Original Research Article

Seroprevalence and Associated Risk factor of Cattle Brucellosis in Al Gadarif state – Eastern Sudan

Comment [n1]: Need to change as indicate in manuscript.

ABSTRACT

Comment [n2]: Need major revision, it is not as per journal format

Aims: This study was conducted to determine the seroprevalence of bovine brucellosis in Al Gadarif state -eastern Sudan- and to evaluate the sensitivity of RBPT and mRBPT, and milk ring test for serodiagnosis of bovine brucellosis.

Study Design: Collect serum and milk samples and apply the recommended tests for diagnosis.

Place and Duration of Study: This cross-sectional study was carried out in Al Gadarif state regional laboratory and Central Veterinary Research Laboratory (CVRL) in 2015.

Methodology: Total of 367 serum samples were collected from 12 localities which include: Al Fao, Fashaga, Baldiat Al Gadarif, Wasat Gadarif, West Gadarif, Basonda, Al Gorisha, Al rhad ,Mafaza , Butana, East Galapat and Gla nahl. Also 100 bulk milk samples have been collected from Baldiat Al Gadarif, West Galapat, Wasat Gadarif and Fashag and subjected to milk ring test. The all collected sera samples were tested for Brucella antibodies using the following serological tests: Rose Bengal Plate Test (RBPT), modified Rose Bengal Plate test (mRBT 1:2 and 1:3) and c.ELISA test, the last test was done for 143 serum samples only.

Results: The overall seroprevalence was 35.7%, 38.4%, 42.2% and 8.4% using RBPT, mRBPT (1:2), mRBPT (1:3) and c.ELISA tests respectively. According to this study the lowest seroprevalence was observed in Al Fao

locality. There is similarity in seroprevalence rate results using the RBPT (1:2) and RBPT (1:3) in Al rahd (43.6%), Al Gorisha (85.7%) and Mafaza (50%) localities.

There were no any association between cattle brucellosis -as risk factor- and the four mentioned test. Using milk ring test the overall prevalence of positive milk samples was 39% (39/100). The highest prevalence was in Fashaga (60%), followed by Wasat Gadarif (46.7%). Baldiat Al Gadarif showed the lowest prevalence (22.9%).

Conclusion:

Serological investigations proved the occurrence of bovine brucellosis in Al Gadarif state -eastern Sudan using the recommended tests.

Keywords: Brucellosis, Seroprevalence, Al Gadarif state, Cattle, serological tests.

INTRODUCTION

Brucellosis is one of the world's major zoonotic diseases [1 ,2]. The disease is characterized by inflammation of the genital organs and fetal membranes, abortion, sterility, and formation of localized lesions in the lymphatic system and joints [3, 4]. In cattle, brucellosis is typically caused by *Brucella abortus* and *B. melitensis* generally [5].

The *Brucella* is small, non-motile, aerobic, facultative intracellular, Gram-negative coccobacilli.

The disease considered endemic in several countries[6].Several researches was done in Al Gadarif state to determine brucellosis seroprevalence among sheep, goats and camels. The Brucella antibodies showed various result rates [7],[8],[9]. El Ansari, *et al* [10], reported a low prevalence of brucellosis in domestic animals,

including goats in Eastern Sudan. . However in Khartoum state the overall seroprevalence rate of brucellosis among cattle was found to be 25.7% [1].

Many tests are used for diagnosis of brucellosis such as Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Milk Ring Test (MRT) complement fixation test and ELISA test [11].

This study was conducted to determine the seroprevalence of bovine brucellosis in Al Gadarif state and to evaluate the sensitivity of RBPT and mRBPT, and milk ring test for serodiagnosis of bovine brucellosis.

MATERIAL AND METHODS

Comment [n3]: Need major revision

Study area

The study was carried out in Al Gadarif state which is in the Eastern part of the Sudan. It lies between 12˚40' and 15˚46' latitude and 33˚30' and 36˚30' longitude. It boarded by Ethiopia from the East part and by Sinaar , Kassala, Khartoum and Gazira states from the other parts.

Samples for serological examinations

Comment [n4]: •Study design/design of experiment: CRD/RBD/cross sectional study/ is not mentioned in the manuscript should be specified.

The standard formula of [12] was used to calculate the sample size (n). A total of 467 random samples consisting of 367 sera and100 milk samples were collected from different cattle breeds with different ages, in different localities of Al Gadarif state.

Blood was collected from each animal aseptically by vein puncture. The samples were left in a refrigerator at 4˚C overnight. Then sera were separated, kept in Eppendorf tubes, and stored frozen till used**.** To collect milk samples, the whole udder was washed and the end of each teat was disinfected with small of alcohol and was kept dry. Then the first two stream of milk were discarded and then sample from all teats was wrinkled directly

into 50ml sterile universal bottles, placed on ice in flask and transferred to Central Veterinary Research Laboratory (CVRL).

