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Sero-Prevalence of Anti-Brucella Antibodies in Goats in El-Gedarif State, Eastern Sudan

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Abstract: *This cross-sectional study was carried out between January and April 2014 with the aim of determining the seroprevalence of anti-brucella antibodies in goats in El-Gedarif state, the Sudan. A total number of 426 serum and 15 milk samples were collected from the study animals from eight different localities. These samples were examined using rose Bengal plate test (RBPT), serum agglutination test (SAT), competitive enzyme-linked immunosorbent assay (cELISA), and milk ring test. An overall seroprevalence of 10.8% (n=26, 95% CI between 7.85 and 13.75) was reported. Of the RBPT-positive samples, SAT confirmed the positivity of 13.0% (n=6). Furthermore, 6.5% (n=6) of the samples tested by cELISA showed positive results. There were no significant statistical differences in the reported seroprevalences among the categories of the investigated risk factors. Location (χ^2=31.62, df=7, p-value=0.001) was associated with RBPT-positive status for brucella in the univariate analysis. Moreover, Basunda locality and animals with no history of abortion had an increased odd of being RBPT positive. It can be concluded that location and history of abortion were risk factors associated with brucella positive status. Further studies investigating brucellosis across the whole state using molecular methods should be carried out to better understand the epidemiology of brucellosis in goats.*

Keywords: *Sero-prevalence, RBPT, brucellosis, goats, Sudan*

1. INTRODUCTION

Brucellosis is a contagious disease of animals and it is transmissible to man (Radostits *et al.*, 2007; Angara and Shuaib, 2015). It is caused by the members of the genus *Brucella* which are small, non-motile, aerobic, facultative intracellular, Gramnegative coccobacilli. *Brucella melitensis, B. abortus, B. suis, B. neotomae, B. ovis,* and *B. canis* are important etiological agents of brucellosis in domestic animals. In sheep and goats, *B. melitensis* and *B. ovis* are the most common etiological agents of brucellosis (Geering *et al.*, 1995; Muma *et al.*, 2006; Musa *et al.*, 2008). Though brucellosis has been eradicated in many developed countries, it remains an uncontrolled problem and endemic in some parts of the world like many African and Asian countries (Refai, 2002; Muma *et al.*, 2006). Transmission of brucellosis between animals typically occurs through contact with infected animals or materials with skin abrasions (Plummet *et al.*, 1998; Lapaque *et al.*, 2005). The ability of brucella species to replicate and persist in host cells is directly associated with their capacity to cause persistent disease and to circumvent innate and adaptive immunity (Greenfield *et al.*, 2002; Fichi, 2003; Young, 2005; Radostits *et al.*, 2007). From public health point of view, brucellosis is considered to be an occupational disease that mainly affects slaughterhouse workers, butchers, and veterinarians (Plummet *et al.*, 1998; Muma *et al.*, 2006; Radostits *et al.*, 2007).

There are many factors that enhance the prevalence of brucellosis in animals (Salih *et al.*, 2016; Wegdan *et al.*, 2016). Prevalence of brucellosis can vary according to climatic conditions, geography, species, sex, and age

(Muma *et al.*, 2006; Abdallah *et al.*, 2015; Salih *et al.*, 2016; Wegdan *et al.*, 2016). Almost all domestic animal species can be affected with brucellosis except cats which are resistant to brucella infection (Muma *et al.*, 2006; Radostits *et al.*, 2007). Considering the damage done by the infection in animals in terms of decreased milk production, abortions, weak off springs, weight loss, infertility and lameness, brucellosis is one of the most serious diseases of livestock. It is also a major impediment for trade in animals and animal products. Death may occur as a result of acute metritis followed by retained fetal membranes (Radostits *et al.*, 2007). Brucellosis in goats in Sudan is not very well studied; only few surveys have been conducted (Ahmed, 2004; Rayas, 2004; Musa, 2006). This study was conducted to determine the seroprevalence of brucellosis in goats in El-Gedarif state.

2. Materials and Methods

2.1. Study Area

El-Gedarif state is located in the Eastern part of the Sudan; longitudes $33^0$30 and $36^0$30' East and latitudes $12^0$40' and $15^0$46' North. It has an area of 75,263 km². The state falls within the Sudano-Sahelian climate zone of Africa. It has an international border with Ethiopia from the East and national borders with Kassala and Khartoum states from the North, with El-Gezira state from the West and with Sinnar state from the South. The total population of goats in El-Gedarif state is about one million head including indigenous ecotypes and exotic breeds as well as their crosses (MARF, 2009). Different animal production systems predominate in El-Gedarif state; such as mixed crop-livestock system, nomadic, and semi-nomadic systems in the region (ILRI, 2009).

