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Evaluation of different serological tests for the diagnosis of human Brucellosis in Sudan

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ABSTRACT:

Objective: Brucellosis is a highly contagious zoonotic disease affecting livestock and human beings. The human disease lacks pathognomonic symptoms and laboratory tests are essential for its diagnosis. However, most tests are difficult to implement in the areas and countries where brucellosis is endemic. In this research the aim was to compare the simple and cheap Rose Bengal Plate Test (RBPT) and the modified Rose Bengal test (mRBPT), with serum agglutination test (SAT) and competitive Enzyme-linked immunosorbent assay (cELISA) in Sudanese citizens with high risk of Brucellosis in Gezira state, central Sudan.

Patients and Methods: Eighty samples were collected from people at risk. Blood for serum samples was collected from arm venous blood using serum vaccutainer tubes with needles and needle holders. Each of the eighty serum samples was used for diagnosis of brucellosis using Rose Bengal Plate test (RBPT), modified Rose Bengal plate test (mRBPT), serum agglutination test (SAT), and competitive Enzyme-linked immunosorbent assay (cELISA).

Results: Serologically, both RBPT and mRBPT gave 7 (8.8%) positive results in comparison to 73 (91.2%) negative results, while the SAT scored 11 (13.8%) positive results in comparison to 69 (86.2%) negative results, and the cELISA scored 8 (10%) positive results in comparison to 72 (90%) negative results.

Conclusions: SAT scored the highest sensitivity although is known for giving both false positive and false negative. cELISA detected more cases than RBPT and mRBPT. More than one test is preferred to be used in endemic areas.

Keywords: Brucellosis, Rose Bengal test, ELISA, SAT.

INTRODUCTION

Brucella species are group of gram-negative bacilli that are transmitted by animals, mainly pigs and ruminants (cows, camels, goats, sheep …etc.). The relatively small coccobacilli chose to be intracellular infectious to humans[1]. Symptoms of brucellosis include fever (in almost all the cases), fatigue, loss of appetite, headache, arthralgia (joint pain), myalgia (muscle pain)[1,2] Due to the non-specificity of these symptoms, the diagnosis of brucellosis is always confirmed by lab diagnosis, and the isolation of the bacteria from the body is the gold standard diagnostic method of diagnosis[3]. Blood being the best isolate, but these bacteria could also be found in the bone marrow, cerebrospinal fluid (CSF), and wounds fluid[3].

Disadvantages of blood culture are the false negative results, and the prolonged time of incubation; most of the cultures need 1-3 weeks for documenting a result[3]. The serology is faster and more accurate[4]. Serology is considered the principal diagnostic method for brucellosis[5].

The Rose Bengal Plate Test (RBPT) is a highly recommended rapid screening test, but the results should always be confirmed by other tests detecting agglutinating and non- agglutinating antibody and by bacteriological culture, particularly in areas where there is a high incidence of animal brucellosis[6,7]. The sensitivity

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of RBPT is over 99%, but it can give false positive reactions with sera from patients infected with *Yersinia enterocolitica* or other cross-reactive organisms and from healthy individuals that have had contact with Brucella species without developing disease[3,7].

The mRBPT was recommended by Blasco et al [6] in 1994 to improve the sensitivity and for confirmation of RBPT when other tests are not available. SAT is also a suitable diagnostic test for human brucellosis, but it results in high percentage of false negatives. Whereas ELISA is widely used for confirmation of RBPT and SAT results[3].

Brucellosis is a highly contagious zoonotic disease affecting livestock and human beings. The human disease lacks pathognomonic symptoms and laboratory tests are essential for its diagnosis. However, most tests are difficult to implement in the areas and countries were brucellosis is endemic. Here, we compared the simple and cheap Rose Bengal Plate Test (RBPT) and the modified Rose Bengal test (mRBPT), with serum agglutination test (SAT) and competitive cELISA, in Sudanese citizens with high risk of Brucellosis, as Sudan is considered a Brucella endemic country[8,9].

MATERIALS AND METHODS

Study description

This was a comparative analytical cross-sectional cohort study, performed in the period between April and October 2011 in Wad Medani city in Gezira State, central Sudan.

The city is surrounded by nomadic areas and owners of cattle, and the Sudan is one of the countries endemic for Brucellosis.

A questionnaire was designed to help in collection of data from people at risk with brucellosis. The questionnaire included: name of the person, age, gender, occupation, risk factor for brucellosis, history of infection with brucellosis, relapses after treatment and if any precaution measures taken to protect from infection with the disease.

