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Sero-prevalence of brucellosis in dairy cattle in Port Sudan: a short communication

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Abstract: This study was carried out to investigate the seroprevalence of brucellosis in dairy cattle in Port Sudan, the Red Sea state, the Sudan. Two hundred and fifteen blood samples were collected from three locations in Port Sudan locality taking the density of animals in each location in account. Sera samples were tested by the following tests: rose Bengal plate test (RBPT), modified rose Bengal plate test (mRBPT), serum agglutination test (SAT) and competitive enzyme-linked immuosorbent assay (cELISA). The seroprevalences were 13.0% by RBPT and 21.0% by mRBPT. Furthermore, SAT confirmed the positivity of 93% of the RBPT- and mRBPT-positive samples with titers ranging from 20 IU/ml to 1488 IU/ml. The cELISA confirmed the positivity of 27, 21, and 25 of the RBPT, mRBPT, and SAT positives, respectively. The results suggest that the mRBPT is more sensitive than the RBPT in identifying brucellosis-positive cattle. Therefore, it is recommended that mRBPT to be used in the routine diagnosis of brucellosis in dairy cattle.
Keywords: Sero-prevalence, brucellosis, dairy cattle, Port Sudan

INTRODUCTION

Brucellosis is a worldwide public health hazard and has an economic importance [1]. Many brucella species have been detected and identified in animals. Brucella abortus, B. melitensis, and B. ovis are responsible of causing brucellosis in cattle, sheep and goats. Other important brucella species that cause the disease in animals include B. canis, B. suis, B. neotomae and B. microti [2].

Bovine brucellosis is included in the list of World Organization for Animal Health) as a notifiable disease as it has the potential to spread worldwide, to cause significant mortality and morbidity within the susceptible populations and to potentially cause the disease in humans [3, 4].

Serological diagnosis of brucellosis began more than 100 years ago with simple agglutination tests. Since then it was realized that the serological tests were susceptible to false negative and false positive reactions resulting from, for instance, exposure to cross-reacting microorganisms [5]. Gall and Nielsen [6] reviewed many publications that investigated the sensitivity and specificity of the diagnostic tests used for detection brucella-seropositive animals. The different tests were then compared to predict the best diagnostic test by summing of the values of the sensitivity and specificity of each test. The average of the values was the final performance index (PI).

Brucellosis has reported in cattle in Sudan and in other animals like sheep and goats, camels and in equines [7, 8]. Angara and Shuaib [9] indicated that brucellosis, if no control strategy is adopted and put in place, can evolve until all animals in a population or a specific geographical area and that the spreading of this disease exert negative impact on the environment and the public health besides the economic losses. The present study was carried out to investigate the seroprevalence of brucellosis in cattle in Port Sudan.

MATERIALS AND METHODS

Study area

The present study was conducted during February 2010 in Port Sudan locality, Red Sea state, the Sudan. The locality is the smallest among the localities of the state and is located at the coast of the Red Sear. The state occupies an area of 218,887 km^2 and is located in northeastern part of the country, bordering Egypt to the north, Kassala state to the south, and River Nile state to the west and the Red Sea to the east. The state is sub-divided into eight localities: Port Sudan, Suakin, Gunub/Aulib, Sinkat, Hayya, Halaib and Tokar/Agig. The overall human population of the state was estimated at 846,113. The animal population in the

state consist of camels, cattle, sheep and goats. The total number of the cattle is 112,700 of which 23,151 are in Port Sudan locality. These cattle are of a local zebu Halfa, Kenana and Butana types and a few Frisian crosses.

Sample size and samples

The sample size was determined according to the standard formula of Thrusfield [10]. Two hundred and fifteen blood samples for serum were collected randomly from the selected animals from the northern, western and southern parts of Port Sudan locality.

The blood samples were taken aseptically from the jugular vein using vacutainer tubes. Following the collection of blood samples, the vacutainer tubes were put in rack then in a refrigerator at 4˚C overnight. After clot formation, the samples were transported to the Veterinary Research Laboratory, Port Sudan, the Sudan, where sera were separated by centrifugation, gently poured into sterile Eppendorf tubes, tested in the same day for anti-brucella antibodies, and the rest was stored at -20 ˚C for further use.

Serological tests

Three serological tests were used for detection of anti-brucella antibodies in serum including rose Bengal plate test (RBPT), standard agglutination test (SAT) and enzyme linked immune-sorbent assay (competitive-ELISA).

Rose Bengal Plate Test (RBPT)

The RBPT was carried out as described by Ferede *et al*. and OIE [2]. It was conducted 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 zero, +, ++ and +++. A score of zero 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.

Modified Rose Bengal Plate (mRBPT)

This was similar to the classic Rose Bengal test but differed in the volume of antigen used which was half or third of the serum volume (antigen to serum was 1:2). This procedure was deemed suitable for detection of weakly positive samples.

Serum agglutination test (SAT)

The SAT was carried out as described by OIE [2]. 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.

Competitive enzyme-linked immunosorbent assay (cELISA)

The 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.

RESULTS

Seroprevalences of anti-brucella antibodies

The estimated seroprevalences were 12.5% (n = 27) and 21.2% (n = 46) using RBPT and mRBPT. SAT confirmed the positivity of 93% (n = 27) of the RBPT- and mRBPT-positive samples with antibody titres ranging between 20 IU/ml and 1488 IU/ml. Furthermore, the cELISA confirmed the positivity of 27, 21, and 25 of the RBPT, mRBPT, and SAT positives, respectively.