Serum samples were examined using RBPT, mRBPT, SAT, and milk ring test was used for testing milk samples.

Comment [n5]: •On what basis? Prevalence/seroprevalence of brucellosis in cattle/ why this Rose Bengal Plate/ Test (RBPT)/ c.ELISA/ Milk Ring Test (MRT) used and should be elaborated as indicated in the manuscript

Rose Bengal Plate Test (RBPT):

This simple agglutination test essentially, consists of mixing equal volume of antigen and serum and observing the agglutination after period of time. This test was done according to [13].

The antigen and tested sera were placed at room temperature, then 25 µl of tested serum and antigen were placed and mixed onto the well of the white enamel plate. The samples with no agglutination (0) were recorded as negative, while any visible coloured agglutination is considered to be positive reaction TheModified Rose Bengal Plate Test (mRBPT) was performed following the procedure described by Blasco *et al*.1994 in which 50 µL(1:2) and 75 µL(1:3) of tested sera was mixed with 25 µL of the antigen. The plates were shaken for 4 min and any agglutination appeared within this time was recorded as a positive reaction.

Competitive Enzyme Linked Immunosorbent Assay (c.ELISA):

The kits were brought from Animal Health Veterinary Labororatry Agency (AHVLA- U.K) and carried out as described by OIE (2022). The interpretation of the results was done by comparing the tested samples with negative and positive controls.

Milk Ring Test (MRT):

The test was performed according to Alton *et al.*,[14]. The test was done by adding 1ml of each milk sample into a sterile test tube. Then 30µl of the stained antigen was added each sample tube, mixed well, and left in a water bath at 37°C for 24 hours before reading the results. Positive samples

showed a blue ring on the top of the sample while the negative remained homogenously blue.

RESULTS

The overall seroprevalence findings of cattle brucellosis revealed 35.7% (131/367), 38.41(141/367), 42.8% (157/367) and 8.4% (12/143) using Rose Bengal test, modified Rose Bengal (1: 2) and modified Rose Bengal(1:3)and ELISA tests respectively.

In this study the highest seroprevalence was in Al Gorisha Locality which was71.4%, 85.7% and 85.7% using the RBPT, mRBPT(1:2) and mRBPT (1:3) tests. While the lowest seroprevalence was found in Al Fao locality with estimated 11.1%, 19.4% and 25% using the above mentioned three tests.

The obtained data in this study showed no significant association between brucellosis with age, using ELISA test, among the screened the seroprevalence rate showed 10.2% and 14.5% in males and females respectively.

The overall prevalence rate among one hundred screen milk the estimated rate was 39%(39/100) using milk ring test. The highest prevalence was in Fashaga (60%), followed by Wasat Gadarif (46.7%). The lowest prevalence was in Baldiat Al Gadarif (22.9%).

Table 1 : Estimated seroprevalence rate in localities of Gadarif state using RBPT

mRBPT (1:2), mRBPT (1:3)

Locality	**Number of tested sample**	**RBPT %**	**m.RBPT(1:2)**	m.RBPT(1:3)
Gla nahl	34	8(23.5)%	8(23.5)%	10(29.4)%

Comment [n6]: •Need revision

Rhad	32	12(37.5)%	14(43.6)%	14(43.6)%
Al Gorisha	28	20(71.4)%	24(85.7)%	24(85.7)%
Wasat Gadarif	30	4(13.3)%	8(26.7)%	10(33.3)%
Fashaga	28	11(39.8)%	11(39.8)%	13(46.4)%
Mafaza	36	17(47.2)%	18(50)%	18(50)%
West Galapat	28	6(21.4)%	9(32.1)%	12(42.9)%
Baldiat Al Gadarif	28	11(39.3)%	13(46.4)%	14(50)%
Al boutana	36	11(30.6)%	14(38.9)%	15(42.7)%
East Galapat	21	7(33.3)%	9(42.9)%	10(47.6)%
Al fao	36	4(11.1)%	7(19.4)%	9(25)%
Basonda	30	6(20)%	6(20)%	8(26.7)%
Total	**367**	**131(35.7)**	**141(38.4)%**	**157(42.8)%**

Table 2 : Estimated Seroprevance rate of cattle brucellosis in both males and females using ELISA and Rose Bengal test

Sex	**Seropositive%**	**Rose bengal**	**Seropositive%**	**ELISA**
Male	6(10.9)%	55	8(14.5)%	55
Female	8(9.1)%	88	9(10.2)%	88

Table 3 : Association of sex -as a risk factor -with the Seropositive results of cattle brucellosis based on sex in Gadarif state

Comment [n7]: •Table 3: Association of sex - as a risk factor -with the Seropositive results of cattle brucellosis based on sex in Gadarif state How??