Eighty samples were collected from people at risk. Blood for serum samples was collected from arm venous blood using serum vaccutainer tubes (Becton Dickinson, Johannesburg, South Africa) with needles and needle holders. The blood samples were preserved on ice using ice boxes, transported to the laboratory, left to clot and the serum samples were separated by centrifugation at 6000 round per minute for six minutes. The serum samples were then kept at -20°C in a deep freeze till needed to examination.

Serological tests

Each of the eighty serum samples was used for diagnosis of brucellosis using Rose Bengal Plate test (RBPT), modified Rose Bengal plate test (mRBPT), serum agglutination test (SAT), and competitive Enzyme-linked immunosorbent assay (cELISA). The RBPT and mRBPT antigens were imported from Spain (VIRCELL®, Santa Fe, Granada, Spain), the SAT antigen, and the ELISA kit were kindly supplied by Alpha laboratories (Eastleigh, Hampshire, UK). The RBPT was performed by adding 25 micro liter (µl) of the antigen to an equal volume of the test serum on an enamel plated plate, mixed, rocked gently for 4 minutes and any degree of agglutination was considered positive or otherwise negative. The mRBPT was performed by adding 25 µl of the RBPT antigen to 75 µl of the test serum and the test was performed similarly to the RBPT. SAT was performed by preparing serial dilutions of each test serum starting with 1:20 (1/20) dilution up to 1:320 (1/320), then an equal volume of antigen was added to each tube, the tubes were incubated at 37°C for 24 hours, and the agglutinations were read for positive or negative results. The titer of (≥ 1/160) was considered positive for brucellosis according to the supplier of antigen. The cELISA kit used contained micro plates pre-coated with *Brucella melitenesis* lipo-polysaccharide (LPS) antigen, monoclonal immunoglobulin G (IgG) antibodies conjugated with a horseradish peroxidase (HRP), a diluting buffer, a washing solution, a substrate and a stopping solution plus positive and negative controls. The test was performed as described by the manufacturer, and then it was read using ELISA reader at a wavelength of 450 nanometers (nm). The calculations were made as recommended and the positive/negative cut off point was 60% of the mean optical density (OD) of the 4 conjugate control wells, which was 0.1335, and any test sample giving OD equal to or below this value was regarded as being positive.

Statistical Analysis

Statistical tests were carried using Statistical Package for the Social Sciences SPSS, version 1 (SPSS Inc. Chicago, IL, USA).

RESULTS

The distribution of the age groups within the 80 persons examined showed that 11 (13.8%) were below 20 years of age, 52 (64.9%) from 20 -50 years and 17 (21.3%) above 50 years. Forty-nine persons (61.3%) were males, and 31 (38.7%) females. Table 1 shows the result of distribution of brucellosis in the different age groups. Of the 80 people examined there were 23 (28.8%) working in farms, 18 (22.5%) in abattoirs and 39 (48%) were veterinarians, student in faculties of animal production, and housewives. There were five (6.3%) of the 80 people who were previously diagnosed positive for brucellosis.

Regarding symptoms, fever was the main complaint in 43 (53.8%), joint pains in 61 (76.3%), and drenching sweating in 48 (60%) of the 80 persons. People who ate raw meat were 31 (38.8%) and who drank raw milk 19 (23.8%) of the 80 persons.

Concerning contacts with animals, 28 (35%) were milk handlers, 23 (28.7%) attended cattle and sheep at labor, 1 (1.3%) was in contact with animal secretions, and 27 (33.8%) had no direct contact with animals (Fig-

Table 1. Prevalence of brucellosis in different age groups of the people examined.

Age group	Frequency	Percent	Positive for the disease	Negatives
< 20	11	13.8	0 (0%)	11 (13.8%)
20 – 30	19	23.8	1 (1.3%)	18 (22.5%)
30 – 40	11	13.8	2 (2.6%)	9 (11.3%)
40 – 50	22	27.5	5 (6.3%)	17 (21.3%)
> 50	17	21.3	0 (0%)	17 (21.3%)
Total	80	100	8 (10%)	70 (90%)