2.2. Study Design and Sampling Strategy

This cross-sectional study was conducted for 4 months, from January to April 2014, using a multistage sampling strategy (Thrusfield, 2007). Eight localities (administrative units) of the localities of El-Gedarif state were randomly or conveniently selected. These were Al-Fao, El-Gadarif, Al-Hawata, Al-Showak, Basunda, Dokah, Al-Guraisha, and Kassab. Within each of the selected localities, peasant association or villages, goat flocks, and individual animals were randomly and/or conveniently sampled (Thrusfield, 2007).

2.3. Sample Size

The standard formula of Thrusfield (2007) was used to calculate the sample size (n) for determining the prevalence of anti-brucella antibodies in goats. Considering all parameters of the formula, n was determined to be 426 animals.

2.4. Collection of Samples

Blood samples were taken aseptically from the jugular vein using vacutainer tubes which were put in rack and left in a refrigerator at 4°C overnight. After clot formation, the samples were transported to the Veterinary Research Laboratory, El-Gedarif, the Sudan, where sera were separated and tested for anti-brucella antibodies on the same day after collection. The rest of the sample was stored at -20°C for further use.

Fifteen milk samples, 10 ml each, were collected after cleaning the teats with water and alcohol into universal bottles. The samples were kept in a refrigerator at 4°C and were tested within 24 hours of collection.

3. Laboratory Procedures

3.1. Rose Bengal Plate Test

The Rose Bengal platetest (RBPT) was carried out as described by OIE (2009). The procedure of the test was as follow: i) serum samples and antigen were brought to room temperature first, ii) then, 25 µl of each serum sample was place on a porcelain plate, iii) an equal volume of antigen was placed near each serum spot, iv) serum and antigen were then mixed thoroughly (using a clean wood rod for each sample) to produce a circular or oval zone approximately 2 cm in diameter, v) the mixture was agitated gently for 4 minutes at ambient temperature on a rocker, and finally, vi) agglutination was immediately read for after that.

The interpretation of the result was done according to the degree of agglutination, which was recorded as 0, +, ++ and +++. A score of 0 indicated the absence of agglutination; a score of + indicated barely visible agglutination; ++ indicated fine agglutination and +++ indicated coarse clumping. Those samples with no agglutination (0) were recorded as negative while other were recorded as positive.

3.2. Serum Agglutination Test (SAT)

The serum agglutination test (SAT) was carried out as described by OIE (2009). To overcome the prozone phenomenon, if any to occur, 7

tubes were used for each serum sample. An amount of 0.8 ml of phenol-saline was placed in the first tube and 0.5 ml in each succeeding one. Then 0.2 ml of the serum sample was transferred to the first tube and mixed thoroughly with the phenol-saline until it the mixture became homogenous. Then an amount of 0.5 ml of the mixture was carried over to the second tube from which, after mixing, 0.5 ml was transferred to the third tube, and so on. This process was continued until the last tube, from which, after mixing, an amount 0.5 ml of the serum dilution was discarded. This process of doubling dilutions resulted in 1:5, 1:10, 1:20, and so on, dilutions in each tube. To each tube, 0.5 ml of antigen was then added at the recommended dilution and the content of the tube was thoroughly mixed. The tubes were then incubated at 37˚C for 20 hours ±1 hour before the results were read. Furthermore, standard tubes were prepared at the time parallel to the test tubes and incubated together. The antigen was diluted by mixing of 2 ml with 2 ml of phenol-saline, then 5 standard tubes were prepared as follow: in the first tube, 1 ml phenol-saline as ++++, in the second tube 0.75 ml phenol saline with 0.25 ml diluted antigen (1:2) as +++, in the third tube 0.5 ml phenol saline with 0.5 ml diluted antigen as ++, in the fourth tube 0.25 phenol saline with 0.75 ml diluted antigen as + and in the last tube 1 ml of diluted antigen as - or negative.

The degree of agglutination was assessed by the amount of the clearing that had taken place in the tubes compared with the standard tubes. 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% clearing as ++, some sedimentation and 25% clearing as +, and no clearing as negative.