Risk factor / sex	No. inspected	Frequency %	Cumulative Frequency %	No. affected (%)	Chi-square	p-value	Odds ratio
Male	55	38.5	38.5	6(10.9)	.127[a]	.776	1.224
Female	88	61.5	100	8(9.1)			

Table 4 : Results of bovine brucellosis examined by (MRT), from four localities in Gadarif State

Locality	Samples	Percentage
Al-Balldia Gadarif	35	8(22.9)%
West Gadarif	25	11(44)%
Wast Gadarif	30	14(46.7)%
Al Fashga	10	6(60)%

DISCUSSION

Comment [n8]: Justification in support of results as indicated in the manuscripts

Brucellosis in dairy cows is a public health hazard to the milkers, nomads, animal owners and their families in contact with the infected animals or their discharges [5, 1].

Many surveys have been carried out to estimate cattle Brucellosis in Sudan. Most of the work was directed towards bovine brucellosis because of the larger number and increase value of cattle. Recently, The Ministry of Animal Resources and Fisheries and Animal owners, paid great attention to goat and cattle production and have already imported foreign breeds to improve the local ones.

As the incidence of brucellosis appears not to be declining on a worldwide basis, rapid and accurate diagnosis is imperative in order to control/eradicate this disease from man, domestic animals and wildlife.

In the current study three types of Rose Bengal tests were used to determine the seroprevalence rate of cattle brucellosis.|However, modified RBPT (1:3) was found to have similar results with m.RBPT (1:2) in some sera samples, different results between RBPT (1:2) and RBPT (1:3) were also recorded. This contradicts of these necessitate more work to explain the reason.

The overall seroprevalence findings of cattle brucellosis revealed 35.7% (131/367), 38.41(141/367), 42.8% (157/367) and 8.4%(12/143) using Rose Bengal test, modified Rose Bengal test(1: 2) , modified Rose Bengal (1:3) and ELISA test respectively.These findings are lower than the prevalence of the disease in Gongli State in south Sudan in which the overall estimate sero-prevalence of bovine brucellosis was 31.0% ,however the findings was significantly higher compared to the findings in Khartoum State 25.7% (n=77) using RBPT and 22.7% (n=68) with a 95% CI from 17.96 to 27.44 using SAT. Out of the 77 RBPT positive sera, 66.2% (n=51) were confirmed to be positive by c-ELISA (95% CI from 55.63 to 76.77)[15, 1].

Comment [n9]: Space

In this study the highest seroprevalence rate was recorded in Al Gorisha localities while the lowest seroprevalence rate were found in Al Fao and Wast Gadarif. These finding could be attributed to the knowledge among cattle

owners who tend to get rid of seropositive cattle. The highest prevalence of positive milk for Brucella using MRT was 14% from the total tested milk (100sample), but it was 46.7% among the milk collected from west Gadarif locality, on the other, the lowest rate was found in Baldia Gadarif and Fashage localities with 8% and 6% respectively.

In this study there no significant association between sex and cattle brucellosis using Rose Bengal test mRBPT (1:2), mRBPT (1:3) and ELISA respectively. On the other hand, Abdallah *et al*. [16] and Wegdan *et al*. [1]; their study revealed that no association between the prevalence of brucellosis in sheep in North Kordofan state and cattle in Khartoum state with most of the risk factors.

Serological investigations have demonstrated that brucellosis is occurring in the Sudan and evidence of infection has been found in large and small ruminants (cattle, sheep, goats, and camels), wildlife and human beings. B. abortus biovars 1, 3, 6 and 7 and B. melitensis biovars 2 and 3 were found to be associated with the disease [11].

The estimated over all seroprevalence rate was 8.4 %(12/143), 35.7 %(131/3) of 38.4% (141/3 67) and 42.8% (157/367) using ELISA and varied types of mRBPT. These estimated findings indicated that Elisa test is more specific than RBPT serological studies were carried out in area of study in sheep, goat, and camels [8, 9, and 7].

Conclusion

The present study concluded that; brucellosis is present in cattle in the study area. And more work must be done to study the prevalence of the disease in other states and other species. The eradication of the disease must be taken inconsideration through screening ,monitoring diagnosis and vaccination programmes.

Comment [n10]: At what P-value mention it

Comment [n11]: Need revision

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Comment [n12]: Rreference should be in journal format

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