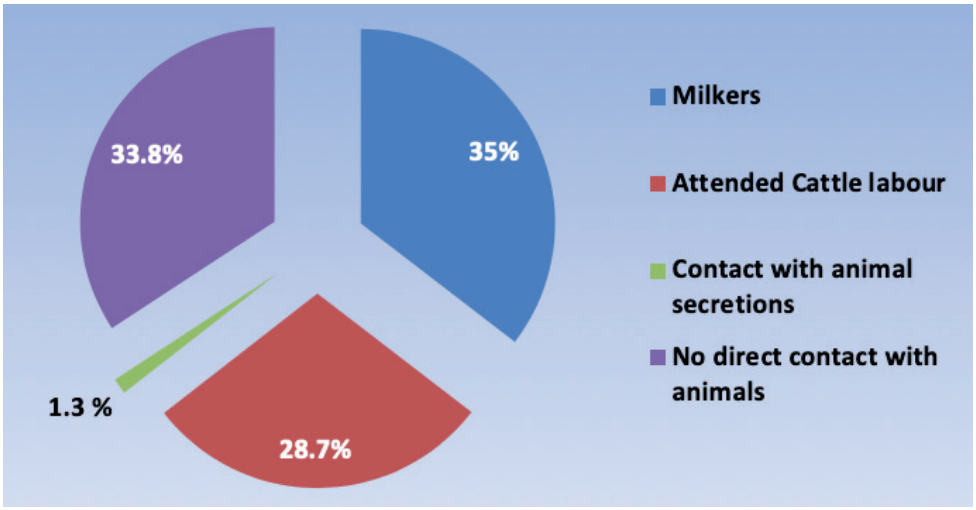

Figure 1. A pie chart presenting the nature of contact with animals, if any, in the study population.

ure 1). The results of examination of the 80 serum samples by RBPT, mRBPT, SAT, and cELISA are presented in Table 2 and Figures 2 and 3.

DISCUSSION

The major findings in this study are: seven of the candidates, resembling 8.8% were positive of Brucellosis by the RBPT and mRBPT, eleven (13.8%) were positive suing the SAT and eight (10%) were positive through the cELISA. These results go with the literature which gives the ELISA a higher sensitivity than Rose Bengal tests, but not higher in specificity than the SAT[3]. On the other hand, SAT is known to give false positive results[10].

In a comparative study conducted by Araj et al[11], it was argued that the ELISA method should be preferred because in chronic and complicated cases since SAT and Rose Bengal tests might miss a serious portion of positive cases. This is not the case here, since all participants were having mild symptoms.

In Sudan, the researches in brucellosis seem to gather both humans and cattle in the same study. Omer et al[9] sampled, in 2010, over 2000 camels and fifty humans for brucellosis by different serological tests. Of the humans, 60% of the local residents were positive for brucella, compared with only 9% of the camel meat handlers were positive. The overall percentage of cELISA detection of brucellosis was higher than RBPT by 2.1%[9], while in our current study, the difference is 1.2%, also in favor of the cELISA. The Omer et al[9] study has the advantage of having a markedly larger sample size, although when considering the human factor, our current study has the larger population. Our study has the advantage of comparing wide variety of serological tests including the relatively expensive cELISA. Also, it targeted a suitable population of Gezira state which is a known focus of Brucella in Sudan. What to be taken against this current study is the small sample size. When comparing the 80 candidates of the study to the total population of Gezira state (5.000.000 citizens), we get a confidence interval of 11, which is a relatively high interval, and

Table 2. Results of examinations of the 80 human sera with different tests.

Test	RBPT	mRBPT	SAT	cELISA
Positive results	7 (8.8%)	7 (8.8%)	11 (13.8%)	8 (10%)
Negative results	73 (91.2%)	73 (91.2%)	69 (86.2%)	72 (90%)

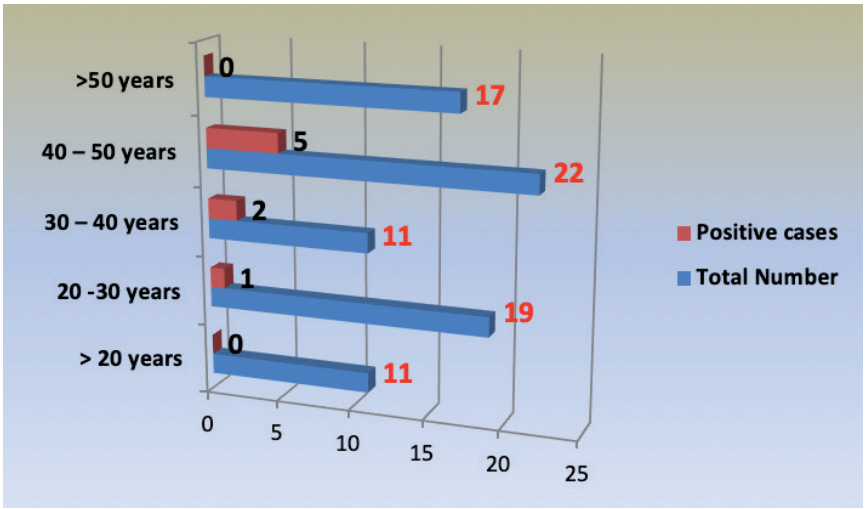

Figure 2. A bar chart showing the ratio of each age group positive cases to the total number of participants.