3.3. Competitive Enzyme-Linked Immunosorbent Assay (cELISA)

The Competitive enzyme-linked immunosorbent assay (cELISA) kit was obtained from the Central Veterinary Laboratory, Weybridge, UK. The test was conducted according to the instructions of the manufacturer. Initially, the diluting buffer, wash solution, stopping solution, conjugate solution, and controls were reconstituted. Test serum samples were added per each well of the microtiter plate which has sixty columns (wells). A volume of 100 µl of the prepared conjugate solution was then dispensed in all wells. It was then shaken for 2 minutes in order to mix the serum with the conjugate solution. The plate was then covered with a lid and incubated at room temperature for 3 minutes. The content of the plate was after that discarded and rinsed 5 times with washing solutions and then dried. Thereafter, 100 µL of the substrate chromogen solution was added to all wells. The plate was kept at room temperature for 10 minutes. The reaction was slowed by adding 100 µl of the stopping solution to each well.

To setup the controls 20 ml of the negative controls was added to well A11, A12, B11, B12, C11, and C12, while another 20 ml of the positive control was added to wells F11, F12, G11, G12, H11, and well H12. D11, D12, E11, and E12 serve as conjugated controls.

The results of the tested samples wells were interpreted by comparing to the control wells as follows: very weak or no color development in the well indicated negative result while a strong color development in wells indicates positive result.

3.4. Milk Ring Test

The milk ringtest was performed according to Alton et al. (1975) by adding1ml of each milk sample into a clean sterile test tube. Then 30µl of the stained antigen was added to the samples, mixed well, and left in a water bath at 37ºC for 24 hours before reading the results. Positive samples showed a blue ring that was formed on top of the sample while the negative remained homogenously blue.

3.5. Data Analysis

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS) for Windows® version 22.0 (SPSS Inc., Chicago, Illinois). Appropriate statistical analyses including descriptive statistics, frequencies and cross-tabbing were obtained for each potential risk factor. Univariate and multivariate analyses by means of the 2-tailed chi-square test and logistic regression model were conducted. Associations in the chi-square test and logistic regression model were deemed significant when $p \leq 0.05$. Age and history of abortion were entered into the final logistic regression model with a $p \leq 0.25$ in the Univariate analysis.

4. RESULTS

4.1. Overall Seroprevalence of Anti-Brucella Antibodies

Overall, 10.8% (n=46, 95% CI between 7.85 and 13.75) of the serum samples had anti-brucella antibodies using RBPT of which 13.0% (n=6) were confirmed to be positive by SAT. The RBPT-positive serum samples plus another 46 randomly selected ones were subjected to c-ELISA. The c-ELISA showed that 6.5% (n=6) were positive. None of the milk samples did test positive for milk ring test.

4.2. Seroprevalences and Risk Factors

Statistical significant differences at *p-value* ≤0.05 were not observed between the seroprevalences of the categories of the investigated risk factors by RBPT (Table 1).

Table1. *Seroprevalences of anti-brucella antibodies in goat in El-Gedarif state (January -April 2014)*

Risk factors	No. tested	No. positive	%	95% CI Lower - Upper
Locality				
Al-Fao	46	3	6.5	0.62 - 12.62[a]
El-Gadarif	102	7	6.9	1.98 - 11.82[a]
Al-Hawata	61	4	6.6	0.37 - 12.83[a]
Al-Showak	20	3	15.9	0.13 - 30.93[a]
Basunda	34	13	38.2	21.9 - 54.53[a]
Dokah	55	7	12.7	3.90 - 21.50[a]
Al-Guraisha	55	4	7.3	0.43 - 14.17[a]
Kassab	53	5	9.4	1.54 - 17.26[a]
Sex				
Male	3	0	0.0	0.00 - 0.00
Female	423	46	10.9	7.93 - 13.87[a]
Age (yrs)				
≤ 2	159	12	7.5	3.41 - 11.6[a]
2 - 4	232	28	12.1	7.90 - 16.3[a]
> 4	35	6	17.1	4.63 - 29.6[a]
Breed				
Baladi	398	44	11.1	8.01 - 14.19[a]
Boier	3	1	33.3	-20.03 - 86.6[a]
Sanien	17	1	5.9	-5.30 - 17.10[a]
Shami	8	0	0.0	00.00 - 00.00
Abortion				
Yes	15	3	20.0	0.24 - 40.24[a]
No	411	43	10.5	7.54 - 13.46[a]
Total	426	46	10.8	7.85 - 13.75

