



## Seroprevalence of Cattle Brucellosis in Al Gadarif State – Eastern Sudan

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### Authors' contributions

This work was carried out in collaboration among all authors. Author HGA designed and wrote the protocol. Authors AAE and HHA supervised and guided the laboratory work. Author HAMAB reviewed the practical part of the study and managed the literature searches. Authors MK and SK helped in practical and result analysis. Author RAHO reviewed and corrected the manuscript. All the authors revised, read and approved the final manuscript.

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### ABSTRACT

**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, 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 study was carried out in Al Gadarif state regional laboratory and Central Veterinary Research Laboratory (CVRL) in 2015.

**Methodology:** A 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 Gadarif, Wasat Gadarif, and Fashaga and subjected to the milk ring test. All collected sera samples were tested for Brucella antibodies using the following serological

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tests: Rose Bengal Plate Test (RBPT), modified Rose Bengal Plate test (mRBPT 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 (11.1%), and the highest seroprevalence was in Al Gorisha Locality (71.4%). There was similarity in seroprevalence rate results using RBPT (1:2) and RBPT (1:3) in Al rahd (43.6%), Al Gorisha (85.7%) and Mafaza (50%) localities. Seroprevalence rate showed 10.9% and 9.1% in males and females respectively. There was no association between sex-as risk factor- and cattle brucellosis. Using the 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:** The serological investigation 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.*

## 1. 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 the 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 is considered endemic in several countries [6]. Several types of research were 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 the diagnosis of brucellosis such as the 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 the milk ring test for serodiagnosis of bovine brucellosis.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in Al Gadarif state which is in the Eastern part of Sudan. It lies

between 12°40' and 15°46' latitude and 33°30' and 36°30' longitude. It is boarded by Ethiopia from the East part and by Sinaar, Kassala, Khartoum, and Gazira states from the other parts.

### 2.2 Samples for Serological Examinations

The standard formula of Thrusfield [12] was used to calculate the sample size (n). A total of 467 random samples consisting of 367 sera and 100 milk samples were collected of different cattle breeds with different sex and 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 amount of alcohol and was kept dry. Then the first two streams of milk were discarded and the 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 the recommended tests of (OIE, 2022) [13], which are RBPT, mRBPT, and the Milk ring test was used for testing milk samples.

### 2.3 Rose Bengal Plate Test (RBPT)

This simple agglutination test essentially, consists of mixing an equal volume of antigen and serum and observing the agglutination after a period of time. This test was done according to (OIE, 2022) [13].

The procedure briefly that; 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 plates were shaken for 4 min. The samples with no agglutination (0) were recorded as negative, while any visible colored agglutination is considered to be positive reaction. The modified Rose Bengal Plate Test (mRBPT) was performed following the procedure described by Blasco et al., [14], 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 that appeared within this time was recorded as a positive reaction.

#### 2.4 Competitive Enzyme Linked Immunosorbent Assay (c.ELISA)

The kits were brought from Animal Health Veterinary Laboratory 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.

#### 2.5 Milk Ring Test (MRT)

The test was performed according to Alton et al., [15]. The test was done by adding 1ml to 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 homogeneously blue.

### 3. 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 the Rose Bengal test, modified Rose Bengal (1: 2), modified Rose Bengal (1:3) and c.ELISA tests respectively.

In this study the highest seroprevalence was in Al Gorisha Locality which was 71.4%, 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 an 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 sex, using the c.ELISA test. Among the screened serum samples the seroprevalence rate showed 10.9% and 9.1% in males and females respectively.

The overall prevalence rate among the one hundred screens milk samples revealed 39% (39/100), using the 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%).

### 4. DISCUSSION

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 [1,5].

**Table 1. Seroprevalence rate within localities of Al Gadarif state using RBPT, mRBPT (1:2) and mRBPT (1:3)**

| Locality           | Number of the tested sample | RBPT %           | m.RBPT(1:2) %     | m.RBPT(1:3) %     |
|--------------------|-----------------------------|------------------|-------------------|-------------------|
| Gla nahl           | 34                          | 8(23.5)%         | 8(23.5)%          | 10(29.4)%         |
| 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)%          |
| <b>Total</b>       | <b>367</b>                  | <b>131(35.7)</b> | <b>141(38.4)%</b> | <b>157(42.8)%</b> |

**Table 2. Seroprevalence rate of cattle brucellosis in both males and females using c.ELISA and Rose Bengal test**

| Sex    | samples | Seropositive% by Rose Bengal | Seropositive% by c.ELISA |
|--------|---------|------------------------------|--------------------------|
| Male   | 55      | 6(10.9)%                     | 8(14.5)%                 |
| Female | 88      | 8(9.1)%                      | 9(10.2)%                 |

**Table 3. Association of sex -as a risk factor -with the seropositive results of cattle brucellosis in Al Gadarif state**

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

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

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

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 test were used to determine the seroprevalence 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 them also recorded. This contradicts of the results 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 the Rose bengal, modified Rose bengal (1:2), modified Rose Bengal (1:3) and c.ELISA tests respectively. These findings are lower than the prevalence of the disease in Gongli state in

South Sudan in which the overall estimated seroprevalence of bovine brucellosis was 31%, however the findings were significantly higher compared to the findings in Khartoum State 25.7% (n=77) using RBPT and 22.7% (n=68) (95% CI : 17.96 to 27.44) using Serum Agglutination test (SAT) [16]. On the other hand seroprevalence estimate of brucellosis in Ethiopia which is the neighbor of Al Gadarif state from east part was 16.3% (95% CI: 12.9-20.5) in cattle [17], this result is obviously lower than the result obtained from the study. 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) in Bahr el Ghazal region, South Sudan [1,15].

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 (p-value .776). On the other hand, Abdallah et al. [18] and Wegdan et al. [1]; their study revealed that no association between sex and the prevalence of brucellosis in sheep in North Kordofan state and cattle in Khartoum state respectively. Serological investigations 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]. These estimated overall findings indicated that the c.ELISA test is specific for bovine species, as which was carried out in the area of study of sheep, goats, and camels [8,9,7]. The highest prevalence of positive milk for *Brucella* using MRT was 39% (39/100) from the total tested milk. On the other hand the highest prevalence was in Fashaga (60%), followed by Wasat Gadarif (46.7%). Baldiat Al Gadarif showed the lowest prevalence (22.9%).

## 5. 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 in to consideration through screening, monitoring, diagnosis, and vaccination programs.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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