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Original Article

Comparison the serologic tests used in the diagnosis of brucellosis; brucellacapt, brucella coombs gel, and brucella coombs tube agglutination tests

Elif Seren Tanriverdi[1], Yucel Duman[1], Harika Gozde Gozukara Bag[2], Mehmet Sait Tekerekoglu[2]

[1]Department of Medical Microbiology, Faculty of Medicine, Inonu University, Malatya, Turkey
[2]Department of Biostatistics and Medical Informatics, Faculty of Medicine, Inonu University, Malatya, Turkey

Abstract

Aim: Brucellosis is the most common bacterial zoonosis in the world and in our country. The definitive diagnosis of the disease is the isolation of the agent in culture, but in routine diagnosis serologic tests are mostly used. In the routine serological diagnosis of brucellosis, rose bengal, standard tube agglutination (STA) and coombs tube agglutination (CTA) tests were used. The aim of this study was to determine the effectiveness of Brucellacapt (BCAP) and Coombs Gel (CJ) tests by comparing with STA and CTA tests.
Materials and Methods: A total of 100 samples (47 positive and 53 negative by CTA test) were included in the study between June 2018 and July 2019. Titters detected as ≥1 / 160 in STA, CTA, BCAP (METSER Brucella test with Coombs, Savas Medical, Istanbul), CJ (ODAK Brucella Coombs Gel test, Toprak Medical, Istanbul) tests were accepted as positive. Cohen kappa (κ) analysis was used to evaluate the consistency between the tests.
Results: Out of 100 samples included in the study were found positive, 20 with STA, 48 with CAP and 53 with CJ tests, respectively. Among the 47 patients who were positive with CTA test, 44 were positive with BCAP and CJ tests, also 2 of them were negative with BCAP and 1 with CJ test. Among the samples found negative with the CTA test, 3 were found positive with BCAP and 7 with the CJ test. STA test was negative in 27 samples that were positive by CTA test. κ = 0.900 for CTA and BCAP, κ = 0.841 for CTA and CJ, κ = 0.860 for BCAP and CJ; showed a high level of agreement. The STA test showed a very low level of agreement with all three methods (κ = 0.440 for CTA with STA, κ = 0.426 for BCAP with STA, κ = 0.363 for CJ with STA).
Conclusion: Compared to CTA testing, the applicability of BCAP and CJ tests is easier. Among the three tests, the CJ test gives the fastest results. In the serologic diagnosis of brucellosis, BCAP and CJ test can be used because of high compatibility with CTA test, and it is thought that the compatibility between the tests should be evaluated with more comprehensive studies.

Keywords: Brucellosis; coombs tube agglutination; brucellacapt; coombs gel test; serology; rose bengal

INTRODUCTION

Brucellosis is a zoonotic disease caused by gram-negative, aerobic, intracellular, Brucella species bacteria. It is transmitted to humans through consumption of infected food, close contact with infected animals, direct contact with infected animal tissues such as the placenta, and inhalation of infected aerosols (1). After 2-3 weeks of incubation, the disease causes a wide range of clinical symptoms and resembles a systemic infection. The most common symptoms are coronary fever, fatigue, night sweats, muscle and joint pain, loss of appetite, weight loss, and headache (2). The disease is endemic in our country and is still a public health problem. Mortality due to brucellosis was last seen in 2008 in our country. According to the data of the Ministry of Health department, the morbidity rate was 13.73 / 100000 in 2008 and it decreased to 7.99 / 100000 in 2017. Control of disease in animals reduces the incidence of brucellosis in humans (3). (T.C. Ministry of Health, General Directorate of Primary Health Care, Zoonotic Diseases Department. Brucellosis data.)

Although culture isolation is the gold standard method in the diagnosis of brucellosis, the success rate is 40-70 %. Therefore, in the laboratory diagnosis of brucellosis, specific antibody determination is usually made in serum (4). Rose Bengal (RB), standard tube agglutination (STA), coombs tube agglutination (CTA) tests are the most commonly used tests in the serological diagnosis of

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Corresponding Author: Yucel Duman, Department of Medical Microbiology, Faculty of Medicine, Inonu University, Trabzon, Turkey, **E-mail:** yucel.duman@inonu.edu.tr

brucellosis. Interpretation of serological tests is difficult if the blocking antibody to brucellosis occurs, such as in endemic areas, chronic brucellosis patients, re-infections and relapses (5, 6). STA titter can be determined below 1/160 in healthy individuals who have had previous brucellosis, chronic brucellosis or re-infection (7). The false negativity rate is high because STA test cannot detect blocking antibodies. In endemic areas CTA testing is performed to detect blocking antibodies, which are difficult to distinguish. CTA test requires long time and experienced technical personnel and therefore it is difficult to apply in routine. Recently, Brucellacapt (BCAP) and Coombs Gel (CJ) tests have been used in the serological diagnosis of brucellosis, which are able to detect blocking antibodies and are easier to administer. The wells in the BCAP assay are coated with Coombs antibodies. In the CJ test, the wells contain a gel matrix containing Coombs antibodies (8). In this study, we aimed to determine the sensitivity and specificity of CJ and BCAP tests used in the serological diagnosis of brucellosis by comparing them with CTA test and to evaluate the usability of these two tests in routine laboratory applications.

