

Brucellosis seropositivity in Ağrı Training and Research Hospital: Rose Bengal and serum tube agglutination test results

Ağrı Eğitim ve Araştırma Hastanesinde bruselloz seropozitifliği: Rose Bengal ve serum tüp aglütinasyon test sonuçları

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ABSTRACT

Objective: Brucellosis is a zoonotic infection that is transmitted from infected animals to humans through the consumption of food products (such as unpasteurized dairy products) or direct contact with tissues or body fluids. It is the most common zoonosis worldwide and is a major public health problem in many developing countries. Knowledge of brucellosis seropositivity and geographic distribution is of great importance for the control of the disease. Thus, it was aimed to examine the Rose-Bengal test (RBT) results and serum tube agglutination (STA) test titers from the serum samples sent to the microbiology laboratory with a preliminary diagnosis of brucellosis and to investigate the seropositivity of brucellosis in our region.

Methods: Between 1 January 2020 and 31 December 2023, 13.588 serum samples sent to the medical microbiology laboratory with preliminary diagnosis of brucellosis from various outpatient clinics or services of Ağrı Training and Research Hospital were included. The results of these samples were obtained from laboratory information management system retrospectively. RBT

ÖZET

Amaç: Bruselloz, enfekte hayvanlardan insanlara gıda ürünlerinin (pastörize edilmemiş süt ürünleri gibi) tüketilmesi veya doku, vücut sıvıları ile temas yoluyla bulaşan zoonotik bir enfeksiyondur. Tüm dünyada en sık görülen zoonozdur ve gelişmekte olan birçok ülkede önemli bir halk sağlığı sorunu olarak kabul edilmektedir. Bruselloz seropozitifliği ve coğrafi dağılımının bilinmesi hastalığın kontrolü için büyük önem taşımaktadır. Bu nedenle bruselloz ön tanısı ile mikrobiyoloji laboratuvarına gönderilen serum örneklerinden Rose-Bengal testi (RBT) sonuçları ve serum tüp aglütinasyon (STA) test titrelerinin incelenmesi ve bölgemizde brusellozun seropozitifliğinin araştırılması amaçlanmıştır.

Yöntem: 1 Ocak 2020 ve 31 Aralık 2023 tarihleri arasında Ağrı Eğitim Araştırma Hastanesi'ne bağlı çeşitli poliklinik veya servislerinden, bruselloz ön tanısı ile tıbbi mikrobiyoloji laboratuvarına gönderilen 13.588 serum örneği dahil edilmiştir. Bu örneklerle ait sonuçlar retrospektif olarak laboratuvar bilgi yönetim sisteminden elde edilmiştir. Çalışmada RBT antijeni (THSK, Türkiye)

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antigen (THSK, Türkiye) and STA standardized suspension of *B. abortus* S99 dead strain (THSK, Türkiye) were used in the study.

Results: A total of 13.588 blood samples with a preliminary diagnosis of brucellosis included in our study. RBT seropositivity was found to be 12.3% (n: 1676). STA was applied to the sample of patients who were RBT positive. In serum agglutination test, brucellosis seropositivity was 4.2% (n: 575) at <1/160 titer and 5.5% (n: 750) at ≥1/160 titer. According to investigated by years, it has been determined that the rate of seropositivity decreased in 2023 years.

Conclusion: In conclusion, a high rate of brucellosis seropositivity was detected in our region. Brucellosis remains an important public health problem in the Eastern Anatolia region. Serological test results contribute to the development of regional epidemiological data and to increase measures. It is important to inform people about brucellosis infection and transmission. Clinicians in our hospital should consider brucellosis in the differential diagnosis.

Key Words: Brucellosis, tube agglutination, Rose-Bengal

ve STA testi için *B. abortus* S99 ölü süşunun standardize süspansiyonu (THSK, Türkiye) kullanılmıştır.

Bulgular: Çalışmamıza brucelloz ön tanısı alan 13.588 hastadan alınan kan örneği dahil edilmiştir. RBT seropozitifliği %12.3 (n: 1676) olarak bulunmuştur. RBT pozitif olan hastaların örnekleme STA uygulanmıştır. Serum aglütinasyon testinde <1/160 titrede %4.2 (n: 575), ≥1/160 titrede ise %5.5 (n: 750) oranında brucelloz seropozitifliği saptanmıştır. Yıllara göre incelendiğinde 2023 yılında seropozitiflik oranının azaldığı tespit edilmiştir.

