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SOJ Dairy and Veterinary Science

Research Article

Detection of Antibodies against Bovine Brucellosis in ElHawata area, ElGadarif State, Sudan

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Abstract

The main objective of this research was to study bovine brucellosis in ElHawata area, ElGadarif State (2007). A total of 121 bovine sera were collected randomly from cattle of different age and breeds. Data regarding the history of the disease (frequent abortion) in the area were recorded. Samples were 1st subjected to serological investigation using Rose Bengal Plate Test (RBPT). Nineteen samples (15.7%) were found positive for antibodies against Brucellosis. The result was confirmed with (competitive ELISA) c-ELISA. The result of both tests was almost identical with one exception (one additional sample was found positive with c-ELISA). Only positive samples were tested with Serum Agglutination Test (SAT). The result confirmed that of c-ELISA and revealed antibody titer ranged between 17-1280. Regardless of age, the disease was detected in all groups, and it was more prevalent in cross breeds. It was concluded that bovine brucellosis is prevalent in ElHawata area, ElGadarif State, Sudan.

Keywords: Brucella, Rose bengal test, competitive ELISA, Serum agglutination test

Introduction

Brucellosis is a zoonotic disease with public health and economic implications. Losses in animal production due to brucellosis include diminution of milk and meat, abortion, infertility, longer calving intervals and higher culling rates.[1] The disease poses great hazard to human health specially in countries where no proper program for disease control and the microbiological quality of milk is rarely checked.

The first isolation of *Brucella* organism from animals was made by Bang[2] who was the first to report contagious abortion in cattle and other animal species and he named his isolate *Bacillus abortus*, which was followed by other names, including *Corynebacterium abortus, Bacterium abortus* and *Alcaligenes abortus*. Meyer and Shaw (1920) suggested the name *Brucella (Br.)* for the genus.

In Sudan the first isolation of *Br. abortus* was made by Bennet[3] in 1943 from a Friesian herd at Bulgravia dairy farm, but the first isolation of *Br. abortus* from local cattle was from aborted case of a cow at Juba dairy farm.[4] Thereafter the disease was detected in many parts of the country following isolation of the causative agent or antibody detection.

Detection of *Brucella* antibodies is a useful method for diagnosis of bovine brucellosis in many countries in the final phase of an eradication program, and it is still used for monitoring when countries are certified officially free of brucellosis.

Although brucellosis was extensively studied in the country, epidemiological data regarding some parts of the country including ElHawata area, ElGadarif State are still lacking.

Quick Response Code:

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Received: 19 March, 2022 **Published:** 30 March, 2022

Citation: Taha Abdelnassir A, Zaki AZS A. Detection of Antibodies against Bovine Brucellosis in ElHawata area, ElGadarif State, Sudan. *SOJ Dairy Vet Sci*. 2022;1(1):1–5. DOI: 10.53902/SOJDVS.2022.01.000502

Materials and Methods

A total of 121 serum samples were collected from dairy cattle in AlHawata area, ElGadarif State during the period between March to April 2007. The examined animals comprised different breeds and different age groups(see tables 1 and 2). Blood samples were taken from cattle as described by Alton.[5] The skin over the jugular vein was rubbed with 70% alcohol and disinfected by the application of tincture of iodine. Then 7ml of blood was withdrawn using a labeled vacutainer. Samples were put in a wire basket under shade, before taken to laboratory with minimum possible shaking. The samples were kept overnight at 4°C then the serum was separated from the whole blood by centrifugation, at 1000 g for 10 minutes, placed in sterile bijou bottles labeled and stored frozen for months.

Table 1: Animals age groups.

Age group	Number	Percent
Less than or equal to 8 years	75	62.0
More than 8 years less than 12 years	32	26.4
More than 12 years	14	11.6
Total	121	100.0

Table 2: Breeds of animals.

Animal breed	Number	Percent
Kenana	66	54.5
Rufaa	21	17.4
Sharig	31	25.6
Cross	3	2.5
Total	121	100.0

Rose Bengal Plate Test (RBPT)

The RBPT antigen was obtained from Central Veterinary Research Laboratory (CVRL), Sudan. The sera and the antigen were brought to room temperature before testing. The test was done as described by Alton[5] Briefly, an amount of 0.03 ml of each serum to be tested to an enamel plate and equal amount of RBPT antigen was added to each serum Both components were mixed thoroughly (using a clean glass or plastic rod for each test) and the mixture was then agitated gently for four minutes, after which the test was immediately read as follows: -

a. Negative when there was no agglutination or clumping, or showing a pattern of dispersed particles without clumps.

b. Positive when there was agglutination, with moderate to large clumps.