Different superscripts indicate significant difference

The highest and lowest seroprevalences among the investigated localities were found in Basunda (38.2%; 95% CI from 21.9 to 54.53) and Al-Fao (6.5%; 95% CI from 0.62 to 12.62) respectively. Moreover, none of the 3 tested samples collected from male animals was positive but 10.9% (95% CI between 7.93 and 13.87) of the samples collected from female animals were positive. The age group > 4 years old had the highest seroprevalence (17.1%, 95% CI between 4.63 and 29.6) among the surveyed different age groups. Baladi, Boier, Sanien, and Shami breeds showed seroprevalences of 11.1%, 33.3%, 5.9%, and 0.0% respectively. While the seroprevalences reported among animals with history of abortion and animals with no history of abortion were 20.0% and 10.5% (Table 1).

4.3. Risk Factors and RBPT-Positive Status

The proportions of sero-positive serum samples varied between the investigated five risk factors. As shown in Table 2, only location (χ^2=31.62, df=7, *p-value*=0.001) was determined to be significantly associated with RBPT-positive status for brucella in the univariate analysis. However, sex, age, breed and history of abortion did not correlate with brucella positive status by using 2-tailed chi-square test.

Table2. *Univariate Association of potential risk factors with RBPT positive status in goats in El-Gedarifstate (January -April 2014)*

Risk factors	No. tested	%	df	χ^2	*p-value*
Locality			7	31.62	0.001
Al-Fao	46	6.5			
El-Gadarif	102	6.9			
Al-Hawata	61	6.6			
Al-Showak	20	15.9			
Basunda	34	38.2			
Dokah	55	12.7			
Al-Guraisha	55	7.3			
Kassab	53	9.4			
Sex			1	0.366	0.545
Male	3	0.0			
Female	423	10.9			
Age (yrs)			2	3.596	0.166
≤ 2	159	7.5			
2 - 4	232	12.1			
> 4	35	17.1			
Breed			3	3.004	0.391
Baladi	398	11.1			
Boier	3	33.3			
Sanien	17	5.9			
Shami	8	0.0			
Abortion			1	1.698	0.193
Yes	15	20.0			
No	411	10.5			

The logistic regression analysis assessed the combined relationship between location (χ^2=31.62, df=7, *p-value*=0.001), age (χ^2=3.596, df=2, *p-value*=0.166), and history of abortion (χ^2=1.698, df=1, *p-value*=0.193). The regression coefficients (Exp(B)) express 'odds ratios' (OR) (= the increased or decreased probability (OR≠1)) of sero-positivity occurrence in comparison to the reference (OR=1). Basunda locality (OR=9.33, 95% CI from 2.767 to 37.39, *p-value*=0.002) and goats with no history of abortion (OR=5.52, 95% CI between 1.091 and 29.60, *p-value*=0.050) were significantly associated with increased odds of being RBPT positive (Table 3). In opposition, age was not significantly associated with increased odds of being RBPT positive (Table 3).

Table3. *Multivariate Association of potential risk factors with RBPT positive status in goats in El-Gedarifstate (January -April 2014)*

Risk factors	No. tested	No. positive	%	Exp(B)	95% CI Lower - Upper	*p-value*
Locality						
Al-Fao	46	3	6.5	ref		
El-Gadarif	102	7	6.9	1.04	0.157 – 3.486	0.703
Al-Hawata	61	4	6.6	1.07	0.206 – 4.583	0.971
Al-Showak	20	3	15.9	2.63	0.478 – 14.44	0.266
Basunda	34	13	38.2	9.33	2.767 – 37.39	0.002
Dokah	55	7	12.7	2.21	0.529 – 9.259	0.277
Al-Guraisha	55	4	7.3	1.10	0.229 – 5.133	0.918
Kassab	53	5	9.4	1.57	0.332 – 6.561	0.608
Age (yrs)						
≤ 2	159	12	7.5	ref		
2 - 4	232	28	12.1	1.43	0.674 – 3.038	0.351
> 4	35	6	17.1	1.06	0.259 – 3.135	0.961
Abortion						
Yes	15	3	20.0	ref		
No	411	43	10.5	5.52	1.091 – 29.60	0.050

5. DISCUSSION

This study showed that the overall seroprevalence of anti-brucella antibodies in goats was 10.8% what means nearly one animal in each eleven animals is seropositive. In addition to that location and history of abortion were found to be associated with brucella positive status.