The RBPT classified 13.0% (n = 26) of the samples from the northern part of the locality as seropositive, 11% (n = 1) from the southern part, and 100% (n = 5) from the western part. By the mRBPT 22% (n = 44), 22% (n = 2) and 0% (n = 0) were showing positive reactions to anti-brucella antibodies from the northern, southern, and western parts of Port Sudan (Table 1).

Table 1: Detection of brucella seropositive cattle by RBPT and RBPT in Port Sudan locality (February 2010)

Area	No. of samples	RBPT	%	mRBPT	%
North	201	26	13.0	44	22.0
South	9	1	11.0	2	22.0
West	5	0	0.00	0	0.00
Total	215	46	13.0	27	21.0

Comparison between the serological tests

The chi square test showed some differences between the tests used in this study with respect to brucellosis sero-positivity in cattle (x^2= 71.7, df=3, P=0.001). As shown in Table 2, subsequent paired comparisons showed significant differences between cELISA and SAT, RBPT and mRBPT, RBPT and SAT, and between mRBPT and SAT. There were no differences between the results obtained by RBPT and cELISA (x^2=1.6, df=1, P =0.200) and mRBPT and cELISA (x^2 = 0.1, df =1, P =0.700).

Table 2: Comparison between the tests used for detection of brucella seropositive cattle in Port Sudan locality (February 2010)

Test	negative	positive	x^2	df	P-value
SAT and cELISA	5* 98	22 22	39.0	1	0.001
cELISA and mRBPT	98 171	22 46	0.1	1	0.700
RBPT and SAT	188 5*	27 22	66.3	1	0.001
mRBPT and RBPT	171 188	46 27	4.3	1	0.040
RBPT and cELISA	188 98	27 22	1.6	1	0.200
RBPT and SAT	171 5*	46 22	42.0	1	0.001

* = original is three

DISCUSSION

Brucellosis is an important infectious bacterial disease, which has been reported in many countries worldwide. The magnitude of the disease is due to its zoonotic nature and the economic losses it causes. Moreover, it is a multi-species disease [11, 7]. McDermott and Arimi [12] and Ziad *et al.;* [13] indicated that prevalence of brucellosis among animals in sub-Saharan Africa is poorly estimated or unknown in some cases. In addition, because of the economic status of most of African countries, control of brucellosis has been very difficult. In the present study, the sero-prevalence of brucellosis in dairy cattle in Port Sudan locality was estimated and some serological tests that are routinely used for the diagnosis of brucellosis were compared. The seroprevalences reported herein were in agreement with the reports of Ali [18] and Wegdan *et al.;* [14]. However, the records of the

Veterinary Research Institute, Port Sudan, showed that 54.6% of the cattle, 9.1% of the camels, 25.0% of the sheep, 31.3% of the goats and 66.7% of the equines in the state were brucella-seropositive in the period between May 1998 and March 2007. Additionally, it was different from the reports of Musa [15], Musa [16], El-Ansary *et al.;* [17], Mohammed [18], Abdel-Gader [19], Mohammed [20], Senein and Abdelgadir [21], Angara *et al.;* [22, 23], Solafa *et al.;* [24], Salman *et al.;* [25], and Hamid *et al.;* [26]. Further different reports were also made in many other countries in Africa, Asia, and South America [27-32]. Poor management, crowding of animals in small and closed farms as well as poor hygienic measures could probably explain the high sero-prevalence of brucellosis in this study. There is no annual vaccination against brucellosis in the state either. The habit of culling of the infected animals by easily selling them to other farms or to other states. Most of the owners and/or herders are not aware about the transmission routes of the disease.

The mRBPT was more sensitive than RBPT but both were less sensitive in comparison to cELISA in this study. Gall and Nielsen [6] found that the buffered antigen plate agglutination test (BPAT) had the highest PI which was 193.1 among the conventional tests, and hence, it was more accurate than the other conventional tests including RBPT (PI = 167.6) and complement fixation test (PI = 172.5). On the other hand, the primary binding assays like fluorescence polarisation assay (PI = 196.4), iELISA (PI = 189.8) and cELISA (PI = 188.2), were more accurate than the conventional tests, except for the BPAT. The cELISA was routinely used as confirmatory tests because of its high sensitivity and specificity in detection of Brucella antibodies [33, 6]. Elisa is the lease sensitive in diagnosis of brucellosis in camels may be due to the morphology of antibodies of camels [34]. This was attributed in part to the instability of some of the antigen preparations used in conventional tests. In addition, MacMillan [35] reported that, perhaps, the RBPT antigen is deteriorating when repeatedly cycled and used between refrigerator and room temperature. Other sources of variation are the concentration of whole cells and the pH of the antigen used in agglutination tests, such as the RBPT and SAT [36]. The cELISA is capable of distinguishing vaccinated animals or animals infected with cross-reacting organisms from naturally infected animals, thereby reducing the number of false positive reactions. Gall and Nielsen [6] suggested that the primary binding tests were price competitive with conventional tests and, therefore, had a better-combined cost/efficiency rating. However, in developing countries, ELISA is expensive to be used as routine test and laboratory infrastructure and trained personnel are another issue. Conventional tests that are highly sensitive, inexpensive and rapid are ideal as screening tests in these countries such as the Sudan and primary binding tests that are ideally sensitive and specific can be used as confirmatory tests.

CONCLUSION AND RECOMMENDATIONS

In conclusion, anti-brucella antibodies are prevalent in dairy cattle in Port Sudan and this might pose a risk to milk consumers and producers. Therefore, it is recommended to raise awareness of the public on brucellosis and its zoonotic dimension. An area-wide survey of brucellosis should be carried out in cattle and other animal species in the Red Sea state.

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