C-ELISA

The test was carried out using a commercial kit (Veterinary Laboratory Agency, UK). The procedure was done as instructed. The conjugate solution was prepared immediately and diluted to working strength with diluting buffer. An amount of 20µl of test serum was added per well and columns 11 and 12 were left for controls. Then 20µl of the negative and positive controls were added to wells A11, A12, B11, B12, C11, C12 and F11, F12, G11, G12, H11 and H12 respectively. The remaining wells of columns 11 and 12 (had no serum) were act as the conjugate controls. Immediately 100µl of the prepared conjugate solution was dispensed. This gave a final serum dilution of 1/6. The plate was then vigorously shaken (on the microtitre plate shaker) for 2 minutes in order to mix the serum and conjugate solution. The plate was covered with the lid and incubated at room temperature (21°C ± 6°C) for 30 minutes on a rotary shaker, at 160revs/min. The contents of the plate were shaken out and the plate was rinsed 5 times with washing solution and then thoroughly dried tapping on absorbent paper towel. The microplate reader was switched on and allowed unit to stabilize for 10 minutes. The substrate and chromogen solution were prepared by dissolving one tablet of urea H_2O_2 in 12ml of distilled water. When dissolved the OPD tablet was added and mixed thoroughly. This took a few minutes; the use of a magnetic stirrer greatly increased the speed with which it dissolved. 100µl of this solution was added to all wells. (This solution was not stored). The plate was left at room temperature for a minimum of 10 minutes and a maximum of 15 minutes. The reaction was slowed by adding 100µl of stopping solution to all wells. Condensation from the bottom of the plate was recovered with absorbent paper towel. Read plate at 450nm in ELISA Reader.

Serum Agglutination Test (SAT)

The test was done according to Alton[5] 0.8ml of phenol saline was placed in the first tube and 0.5ml in each succeeding tube. 0.2ml of the serum under test was transferred to the first tube and mixed thoroughly with the phenol saline already there. 0.5ml of the mixture was carried soon over the second tube. This process is continued until the last tube, from which after mixing, 0.5ml of dilution was discarded. This process of doubling dilution results in 0.5ml of dilutions 1:5, 1:10, 1:20 and so on in each tube. To each tube 0.5ml of antigen was then added at the recommended dilution and the contents of the tube were thoroughly mixed, thus giving final serum dilution of 1:10, 1:20 etc...The tubes were then incubated at 37°C for 20 hours before the results are read.

The degree of agglutination was assessed by the amount of clearing that has taken place in the tube as compared with a standard tube. The tubes were examined, without being shaken, against a black background, with a source of light coming from above and behind the tubes. Complete agglutination and sedimentation with water-clear supernatant was recorded as (++++), nearly complete agglutination and 75% clearing as (+++), marked agglutination and 50% clearly as (++), some sedimentation and 25% clearing as (+), and no clearing as standards were prepared at the time the tests were done and incubated with them. The antigen was diluted by mixing 2ml of antigen, diluted as for the test, with 2ml of phenol-saline. Reagent obtained Veterinary Laboratory Agency, UK

Results and Conclusion

Serological investigation

Results are shown in tables 3, 4 & 5. All serum samples were investigated using RBPT and cELISA. 19 samples (15.7%) were found positive with RBPT (Table 3) and 20 samples (16.5%) were found positive with cELISA (Table 4). The result of the two tests was identical except in one sample which was found positive with cELISA and negative with RBPT see (Table 5). Only positive samples were subjected to further investigation using SAT. Results are shown in (Table 6). All samples were found positive including the sample which gave different reading with RBPT and c-ELISA they had antibody titer ranged between 17-1280.

Table 3: Result of Rose Bengal test.

	Number	Percent	Valid Percent	Cumulative Percent
Negative	102	84.29752	84.29752	84.29752
Positive	19	15.70248	15.70248	15.70248
Total	121	100	100	100

Table 4: Result of ELISA.

	Number	Percent	Valid Percent	Cumulative Percent
Negative	101	83.47107	83.47107	83.47107
Positive	20	16.52893	16.52893	16.52893
Total	121	100	100	100

Table 5: cross-tabulation of ELISA and Rose Bengal Result.

	Results of Rose Bengal test		Total
	Negative	Positive	
Results of the Negative	101	0	101
ELISA Positive	1	19	20
Total	102	19	121

Effect of age

Results are shown in Table 7, Table 8 and Figure 1. The two tests showed that 17.3% of animals ≤ to 8 years old were found positive in contrast to 21.4% of animals ≥12 years old were found positive. The highest antibody titer was detected in animals ≥ to 8 years old. In addition, most of animals with low antibody titer were also detected in animals ≥8 years old Figure 2.

Table 6: Antibody titer for brucellosis using SAT.

