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Evaluation of an Immunocapture-Agglutination Test (Brucellacapt) for Serodiagnosis of Human Brucellosis, Ilam, Iran

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Abstract: Brucellosis is common health problem in some Middle Eastern, Mediterranean countries and Iran. The present investigation was carried out to investigate the prevalence of brucella antibodies through Rose Bengal test (RBT), Wright and Coombs and comparison with Brucella Capt Test. A total of 754 different suspected to brucellosis were tested during the period from March 2008 to February 2009. They assayed by Brucellacapt, Coombs tests and SAT. Our results had shown that of 754 serum samples, 125 samples were positive by Rosbangal test. Thus, frequency of brucellosis by Rosbangal test was 16.5%. The results in 1/40 and 1/80 were equal for Brucellacapt and Coombs tests and different for SAT. The other titers results were different for all testes which used in our study. The results from the present study showed a high sensitivity and specificity of Brucellacapt for the diagnosis of human brucellosis.

Key words: Brucellacapt · Brucellosis · Iran

INTRODUCTION

Brucellosis is a zoonotic disease caused by Gram-negative bacteria brucella that are pathogenic for a wide variety of animals and human beings[1]. It is an emerging disease since the discovery of *B. melitensis* as the cause of Malta Fever by Bruce in 1887 and the isolation of *B. abortus* from aborted cattle by Bang in 1897 [2]. The importance of brucellosis is not known precisely, but it can have a considerable impact on human and animal health, as well as socioeconomic impacts, especially in which rural income relies largely on livestock breeding and dairy products [3].

Human brucellosis is caused by exposure to livestock and livestock products. Infection can result from direct contact with infected animals and can be transmitted to consumers through raw milk and milk products. In humans, the symptoms of disease are weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly and night sweats [4]. Brucellosis is common health problem in some Middle Eastern, Mediterranean countries [5, 6] and Iran. Brucellosis is an infectious disease caused by various Gram-negative bacteria of the genus *Brucella* spp. This disease is the cause of significant economic losses in livestock production due to reproductive disorders and reduced production of affected animals. The prevalence rate of brucellosis in different parts of Iran varied from 1.5 up to 107.5 per 100000. The highest levels of infection appeared in Hamedan with 107.5, Kurdistan with 83.5, Azarbaijan Gharbi with 71.4 and Zanjan with 67.1 per 100000 people [7-9]. Thus, its prevention, control and eradication are a major challenge for public health program.

Many serological tests have been used for the diagnosis of human brucellosis. The most commonly used tests are the serum agglutination test (SAT), the Coombs anti-*Brucella* test, the Rose Bengal test. These present technical difficulties since they require skilled personnel and high-cost material. Also, interpretation of enzyme immunoassay results is difficult due to the variability of antigens and technical procedures employed. Among the techniques used for the diagnosis of human brucellosis, SAT and the Coombs test are most often used and their performance in disease diagnosis and during disease evolution has been studied thoroughly. However, their evaluation is sometimes uncertain and the

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interpretation of SAT titers of >1/160 is problematic in areas of endemicity, since low SAT titers may be present in healthy people who previously suffered the disease [10], in patients during the first stage of the infection [11] and in patients suffering chronic brucellosis or a relapse. Diagnosis of a relapse is particularly difficult and is most often based on the presence of high titers in the Coombs test.[10] However, this is a long and technically difficult test, requiring skilled personnel and so it is not routinely performed in many clinical laboratories. The convenience of using Brucellacapt, a new serological test for the diagnosis of human brucellosis based on immunocapture- agglutination.

The present investigation was carried out to investigate the prevalence of brucella antibodies through Rose Bengal test (RBT), Wright and Coombs and comparison with Brucella Capt Test.

MATERIALS AND METHODS

A total of 754 different suspected to brucellosis was tested during the period from March 2008 to February 2009.

Collection of Blood and Preparation of Sera: About 5-7 ml of blood was collected by using a sterile disposable syringe and needle. Then the sera was prepared by centrifugation as per standard procedure and stored in vials at -20°C until used.

Serological Methods: For serology, blood samples were centrifuged (3000×*g* for 10 min) and the serum divided into aliquots and stored at -20°C until needed. All sera were evaluated using the Rose Bengal Test, Serum Agglutination Test, Coombs' Test and Brucella Capt Test.

Brucellosis was diagnosed on the basis of clinical evidence, a SAT titer of >1/160, or a fourfold rise in SAT or Coombs test titers between two samples collected within 15 to 30 days of each other.

Serum Agglutination Test (SAT): The assay was performed as described by Alton *et al*. [12] Briefly, 0.5 ml of brucella SAT antigen was added to 0.5 ml of each serum sample, diluted serially from 1:5 to 1: 640 in physiological saline solution and mixed thoroughly. SAT reactions were read after a 24-h incubation at 37°C. The agglutination ++ and stronger, observed in sera at dilution 1:20 and higher, was considered to be positive.

Rose Bengal Test (RBT): For RBT, the procedure of Baek *et al*. [13] was followed. Briefly 30 µl of serum was mixed with equal volume of antigen on a white enamel plate circled approximately 2 cm in diameter with manicure. The mixture was rocked gently for 4 min at room temperature and then observed. Any sign of agglutination was considered positive.