MATERIALS and METHODS

In our study, a total of 100 samples (47 positive and 53 negative by CTA test) were included in the brucellosis suspected serum samples sent to our microbiology laboratory between June 2018 and July 2019. All samples were titrated in STA, CTA, BCAP (METSER Brucella test with Coombs, Savas Medical, Istanbul), CJ (ODAK Brucella Coombs Gel test, Toprak Medical, Istanbul) tests. Dilutions were made up to 1/5120 ratio and titters detected as 1/160 and above were accepted as positive. All tests were performed after the serum diluents and brucella antigens were brought to room temperature.

STA test was performed as follows: 950 µL was added to the first tube and 500 µL of saline was added to the others, and 50 µL of patient serum was added to the first tube and mixed. 500 µL was taken from this tube and serial dilution was performed and 500 µL was expelled from the last tube. Then 500 µL Brucella abortus antigens (Linear Chemicals) were added to all tubes. The tubes were shaken and mixed and evaluated after a 24-hour incubation at 37ºC. The highest serum dilution with 50% agglutination was considered agglutinating titter (7, 9).

The CTA test was performed as follows: STA test tubes were washed three times with phosphate buffered saline (pH 7.2) and centrifuged 3 times at 3000 rpm for 20 minutes. After the last wash, the bacteria were suspended in 1 ml of phosphate buffered saline and 0.05 ml of pre-standardized anti-total human immunoglobulin (Lab21 Healthcare) was added to each tube. The tubes were mixed and incubated at 37 ° C for 24 hours (9).

The BCAP test was performed according to the recommendation of the company: 95 µl of the first well, 50 µl of the diluent in the other wells, and 5 µl of patient serum was added to the first well and mixed. 50 µl was taken from this well and the last 50 µl was thrown out by serial dilution. 50 µl of brucella antigen was then added to all wells. The plates were covered with adhesive tape so that the liquid in the wells did not dry and incubated in a humid environment for 18-24 hours at 37 ° C. Results were evaluated visually after incubation. Evaluation; if there are no brucella antibodies, the antigens collapse to the bottom without attaching to the wall; brucella antibodies, if present, were seen as a homogeneous blue image attached to the inner surface of the wall.

The CJ test was performed according to the recommendation of the company as follows: 100 µl of the first well, 50 µl of the other diluent was added and 5 µl of patient serum was added to the first well and mixed. 50 µl was taken from this well and serial dilution was performed and 50 µl was thrown out from the last well. Then, 50 µl of brucella antigen was added to all wells and mixed and the plates were covered and incubated at 37 ºC. After incubation, the plate was shaken well and 50 µl of the well was pipetted into the micro-tube in the gel matrix. The micro-tubes were centrifuged for 20 minutes at 37 °C after 20 minutes at the appropriate cycle recommended by the manufacturer. Results were evaluated visually. Evaluation; In the absence of antibody, the precipitation of pink brucella antigens at the bottom of the tube was considered negative, and in the presence of antibody, the presence of the pink antigen and antibody complex on the gel was considered positive.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows version 22.0. Cohen kappa (κ) coefficient was used to determine the agreement between the methods. Significance level was accepted as 0.05 in all analyses.

RESULTS

A total 47 positive (≥ 1/160) and 53 negative found samples (0-1/160) by CTA test were included in the study. 100 patient samples were evaluated by STA, BCAP and BCGT methods. Twenty of the samples were positive with STA, 48 with BCAP and 53 with CJ test, respectively. The number of samples detected as positive in all three CTA, BCAP and CJ tests was 44 (Table 1). The number of samples that the two tests found positive together was 20 for CTA and STA tests, 46 for CTA and CJ tests (Table 2), and 45 for CTA and BCAP tests (Table 3). Of the 47 patients who were positive by CTA test, 27 were negative by STA, 2 by BCAP, and 1 by CJ test. Of the 53 patients who were found to be negative with CTA test, 3 were positive with BCAP and 7 with CJ test. κ = 0.900 for CTA and BCAP, κ = 0.841 for CTA and CJ, κ = 0.860 for BCAP and CJ tests (Table 4); showed a high level of agreement. On the other hand, STA test showed a very low level of compliance with all three methods due to the high false negative rate. κ = 0.440 for STA with CTA, κ = 0.426 for STA with BCAP, κ = 0.363 for STA with CJ.