Titer	No of samples	Percent	Valid Percent	Cumulative Percent
17	1	0.826446	5	5
20	2	1.652893	10	15
67	1	0.826446	5	20
80	4	3.305785	20	40
106	2	1.652893	10	50
160	2	1.652893	10	60
186	1	0.826446	5	65
212	1	0.826446	5	70
320	1	0.826446	5	75
372	2	1.652893	10	85
640	1	0.826446	5	90
744	1	0.826446	5	95
1280	1	0.826446	5	100
Total	**20**	**16.52893**	**100**	
Missing System	101	83.47107		
Total	**121**	**100**		

Effect of animal's breed

Results are shown in Tables 9, Table 10 Among the investigated animals, the cross animals were the most frequently affected group (33.3%).

Table 7: Result of RBPT in different age group.

		Result of Rose Bengal test		Total
		Negative	Positive	
Age group Less than or equal to 8 Years	Count % Within age group	62 82.7%	13 17.3%	75 100.0%
Age group More than 8 Years less than 12 Years	Count % Within age group	28 87.5%	4 12.5%	32 100.0%
Age group More than 12 Years	Count % Within age group	12 85.7%	2 1t.3%	14 100.0%
Total	Count % Within age group	102 04.3%	19 15.7%	121 100.0%

Table 8: Result of C-ELISA in different age group.

		Result of Rose ELSIA		Total
		Negative	Positive	
Age group Less than or equal to 8 Years	Count % Within age group	62 82.7%	13 17.3%	75 100.0%

Age group More than 8Years less than 12 Years	Count % Within age group	28 87.5%	4 12.5%	32 100.0%
Age group More than 12 Years	Count % Within age group	11 78.6%	3 21.4%	14 100.0%
Total	Count % Within age group	101 83.5%	20 16.5%	121 100.0%

Table 9: Result of RBPT in different animal breed.

		Result of Rose Bengal test		Total
		Negative	Positive	
Breed Kenana	Count % within Breed	56 84.8%	10 15.2%	66 100.0%
Breed Rufaa	Count % within Breed	17 81.0%	4 19.0%	21 100.0%
Breed Sharig	Count % within Breed	27 87.1%	4 12.9%	31 100.0%
Breed Cross	Count % within Breed	2 66.7%	1 33.3%	3 100.0%
Total	Count % Within Breed	102 84.3%	19 15.7%	121 100.0%

Table 10: Result of cELISA in different animal breeds.

		Result of ELISA		Total
		Negative	Positive	
Breed Kenana	Count % Within Breed	55 83.30%	11 15.20%	66 100.00%
Breed Rufaa	Count % Within Breed	17 81.00%	4 19.00%	21 100.00%
Breed Sharig	Count % Within Breed	27 87.10%	4 12.90%	31 100.00%
Breed Cross	Count % Within Breed	2 66.70%	1 33.30%	3 100.00%
Total	Count % Within Breed	101 83.50%	20 16.50%	121 100.00%

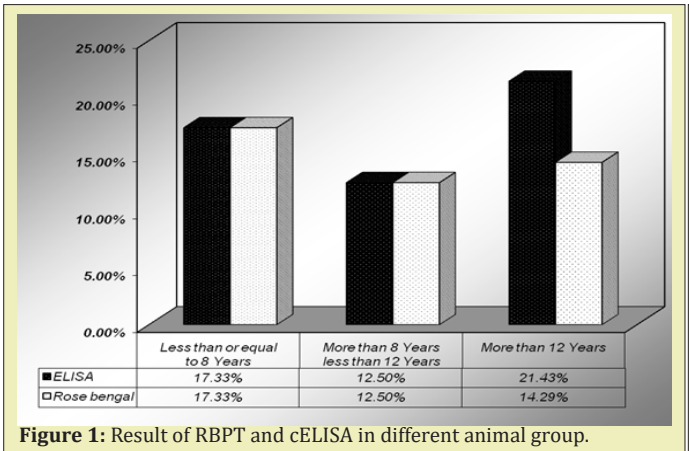

Figure 1: Result of RBPT and cELISA in different animal group.

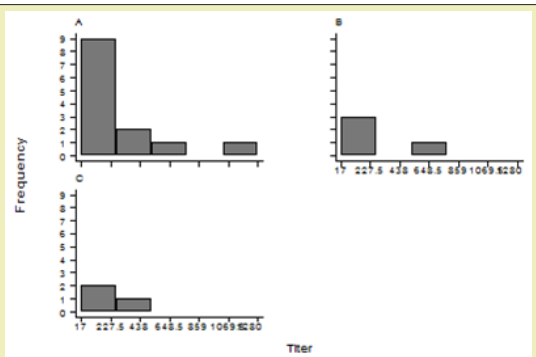

Figure 2: A: Distribution of antibody titre in animals less than or equal to 8 Years; B: Distribution of antibody titre in animals more than 8 Years less than or equal to 12 Years; C: Distribution of antibody titre in animals more than to 12Years.

