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Detection of Human Brucellosis by Brucellacapt and Rose Bengal Test in the Endemic Area

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Abstract: Although brucellosis is the most common zoonosis in the world, it remains an infectious disease that has not received sufficient attention. There are a few tests detecting brucellosis by serum. Rose Bengal Test is an advantageous one. Our aim with this study was to compare Rose Bengal and Brucellacapt tests in serum samples and draw attention to the advantages of the test. Between June 2019 and June 2021, 7827 serum samples sent to the public health laboratory with a provisional diagnosis of brucellosis were evaluated. The Rose Bengal and Brucellacapt test (Vircell, Spain) was used to diagnose infection. Samples with antibody titer ≥1/160 were considered positive. Of the 7827 samples included in the study, 354 (4.6%) of the total 7677 serum samples tested were RBT positive, 118 (3.1%) of the 3776 samples tested were BCAP positive, and 118 (3.3%) of the 3626 samples tested were both RBT and BCAP positive. It was found that seropositivity was significantly higher in female patients ($p<0.10$). RBT and BCAP test positivity were most frequently observed in the 25-34 year age group. Considering brucellosis cases in terms of seasonal changes; 10.7% of cases were found in spring, 52.4% in summer, 20.9% in fall, and 16% in winter. In suspected cases, RBT is still considered the ideal detection method because it is easy to use, inexpensive, sensitive, and provides rapid results. It was concluded that the BCAP test is suitable for diagnosis due to its ability to detect blocker and high titer antibodies. ©2023 NTMS.

Keywords: Brucellacapt; Brucellosis; Endemic Area; Public Health; Rose Bengal; Seroprevalence.

1. Introduction

Brucellosis is an ancient disease that remains the most common zoonosis in the world. *Brucella* spp. Gram-negative coccobacilli are defined as non-sporulating immotile, pleomorphic, facultative intracellular bacteria. The species *B. melitensis* and *B. abortus* are most commonly isolated in our country [1]. The main methods of transmission of brucellosis are direct contact with the blood or uterine fluids of sheep, goats, cattle, pigs, and camels, as well as ingestion of raw animal products that have been contaminated and unpasteurized milk. In addition, brucellosis is one of the most common laboratory-acquired bacterial infections worldwide [2]. In our country, brucellosis cases occur more frequently in the Eastern and

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Southeastern Anatolia regions, where animal husbandry is intensive [3]. According to the data of the Public Health Institution of Turkey, the number of brucellosis cases in our country as of 2017 is 6457 and the morbidity rate is 7.99 per 100,000. According to the same data, the last case of death caused by brucellosis was observed in 2008 [4]. The disease can affect any organ or system of the body. The symptoms are numerous and nonspecific and include fatigue, weakness, back or joint pain, sweating, anorexia, weight loss, and depression. Fever, mild lymphadenopathy, hepatomegaly, or splenomegaly are the physical findings that may be seen [5].

The diagnosis of brucellosis is usually made by bacteriologic and serological methods. Cultures, Rose Bengal agglutination test (RBT), standard agglutination test (SAT), enzyme-linked immunosorbent test (ELISA), Coombs test, and Brucella Capt (immunocapture-agglutination).Technique are used for serological diagnosis, and polymerase chain reaction (PCR) and real-time PCR are used for molecular diagnosis [6]. Blood, bone marrow, and tissue cultures are used as the gold standard for the diagnosis of brucellosis [7]. The method is invasive and has low sensitivity. For these reasons, clinicians prefer methods that provide indirect evidence of brucellosis rather than these invasive methods [8]. The Rose Bengal test is commonly used as a screening test for brucellosis, but it is not sufficient by itself for diagnosis because of its high false positivity rate. Brucellacapt testing may help detect disease in patients with a long evolutionary time that cannot be detected by SAT. After a successful treatment, a decrease in specific antibody titers is more rapid in Brucellacapt than in the other tests [9].

This study aimed to retrospectively investigate and compare serum samples, RBT, and Brucellacapt (BCAP) test results from patients with a prior - diagnosis of brucellosis sent to the public health laboratory in our province, which is a highly endemic region for brucellosis (The number of reported cases in 2017 was 6404) [14].

2. Material and Methods

Ethical approval for this registration was granted by the Van Regional Training and Research Hospital Clinical Research Ethics Committee, dated 30.09.2021 with the decision number 2021/17.

2.1. Patients

In our study, patients sent from family medicine and community health centers to our public health laboratory between June 2019 and June 2021 were retrospectively included. RB and BCAP results of 7827 cases, demographic information of patients, and seasonal distribution of cases it has been taken into consideration.