Brucellosis is one of the important infectious diseases of farm animals. It causes significant economic losses in livestock industry. Besides, it is also one of the most common zoonoses worldwide (Radostits *et al.*, 2007; Wegdan *et al.*, 2016). The reported overall seroprevalence in the present study is different from previous reports which were ranging from 0.3% to 4.0%. These reports were made in different parts of the Sudan (El-Ansary *et al.*, 2001; Ahmed, 2004; Rayas, 2004; Musa, 2006; Ali, 2011). By means of passive surveillance at El-Gedarif Veterinary Clinic, El-Gedarif, a very low seroprevalence of 0.1% (n=69) was noted in a period of 3 years (between 2009 and 2012). During the same period, as low as 0.7% (101/14789) of the male goats destined for export to international markets in the Gulf countries such as Saudi Arabia were found positive for anti-brucella antibodies. These animals were tested at the Veterinary Research Laboratory, El-Gedarif, the Sudan (Anon, 2012). Seroprevalence of anti-brucella antibodies in other animal species like sheep, cattle, and camels varied from 2.5% to 32.0% in different parts of the sudan (Abdallah *et al*., 2015; Wegdan *et al*., 2016; Miada *et al*., 2016; Mahasin *et al*., 2017). In same settings in some parts of Africa, very low seroprevalences of brucellosis in goats were reported. The findings of Muma *et al*. (2006) in Zambia are the best example for such very low seroprevalences. All investigated goats showed no positive reaction against anti-brucella antibodies (Muma *et al.*, 2006). Variations in the seroprevalence of anti-brucella antibodies could possibly be attributed to some factors such as the number of investigated samples and the practiced animal production system in the investigated area. The larger number of investigated animals, the higher chance of finding seropositive animals. This can clearly be seen when the seroprevalences reported in this study for male animals and Shami breed were compared with the seroprevalences reported in female animals and other breed. For male animals and Shami breed only 3 and 8 samples were investigated respectively and none of them was positive. In addition, all samples tested for milk ring test were negative. Furthermore, when the practiced animal production system allows close contact between animals as well as free movement of animals or when good management practices and sanitary measures are not very strictly followed brucellosis can easily spread among goats (Mahasin *et al*., 2017). In this study location and history of abortion were factors that enhance the prevalence of brucellosis in goats. However, Abdallah *et al*. (2015) and Wegdan *et al*. (2016) indicated that there were no risk factors that were found to be associated with the prevalence of brucellosis in sheep in North Kordofan and cattle in Khartoum with exception of age, milking method, and breeding.

Brucellosis is a worrying problem all over the Sudan (Musa *et al.*, 2008). The situation of the disease is alarming although it exerted efforts of the government of the Sudan with the assistance of international organizations during the period 1978-1986 to launch National Training Brucella Program supplemented by training aboard (Musa *et al.*, 2008). Since then many investigators discussed the problems of brucellosis seriously and engaged in intensive reach throughout the country.

The observation that anti-brucella anti-bodies are more prevalent in animals with no history of abortion could be explained by the numbers of samples investigated for animals with and animals with no history of abortion. Only 15 samples were investigated for animals with history of abortion while 411 samples investigated for animals with no history of abortion. Another possible explanation could be that the clinically diagnosed abortion in the tested cases was not due to brucellosis. Nonetheless, cases of abortion associated with serologically positive goats were noticed in different Veterinary Clinics in the state. Numbers of abortion cases were more frequently diagnosed amongst foreign breeds rather than among local or cross-breed goats (Anon, 2012). Because of the increased influx of goats and sheep to the state for export, in addition to the continuous uncontrolled movement of animals for grazing in the state, as well as absence of control measures prevalence of brucellosis is escalating in the state.

Screening of the collected serum samples using RBPT, determining antibody titers of the RBPT-positive samples using SAT besides using cELISA to confirm the positivity of samples with anti-brucella antibodies were among the

strengths of the present study. However, one important limitation of the study is that only few number of milk samples were investigated. Therefore, no conclusions could be made from this result. Not using molecular methods in the study is another limitation.

In conclusion, the seroprevalence of anti-brucella antibodies reported in goats in El-Gedarif state using RBPT is higher than the seroprevalences reported in other parts of the country. Moreover, the seroprevalences reported for risk factors were not significant. Location and history of abortion were found to be associated with brucella positive status. Further studies investigating brucellosis across the whole state using molecular methods should be carried out to better understand the epidemiology of brucellosis in goats. Raising the awareness of goat owners and herders on the routes of transmission of brucellosis and its public health importance is warranted.

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