Relation between brucellosis and history of abortion

Among the investigated animals Seventeen cows had a history of abortion. Only 5 of which were found positive for Brucellosis See Figure 3. In the current study the presence of bovine brucellosis in ElHawata area, ElGadarif State, Eastern Sudan was investigated for the first time. Twenty samples (16.5%) out of 121 bovine sera were found positive for brucellosis. This finding was comparable to other studies, Daffalla,[4] Fayza[6] and Musa[7] who reported that the prevalence rate for brucellosis was 10.7% (in ElGazira), 15.73% (Khartoum State) and 13.7% (in Darfur States) respectively. Possible reasons for the spread of the disease in ElHawata area include lack of control measures (isolation of infected animals, vaccination or stamping out of infected cattle), the nomadic nature of the husbandry method and continuous movement of cattle from one locality to another exposing them to infection specially at water pools, lack of awareness between nomadic owners who for example screen cattle for brucellosis for export and retain the positive ones within their herds.

RBPT is the only serological test used in Sudan for routine diagnosis of brucellosis. The test is considered by the World Organization for Animal Health (OIE) as a suitable screening test for the diagnosis of the disease. However, positive reactors could be retested using a suitable confirmatory or complementary method. In this study, the result of RBPT was confirmed with c-ELISA and further with SAT. The test was found sensitive as 99.2% of results agreed with that of c-ELISA and SAT. C-ELISA was found more sensitive than RBPT (table 5) for screening of animals in this study for brucellosis and this agreed with that of Mukhtar (2006). Antibodies against Brucellosis were detected in all animal groups. However, the higher percentage of affected animals were observed in cows ≥ 12 years old. This result was similar to that of Raias[9] who found that the old animals are susceptible to the disease more than young ones. This could be explained by the fact that once an animal is infected, it cannot be treated. In addition, infected older animals may not abort (i.e. showing no clinical signs of the disease) and accordingly remain hidden in the herd (carriers) and represents a source of infection to others. The highest titer was detected in animals ≥8 years old & this may probably indicate recent infection.

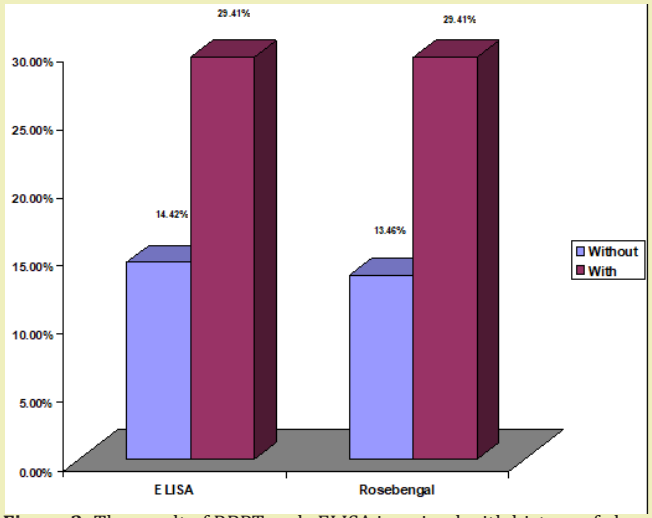

Figure 3: The result of RBPT and cELISA in animal with history of abortion.

Four different cow breeds were included in the study and they represent breeds in ElHawata area. The highest percentage of infected animals was detected in cross breed although the number of samples from this breed was small. This may be attributed to the fact that local breeds (zebu cattle) are generally resistant to diseases in the tropics compared to foreign ones. Only 29.4% of cows with history of abortion were found positive for brucellosis. This finding can be explained by the fact that most infected animals abort only once, and subsequent pregnancies are usually normal. In addition, Abortion may result from a broad range of causes; it could be due metabolic or hormonal abnormalities, nutritional deficiencies, trauma, toxicities, or infectious processes.[10]

Conclusion

It was concluded from this study that bovine Brucellosis was present in El Hawata area, ElGadarif State, Sudan and affects cattle of different age and breed groups.

It is recommended that:

1. The distribution of the disease in all Sudan States should be studied.
2. Proper control measure should be followed to reduce the infection rate.
3. Education of people like animal owners, nomads and abattoir workers is essential to increase their awareness to avoid the spread of the disease and secure the public health.

Acknowledgments

Thanks, and praise the Head and Staff of the Department of Brucella, Central Veterinary Research Laboratory, Sudan, for generous help. The technical help, advice and encouragement of my colleagues Rashid EE, Hind M, Mohammed B, Huda OA, Huda AE, Amar AH, Sara A, also acknowledged. I am greatly indebted to the staff of the Veterinary Office in ElHawata area, ElGadarif State.

Funding

None.

Conflicts of Interest

Author declares that there is no conflict of interest.

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