2.2. Analyses

Patient blood samples were centrifuged at 4000 rpm for 10 minutes, and their serums were separated. For RBT, RBT antigen (50 µL) was mixed with patient serum (50 µL), and agglutination formation was observed by rotating for 4 minutes. Samples with agglutination were classified as positive, and those without agglutination were classified as negative.

BCAP test; microagglutination test according to the Sandwich-Elisa model for the determination of Brucella antibody titer and the determination of total Brucella (IgM/IgG/IgA). All reagents, test plates, and serum samples were brought to room temperature before testing. Eight wells to A-H, including positive and negative controls, were placed on the plate layer for titration 95 µL of special dilution was added to the first well (A) and 50 µL of special dilution was added to the other wells. 5 µL of the positive/negative control serum was added to the wells for the positive and negative controls. 5 µL of the patient serum was added to the first well and mixed three or four times with a micropipette. 50 µL of this sample was added to well (B), and the same procedure was continued until well H. At the end of this procedure, 50 µL of antigen was added to all wells, and the plating layer was mixed with circular movements. The plate was sealed with protective tape in a special box and incubated for 18 to 24 hours at 37°C with moist cotton. The results were evaluated to a titration of 1/1280, where the first well was 1/40. The shape of a blue dot in the well was considered negative and the homogeneous image of the well was considered positive. Antibody titers >1/160 were considered positive.

2.3. Statistical Analysis

In the analysis of the results obtained in the study, the program "IBM SPSS Statistics for Windows, Version 20.0 software (IBM Corp., Armonk, NY, USA)" was used for the analysis. Descriptive statistics for quantitative variables (mean, standard deviation, largest, smallest), frequency tables for qualitative variables (number, n; percentage, %), cross tables, and the chi-square test and Fisher exact test for independent groups were used. Statistical significance was assessed using a margin of error of <0.10 and a confidence level of 0.90.

3. Results

This study includes 7827 suspected brucellosis patients, 5640 (72.1%) were female and 2187 (27.9%) were male. The mean age was 39.06±16.70 (38.97±16.18 for women; 39.30±17.96 for men; age range 1-99). In the samples, both tests were not performed simultaneously. In 7827 serum samples, 7677 (98.08%) RBT, 3776 (48.24%) BCAP, and 3626 (46.32%) samples were tested for both RBT and BCAP (Figure 1).

Looking at the RBT and BCAP test positivity rates of cases by sex, we found that 67.5% and 62.7% of cases involved women, respectively. These rates were higher than those of men (32.5% and 37.3%, respectively), and this fact was considered statistically significant (RBT: p=0.045, BCAP: p=0.092) (Table 1).

Table 1: Distribution of Rose Bengal Test and Brucellacapt Test seropositivity by gender.

	Rose Bengal Test				Brucellacapt Test			
	Negative		Positive		Negative		Positive	
Gender	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Female	5308	72.5	239	67.5	2559	70	74	62.7
Male	2015	27.5	115	32.5	1099	30	44	37.3
Total	7323		354 (4.6%)		3658		118 (3.1%)	

Figure 1: Distribution of Brucella test species.

When the RBT and BCAP positivity rates were deciphered according to the age groups of the cases, a statistically significant difference was found between the groups (RBT: p=0.080, BCAP: p=0.060). RBT and BCAP test positivity were most frequently observed in the 25-34 age group, with 23.2% and 24.6%, respectively (Table 2).

Table 2: Distribution of Rose Bengal Test and Brucellacapt Test seropositivity by age group

Age	Rose Bengal Test				Brucellacapt Test			
	Negative		Positive		Negative		Positive	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
<18	596	8.1	45	12.7	270	7.4	15	12.7
18-24	898	12.3	47	13.3	431	11.8	21	17.8
25-34	1736	23.7	82	23.2	827	22.6	29	24.6
35-44	1484	20.3	70	19.8	753	20.5	18	15.2
45-54	1091	14.9	43	12.1	573	15.7	12	10.2
55-64	928	12.7	39	11	500	13.7	14	11.9
>65	590	8	28	7.9	304	8.3	9	7.6
Total	7323		354		3658		118	

Positivity for both tests was detected in 354 (4.6%) of 7677 serum samples with RBT, 118 (3.1%) of 3776 samples with BCAP testing, and 118 (3.3%) of 3626 samples with both RBT and BCAP testing (Table 3). Of the 118 samples with positive BCAP test, agglutination was detected at 1/320 titer in 93 (78.81%) and 1/160 titer in 25 (21.19%) in 93 (21.19%). There is a relationship between the Dect and BCAP test results at the level of 0.75 level and this relationship was found to be statistically significant (p=0.000). When Brucella cases were evaluated in terms of seasonal changes, 10.7% of cases were detected in spring, 52.4% in summer, 20.9% in autumn, and 16% in winter (Figure 2).