Coombs Test: The Coombs test was carried out with the SAT tubes by washing three times with phosphate-buffered saline (pH 7.2) by centrifugation at 3,000 3 *g* for 20 min. After the last wash, the bacteria were suspended in 1 ml of phosphate-buffered saline and 0.05 ml of previously standardized anti-total human immunoglobulin (Sanofi Pasteur) was added to each tube. The tube contents was mixed and incubated at 37°C for 24 h [13].

Brucella Capt Test: The Brucellacapt Test (Vircell SL) was performed as specified by the manufacturer.

Briefly, 0.050-ml samples of serum dilutions were added to wells of a U-bottom microtiter plate coated with anti-total human immunoglobulin. Then 0.050 ml of an antigen suspension was added to all the wells. The plates were sealed with adhesive tape and incubated at 37°C for 24 h in a dark humid chamber. Positive reactions show agglutination over the bottom of the well. Negative reactions are indicated by a pellet at the center of the bottom of the well.

RESULTS AND DISCUSSION

Our results had shown that of 754 serum samples, 125 samples were positive by Ros Bangal Test. Thus frequency of brucellosis by RBT was 16.5%. All the initial sera from the 125 patients gave titers of >1/40 in the Brucella Capt, Coombs Tests and SAT. All the initial sera from the 125 patients gave titers > 1/80 for Brucella Capt while only 123 (99%) were positive in the SAT at the same titer. In titer of 1/160 91, 90 and 80% of sera were positive in Brucellacapt, Coombs tests and SAT, respectively. Results had shown in Tables 1-4.

Brucellacapt, Coombs Tests and SAT had 64, 55 and48% in titer of 1/320, respectively. in titer of 1/640 percentage of positive sera were as an equal for Brucella Capt and Coombs tests (31%) while this was 20% for SAT but in titer of 1/1280 results were different and were 12%,9% and 4.8% for Brucellacapt and Coombs tests and SAT, respectively.

Table 1: Comparison between capt SAT and coombs test

	1/40		1/80		1/160		1/320		1/640		1/1280		Total	
SAT	2	1.6%	20	16.0%	35	28.0%	43	34.4%	19	15.2%	6	4.8%	125	100%
Coombs test	0	0.0%	13	10.4%	39	31.2%	35	27.0%	27	21.6%	11	8.8%	125	100%
Brucellacapt test	0	0.0%	12	9.6%	34	27.0%	41	32.8%	24	19.2%	14	11.2%	125	100%

Table 2: Results of SAT in different titer

SAT	1/40	1/80	1/160	1/320	1/640	1/1280
125 (100%)	+					
123 (99%)	+	+				
103 (83%)	+	+	+			
68 (48%)	+	+	+	+		
25 (20%)	+	+	+	+	+	
6 (4.8%)	+	+	+	+	+	+

Table 3: Results of Brucellacapt test in different titer

Brucellacapt test	1/40	1/80	1/160	1/320	1/640	1/1280
125 (100%)	+					
125 (100%)	+	+				
113 (91%)	+	+	+			
79 (64%)	+	+	+	+		
38 (31%)	+	+	+	+	+	
14 (12%)	+	+	+	+	+	+

Table 4: Results of Coombs test in different titer

Coombs test	1/40	1/80	1/160	1/320	1/640	1/1280
125(100%)	+					
125(100%)	+	+				
112 (90%)	+	+	+			
73(55%)	+	+	+	+		
38 (31%)	+	+	+	+	+	
11 (9%)	+	+	+	+	+	+

The results from the present study showed a high sensitivity and specificity of Brucella Capt for the diagnosis of human brucellosis both in the first stages of the disease and in cases with long evolution as well as in relapses and re infections. All the initial sera from patients with brucellosis included in the study had Brucella Capt and Coombs titers of >1/80, while only 99% of them were SAT positive. However, problems in the interpretation arose with the use of a 1/80 diagnostic titer, especially in areas of endemicity, whereas the prevalence of anti-*Brucella* antibodies is high due to previous episodes of brucellosis or exposure to infected animals in a high proportion of the population [14].

The definition of a diagnostic titer, indicative of an active infection, has not been possible in human brucellosis, even in tests such as the Coombs test and SAT, which have been in use for a long time [15]. Most authors [16] consider a SAT titer of >1/160 to be indicative of active brucellosis. However, active brucellosis cannot be excluded in patients with lower SAT titers, especially during the first stage of the infection, in chronic brucellosis and in relapses [17]. In the present study, nearly 48% of the initial sera from infected patients showed SAT titers of, 1/160. This implies a serious limitation for disease diagnosis, especially since prompt treatment is very important for a good prognosis [18]. Our study shows that titers of >1/320 in Brucella Capt and in the Coombs test and >1/40 in SAT indicated the existence of brucellosis with a high degree of probability and titers lower than these allow us to eliminate the possibility of brucellosis in the majority of cases.

Finally, the study shows very good correlation between the Brucella Capt and Coombs tests, with a good concordance between titers obtained by both tests. Similar results were found by Gomez *et al.* [19] with sera from brucellosis patients or uncon- firmed but suspected brucellosis patients. In conclusion, our result showed that the diagnostic efficiency of Brucella Capt is equal to that of the Coombs test. Nevertheless, a lower correlation and concordance were found between Brucella Capt and SAT.

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