Table 1. Serological distributional characteristics of the groups

Titters	STA, n	CTA, n	CJ, n	BCAP, n
0	65	44	36	46
20	0	0	1	0
40	10	3	7	2
80	5	6	3	4
160	6	10	7	6
320	8	7	11	15
640	5	14	14	15
1280	1	14	16	10
2560	0	1	5	2
5120	0	1	0	0

Table 2. Relation between the titters of the CJ and CTA test

		Coombs tube agglutination test									
		0	20	40	80	160	320	640	1280	2560	5120
Coombs gel test	0	36									
	20	1									
	40	3			3	1					
	80	2		1							
	160	1		1		3		1			
	320	1		1		2	1	3	2		
	640					2	5	5	1		
	1280					2	1	4	8	1	
	2560							1	3		1
	5120										

Table 3. Relation between the titters of the BCAP and CTA test

		Coombs tube agglutination test									
		0	20	40	80	160	320	640	1280	2560	5120
Brucellacapt test	0	41		2	3						
	20										
	40	1		1							
	80	1			1	2					
	160					5	1				
	320	1			1	2	6	5			
	640				1	1		8	4	1	
	1280								10		
	2560							1			1
	5120										

Table 4. Relation between the titters of the CJ and BCAP test.

		Coombs tube agglutination test									
		0	20	40	80	160	320	640	1280	2560	5120
Brucellacapt test	0	36	1	5	2	1	1				
	20										
	40			1	1						
	80					3	1				
	160			1		2		2	1		
	320						6	6	3		
	640					1	2	6	4	2	
	1280						1		8	1	
	2560									2	
	5120										

DISCUSSION

Since the clinical symptoms of brucellosis are diverse, the laboratory plays an important role in the diagnosis of the disease. Although culture is the gold standard method for diagnosis, it is a time-consuming method and the detection rate is between 40% and 70% (4). Serological methods are often preferred in the diagnosis of brucellosis because it is faster and safer than producing the causative agent in culture. Serological tests are important in the diagnosis and follow-up of the disease. At routine serological diagnosis of brucellosis, firstly screening is performed with RB test. The samples with positive RB test are titrated by STA and CTA tests (10). Antibodies to the lipopolysaccharide layer, which play an important role in virulence of the bacteria, are detected in the STA test. STA testing is widely used all over the world, but false negative rates are high due to blocking antibodies or prezone events, especially in endemic areas. CTA testing can be used to detect blocking antibodies (7).

In our study, titters 1/160 and above were accepted as positive for STA, CTA, CJ, BCAP tests. In this study, 47 samples that we found positive by CTA test and 53 samples that we found negative were included. Two of the 47 samples that were positive by CTA test were negative by BCAP. Three out of 53 patients who were found to be negative by CTA test were positive by BCAP. In our study, for CTA and BCAP was found to be κ=0.900 and showed excellent agreement. Gomez et al. found a direct correlation between BCAP and CTA test results (11). Close samples were obtained in one or two dilution intervals in CTA test titters with BCAP in positive samples. In the study conducted by Aliskan et al., 25 blood culture positive patients and 31 healthy control samples were studied; When compared with blood culture, sensitivity and specificity were found to be 92% and 100% in BCA and CTA tests, and it was found to be consistent with the data in our study (12). Serra et al. Reported that the sensitivity and specificity of BCAP and CTA tests were similar and could be used in the diagnosis and treatment of brucellosis (13). In the study of Casanova et al., BCAP test showed high sensitivity and specificity in the diagnosis of brucellosis. In contrast to our study, it has been reported that CTA testing cannot replace low-affinity antibodies in some cases of relapse and chronic brucellosis patients (14). In the study of Orduna et al., the sensitivity of BCAP test was 95.1%, the sensitivity of CTA test was 91.5%, and the sensitivity of STA test was 65.8%. Titrations were higher in BCAP than CTA and STA (6). In our study, there was no elevation in BCAP test titters.

One of the 47 patients found positive by CTA test was negative by CJ test, and seven of 53 patients who were found to be negative by CTA test were positive by CJ test. CTA was determined as κ = 0.841 for CJ and excellent fit was determined. In the study of Irvem et al., the kappa value of the CJ test and CTA and BCAP tests were calculated as 0.977 and showed a perfect fit similar to our study (15). In another study, Koroğlu et al. also found excellent agreement between tests (16).

CONCLUSION

In our study, BCAP and CJ tests were found to be in perfect agreement with CTA test. It has been found that the applicability of the BCAP test is quite easy compared to the CTA test, but it is not advantageous in terms of time as it results in 18-24 hours. It was found that the CJ test was easier to perform than the CTA test, and it gave results in a very short time such as half an hour. The interpretation of the results in the CJ and BCAP tests is less subjective than the CTA test. Brucellosis is still an endemic disease in our country. CTA test is very laborious, but BCAP and CJ tests can be preferred because of its easy applicability. In order for the CJ test to be routinely implemented, more patients should be supported by a number of studies.

Conflict of interest : The authors declare that they have no competing interest.

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