4. Discussion

In our study, 4.6% RBT positive, 3.1% BCAP positive, and 3.3% both RBT and BCAP positive results were detected. The final diagnosis of brucellosis is made by bacteriologic methods. However, serological diagnostic methods are among the priority choices for the diagnosis of brucellosis, because of the long time required to grow bacteria, the variability in production rates, the risk of contamination in culture procedures related to the medium, and the fact that blood cultures cannot be performed in every health center [10, 11]. Although gold standard methods existed for the diagnosis of brucellosis, culturing Brucella species is time-consuming and not always possible to produce.

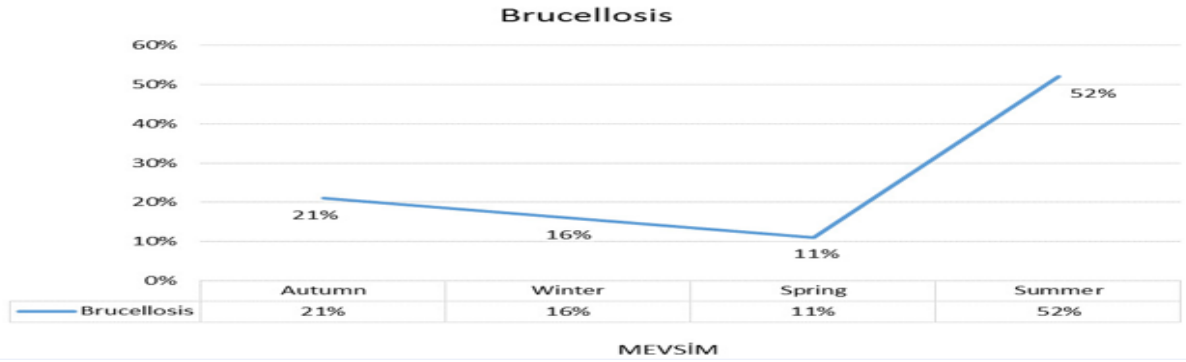

Figure 2: Seasonal distribution of Brucella seropositivity

Table 3: Comparison of Rose Bengal Test and Brucellacapt Test.

		Brucellacapt Test	
		Negative n (%)	**Positive n (%)**
Rose Bengal Test	**Negative**	3420 (94.3%)	0
	Positive	88 (2.4%)	118 (3.3%)
Total		3508	118

χ^2:2024.926; Phi:0.747; p:0.000.

Reasons for this may include misdiagnosis of the patient and thus incorrect treatment, the number of bacteria in the blood, late diagnosis, or in adequate laboratory facilities [12]. Serological tests are frequently preferred in routine laboratories because they are faster, more reliable, and more sensitive in diagnosing brucellosis [13]. According to the statistics of the Ministry of Health for 2017, the number of active brucellosis cases in our country is 6457, with Kars (82.4), Iğdır (76), Hakkari (62.4), Bitlis (61.2), Batman (60), Van (57.6) and Siirt (45) reported as the provinces with the highest incidence of brucellosis [14]. In this study, which is an endemic area for brucellosis in our province and around the comparison of studies has been done with the reason that the test BCAP, and RBT with the highest incidence of brucellosis in our lab previously watched the province and neighboring provinces in the various family medicine and community health centers and patient samples sent to questionable for the detection of brucellosis in BCAP, and RBT tests have been made.

The RBT is the leading serological test for the diagnosis of brucellosis in endemic regions. The RBT has a high diagnostic value in patients with no contact or history of disease, but a lower value in cases of previous or recurrent infections [15]. For this reason, diagnosis with RBT alone is not sufficient for initial or recurrent infections in Brucella endemic areas.[16] The BCAP test, which is considered one of the current serological detection methods, is based on the "sandwich ELISA" method. In this test; the wells, of the plate are coated with antibodies (Coombs antibodies) developed against human IgG, IgM, and IgA antibodies. Thanks to the BCAP test, all three antibodies can be detected in the patient's serum sample. The titers of this test are a good indicator of infection activity, regardless of the stage of brucellosis. BCAP titers show a marked decrease after successful antibiotic therapy compared with standard tube agglutination titers. After a successful treatment, a decrease in specific antibody titers can be reversed after treatment because Brucellacapt is faster than other tests [17].