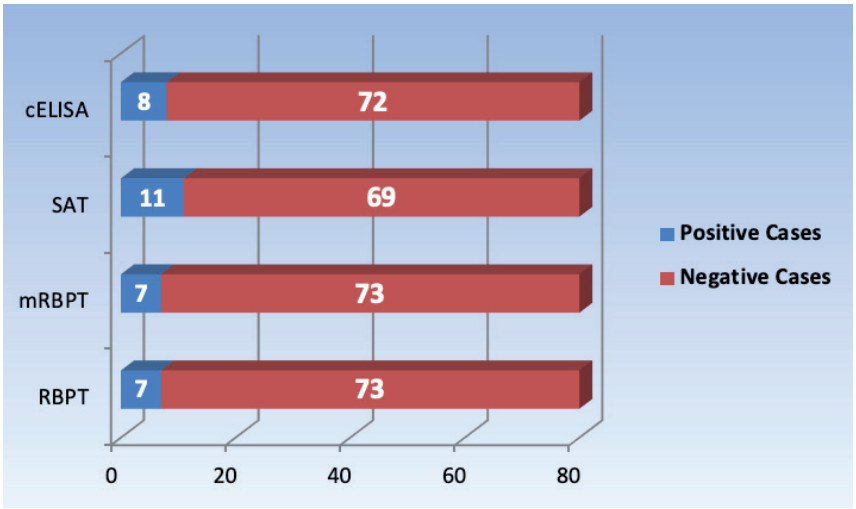

Figure 3. A bar Chart showing the positive cases within the total population of research candidates, with respect to each serology test; cELISA: competitive ELISA, SAT: serum agglutination test, RBPT: Rose Bengal plate test, mRBPT: modified rose Bengal plate test.

this decreases the power of the study. However, due to the limited budget and timing of the study, eighty candidates seem acceptable.

CONCLUSIONS

This was a comparative cross-sectional cohort study performed in Sudanese citizens residing the Gezira state in and the surroundings of Wad Medani city. Four serological tests were performed for each candidate: RBPT, mRBPT, SAT, and cELISA. According to the findings, RBPT and mRBPT had showed fewer positive cases than the cELISA. Although the SAT is less specific than RBPT and cELISA, it scored the highest positive results,; however, this test is known in literature to give both false positives and false negatives. Authors recommend the use of more than one serological test to diagnose and follow-up human brucellosis in Sudan.

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Conflict of Interests:
Authors have no conflicts to declare

REFERENCES

1. Ryan KJ, Ray CG (editors). Sherris Medical Microbiology: an introduction to infectious diseases. 4th ed. New York, NY: McGraw-Hill Inc; 2004.
2. Lopez-Goni I, O'Callaghan D (editors). Brucella: Molecular Microbiology and Genomics. Blackwells, UK: Caister Academic Press; 2012.
3. Corbel MJ (Principal author), Elberg SS, Cosivi O (editors). Brucellosis in humans and animals. Geneva, Switzerland: WHO Press; 2006. URL: https://www.who.int/csr/resources/publications/Brucellosis.pdf

4. Alıskan H. Diagnostic value of Culture and serological methods in human brucellsis. Mikrobiol Bult. 2008; 42: 185195.
5. Galińska EM, Zagórski J. Brucellosis in humans – etiology, diagnostics, clinical forms. Ann Agri Env Med. 2013; 20: 233-238.
6. Blasco JM, Garin-Bastuji B, Marin CM, Gerbier G, Fanlo J, Jimenez de Bagues MP, Cau C. Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of Brucella melitensisinfection in sheep and goats. Veterinary Record 1994; 134: 415-420.
7. Díaz R, Casanova A, Ariza J, Moriyón I. The Rose Bengal Test in Human Brucellosis: A Neglected Test for the Diagnosis of a Neglected Disease. PLoS Negl Trop Dis 2011; 5: e950.
8. Musa MT, Eisa MZM, El Sanousi EM, Abdel Wahab MB, Perrett L. Brucellosis in Camels (Camelus dromedarius) in Darfur, Western Sudan. J Comp Pathol 2008; 138: 151-155.
9. Omer MM, Musa MT., Bakhiet MR., Perrett L. Brucellosis in camels, cattle and human: association and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Rev Sci Tech Off Int Epiz 2010; 29: 663-669.
10. Osaba AO, Balkhy H, Memish Z. Diagnostic value of Brucella ELISA IgG and IgM in bacteremic and non-bacteremic patients with brucellosis. J Chemother 2001; 13: 54-59.
11. Araj GF, Kattar MM, Fattouh LG, Bajakian KO, Kobeissi SA. Evaluation of The PANBIO Brusella immunglobulin G and IgM enzyme-linked immunosorbent assays for diagnosis of human bruscellosis. Clin Diagn Lab Immunol 2005; 12: 1334-1338.