individuals working with sheep and goat (Table 7).

**Table 7: Compare the prevalence of Brucellosis between small ruminants and humans in farms and slaughterhouses.**

Source of sample	Sheep sera	+ve	%	Goat sera	+ve	%	Human sera	+ve	%
Slaughterhouses	50	0	0	50	2	4	10	0	0
Farms	150	3	2	150	5	3.3	30	1	3.3
<b>Total</b>	<b>200</b>	<b>3</b>	<b>1.5</b>	<b>200</b>	<b>7</b>	<b>3.5</b>	<b>40</b>	<b>1</b>	<b>2.5</b>

Prevalence of Brucella antibodies in sheep, goats and humans who were in close contact with tested animals in Sinnar State by RBPT, was 1.5% in sheep, in goats was 3.5% and in humans who were in direct contact with sheep and goats was 2.5%. In Sinnar locality the seroprevalence of Brucella was 1.7% in both sheep and goats. Also, prevalence in samples collected from Al Suki and Abu Hejar locality was 5% in the two species. Abu Hejar showed prevalence of 20%

Locality	Sheep sera	+ve	%	Goat sera	+ve	%	Human sera	+ve	%	significance
Sinnar	60	1	1.7	60	1	1.7	10	0	0	<b>.001</b>
Singa	20	0	0	20	0	0	5	0	0	
E. Sinnar	40	0	0	40	1	5	5	0	0	
Al Suki	20	1	5	20	1	5	5	0	0	
Al Dindir	20	0	0	20	2	10	5	0	0	
Abu Hejar	20	1	5	20	2	10	5	1	20	
Al Dali	20	0	0	20	0	0	5	0	0	
<b>Total</b>	<b>200</b>	<b>3</b>	<b>1.5</b>	<b>200</b>	<b>7</b>	<b>3.5</b>	<b>40</b>	<b>3</b>	<b>2.5</b>	

in humans (Table:8). The differences in prevalence were statistically significant with  $p \leq 0.05$ . (Table 8).

**Table 8: Prevalence of ovine, caprine, and human brucellosis in the localities of Sinnar State**

## DISCUSSION

This study was conducted to estimate the prevalence of brucellosis in animals and humans who were in close contact with them (Milkmen, herdsman and slaughterhouses' workers), using the one health approach.

In Sinnar State there are poor records about brucellosis in both humans and animal clinics. Some of the visited farms such as Singa, Al Dindir and Al Dali localities were negative for Brucellosis especially in sheep and goats. This may be attributed to the continuous replacement of animals within the herd and the appropriate buffer zone of farms from each other. These factors may have led to the reduction of infection and hence the prevalence rate. On the other hand, 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 usually cross and enter the Sudanese border (Hundum and Regasse, 2009).

The overall prevalence of animal brucellosis in Sinnar State was 4.5%. Similar results were reported by El-Ansary et al. (2001) in eastern Sudan who found 4% of goats' sera, 1% of sheep sera, 5% of cattle sera and (1%) in human that included butchers, slaughterhouse workers, milkers and cow attendants were serologically positive to brucellosis, but the present study reported a significantly higher prevalence in humans (10%) in Sinnar State. Miller et al. (2015) reported a prevalence of 14% in cattle serum, 17% in goat serum and 11% of human serum samples in Uganda. This prevalence disagreed with the prevalence recorded by this study in animal sera, but almost in agreement with the findings in the human sera (10%). These differences may be due to the differences of study area, breeds of animals and sample size.

In the present study, the overall seroprevalence of brucellosis was 6.4% in cattle which was lower than the prevalence reported in human which was higher than (4.5%) in cattle and (6%) in contact human reported by Nahar and Ahmed (2022) in Bangladesh.

In dairy farms, seroprevalence of bovine brucellosis reported in cows was higher than in bulls. This may be because males were kept for shorter periods of time in herds than females, so the chance of their exposure to infection is lower. Naturally, females experience comparatively greater physiological stress during pregnancy and lactation making them

more susceptible to infection (Wadood et al., 2009). While the prevalence in farmers was 11.3% and 11.1 in slaughterhouses' workers, the higher prevalence among farm workers compared to slaughterhouse workers may be due to the higher prevalence of infection in dairy farm cows than in bulls prepared for slaughter, and usually dairy workers spend more time in the farm which may expose them to contract the infection either by direct contact or from consuming raw milk.

Human brucellosis is a severely debilitating disease that requires prolonged treatment with a combination of antibiotics. Brucellosis was shown to be associated with exposure to aborted farm animals in the household and consumption of raw milk from bazaars or neighbors (Kozukeev et al., 2006). The duration and the long convalescence mean that brucellosis is an important economic as well as a medical problem for the patient because of time lost from exhibiting activities (Corbel, 2006). Information about human brucellosis in Sudan is insufficient and sporadic. The prevalence of brucellosis was highest in animal owners (14.29%) than butchers (0.0) as reported by Nahar and Ahmed (2022) which is in disagreement with the current study which reported 11.3% in farmers and 11.1 in slaughter house workers. The differences between the two studies may be due to unsafe handling of infected animals and materials and lack of awareness of both farmers and slaughterhouses' workers in Sinnar State.

The prevalence of Brucellosis in humans who were in contact with cattle was higher than those who were in contact with small ruminants (11.3% and 2.5%), respectively. This agrees with Zhen et al., (2013) in China who reported 15.9% and 8.3%, respectively. Zhen et al., (2013) considered the contact with animals to be the main risk factor of human brucellosis.

According to sex of cattle (cows and bulls), the prevalence in Sinnar State was 10.4% in cows and 2.5% in bulls when Rose Bengal Test was used. This difference was statistically significant with  $p \leq 0.05$ , using one way analysis of variance. This finding was higher than what observed by Nahar and Ahmed (2022) in Bangladesh (5.04% in cows), while in bulls there was an agreement between the two studies (2.44%) and (2,5%). This may be due to age factor, as high prevalence depends on multiparous status which 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).

Percentage of human brucellosis antibodies in the current study was 22.2% when confirmed by ELSIA. All two samples which were collected from humans who were in close contact with cattle in Abu Hejar locality were positive with RBPT and ELISA, the prevalence was 100%. And in Sinnar, Singa and East Sinnar localities there were no human reactors. Also, Abu Hejar locality showed the highest rate in human who were in close contact with small ruminants, one sample was positive with RBPT and ELISA the prevalence was 100%.

## CONCLUSION

The high rate of infection in humans is a significant threat to public health. Based on the concept of One Health, controlling disease in animals is the best way to control and preserve human health.

## ACKNOWLEDGMENT

Authors would like to thank the staff members of College of Veterinary Medicine, University of Bahri and Sinnar Veterinary Researches Laboratory, Sudan for their cooperation and support.

## Competing Interest

The authors declare that they have no competing interests.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for profit sectors.

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**CITATION**

M. Y. A. Rahama, Adil M.A. S., E. M. ElSanousi, & E. A. Mustafa. (2023). Brucellosis in Sinnar State, Sudan: A One Health Approach. Global Journal of Research in Agriculture & Life Sciences, 3(5), 43–50. <https://doi.org/10.5281/zenodo.10041822>