In our study, RBT positivity was detected in 4.6% of serum from 7677 patients with suspected brucellosis. In some studies conducted in our country to determine the seroprevalence of brucellosis; RBT positivity was found to be 4.8% in Afyon, 4.2% in Manisa, and 3.6% in Isparta and Kırşehir [18-21]. Our study is largely similar to existing studies in terms of RBT positivity. Although the province where the study was conducted and its surroundings are located in an endemic region in terms of brucellosis, it is expected to be higher. However, in contrast to the studies in which high positivity was found, lower positivity was found in our study because the serum samples tested were not possible patient serum samples but serum samples sent for general screening purposes from primary health care facilities.

From December 2018 to December 2019, BCAP positivity was detected in the serum of 18 patients (3.85%) in 467 patients sent from various outpatient clinics or services with suspected brucellosis in a study conducted in Istanbul. Infection was observed more frequently in the 0 to 18-year-old age group and persons older than 50 years. In addition, seropositivity was found to be significantly higher in male patients [22]. In our study, 3.1% of the serum from 3776 patients with suspected brucellosis was positive by BCAP test. Considering the age groups, the most common positivity in our study was in the 25 to 34-year-old age group (24.6%). In contrast to other studies, our study shows that the positivity rates of RBT and BCAP tests

according to sex, are 67.5% and 62.7% in women, respectively [22, 23]. Although brucellosis is a disease that does not differ by age and sex, it is expected that positivity rates in our study are higher, especially in our region where the incidence is high due to occupational risk, in women who take care of animals and spend more time producing of milk and dairy products. In another study evaluating RBT, SAT, and Brucella capt results, the RBT screening test was found positive in 115 (10.8%) of 1060 serum samples. When BCAP results were evaluated, 86 (74.8%) samples were found to be agglutinated with a titer of 1/320 or more. The positivity rate for both tests was lower in women than in men. The age and seropositivity rates for both RBT and BCAP were higher in the age group of 55-64 years [23]. In another study conducted by Kaya et al. determined the positivity rates of 74 (74.0%) RBT and 84 (84.0%) BCAP in the serum samples of 100 patients with suspected brucellosis.[24] In another study conducted by Karameşe and Acar, RBT and BCAP tests were applied to 107 serum samples sent by internal medicine, infectious diseases, and pediatrics clinics with a provisional diagnosis of brucellosis. According to the data obtained, the RBT test was positive in 96 (89.7%) of 107 patients, and the BCAP test was positive in 102 (95.3%) [25].

In a study examining the distribution of STA test positivity rates by months, it was seen that it increased in March, peaked in August, and decreased to the initial rates as of October. The difference between August and September, when the positivity rates are the highest, was found statistically significant. To interpret it seasonally; the increase that begins with spring peaks at the end of summer and the beginning of fall. In our study, brucellosis cases were evaluated in terms of seasonal changes; 10.7% in spring, 52.4% in summer, 20.9% in fall, and 16% in winter. Thus, this study, conducted in the same endemic region as ours, and our study have similarities in terms of seasonal positivity. It is hypothesized that these seasonal changes are due to the periods when livestock production is intensive and at the same time, more raw milk and dairy products are consumed.

5. Conclusion

Diagnosis of brucellosis is important in endemic areas. Therefore, correct detection methods should be preferred to make the correct diagnosis. In suspected cases, RBT can be considered an ideal detection method because it is easy to use, inexpensive, sensitive, and provides rapid results. On the other hand, it was concluded that the BCAP test would be suitable for diagnosis due to its ability to detect blocking antibodies and to detect high antibody titers.

Although significant success has been achieved with the national mobilization initiated in 1984 in our country to control and eradicate Brucellosis in humans and animals, the eradication of this disease can be made possible by comprehensive support of this mobilization. Especially in the rural areas of our country, it is not possible to have the animals controlled by veterinarians. To reduce the frequency of transmission of brucellosis in endemic regions, it is important to inform the people living there that they should not be consuming raw milk and dairy products as often as necessary.

Limitations of the Study

The main limitation of this study was its retrospective design. The relatively small sample size and lack of a multicenter study were also limiting factors. In addition, the inability to include blood culture results in the study was a limiting factor for our study.

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Conflict of Interests

The authors declare that there is no conflict of interest and no financial support was provided for the study.

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Author Contributions

Concept-EA; Design-YD; Supervision-EA, YD; Resource-YD; Materials-SK, AFG; Data Collection and/or Processing-SK, AFG; Analysis and/or Interpretation-EA; Literature Search-YD; Writing-YD; Critical Reviews-EA, YD.

Ethical Approval

Ethical approval for this study was granted by the Van Regional Training and Research Hospital Clinical Research Ethics Committee with decision number 2021/17 on 30 September 2021.

Data sharing statement

The material used in the study and without the permission of the authors.

Consent to participate

Not applicable.

Informed Consent

The authors accept their responsibilities in the study. There is no conflict of interest between the authors.

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