Sonuç: Sonuç olarak, bölgemizde brucelloz seropozitifliği yüksek oranda saptanmıştır. Brucelloz, Doğu Anadolu bölgesinde önemli bir halk sağlığı sorunu olmaya devam etmektedir. Bu nedenle elde edilen serolojik test sonuçları bölgesel epidemiyolojik verilerin oluşturulmasına ve brucelloz bulaşı açısından önlemlerin artırılmasına katkı sağlayacaktır. Brucelloz enfeksiyonu ve bulaş yolları hakkında halkın bilgilendirilmesi önemlidir. Hastanemizde ayırıcı tanıda klinisyenler brucellozu göz önünde bulundurmalıdır.

Anahtar Kelimeler: Brucelloz, tüp aglütinasyon, Rose-Bengal

INTRODUCTION

Brucellosis is one of the most common zoonoses transmitted from animals, and in endemic areas and brucellosis poses a serious public health problem. *Brucella* species is a bacterial disease that often infects cattle, pigs, goats, sheep and dogs (1). Humans usually transmit the disease through direct contact with tissues or body fluids from infected animals, or indirectly through the consumption of contaminated animal products or inhalation. It is caused by the consumption of unpasteurized milk or dairy products from infected goats or sheep frequently.

Worldwide, it is considered a serious public health threat, with approximately 500,000 new cases of brucellosis per year (2). The Mediterranean Basin, South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East are among the high-risk areas. Those who travel to these areas and especially some occupational groups (veterinarians, hunters, laboratory workers) are at risk. In our country, brucellosis is endemic in the Eastern and Southeastern Anatolia regions where animal husbandry is intense.

B. abortus, *B. melitensis*, *B. canis*, *B. suis*, *B. pinnipedialis*, *B. ceti* species are known to cause

disease in humans (3, 4). The gold standard method in the diagnosis of brucellosis is culture. In the serological diagnosis, such as slide agglutination test, standard tube agglutination test, Coombs test, various solid phase methods for the detection of antibodies (ELISA, chemiluminescence, immunochromatography) and tests based on antibody capture (immunocapture) are used for diagnostic purposes (5).

The prerequisite for the continuation of a comprehensive study aimed at combating, preventing and controlling the disease in our country and around the world is epidemiological data, especially on the regional distribution of the disease. Knowledge of brucellosis seropositivity and geographic distribution is of great importance for the control of the disease (6). Therefore, it was aimed to examine the Rose-bengal test (RBT) results and serum tube agglutination (STA) test titers from the serum samples sent to the microbiology laboratory with the suspicion of brucellosis and determine the seropositivity of brucellosis in our region.

MATERIAL and METHOD

Between 1 January 2020 and 31 December 2023 years, a total of 13.588 serum samples were sent to the medical microbiology laboratory with the suspicion of brucellosis from various outpatient clinics or services of Ağrı Training and Research Hospital. These samples were retrospectively evaluated using the laboratory information management system over a four-year period. Demographic characteristics such as applied departments, age, gender were evaluated.

In the study, blood samples were centrifuged at 3000 g for 10 minutes. One drop (50 µL) of serum was placed into a circle on the test card. One drop of positive control serum and one drop of negative control serum were dispensed into two additional circles. Rose Bengal antigen was added one drop (50 µL) of to each circle into the serum. Rose Bengal test antigen (THSK, Türkiye) was mixed with serum and rotate the card for 4 minutes. Samples with

agglutination observed were considered positive.

Serum tube agglutination test was applied to the serum of the patients who were found positive with Rose Bengal test as a screening test. Standardized suspension of *B. abortus* S99 killed strain (THSK, Türkiye) was used in STA test. Dilutions from 1/20 titer to 1/1280 titer were studied with STA test. In the STA test; antigen and serum were incubated in a tube at 37°C for 24 hours. After incubation, visible agglutination at the bottom of the tube was considered a positive result. Titers of 1/160 and above were evaluated as seropositive by STA method.

Descriptive statistical analyses were performed, including frequency (n), percentage (%), mean (\bar{x}), median, standard deviation, and minimum and maximum values.

The study was approved by the Ağrı İbrahim Çeçen University Scientific Research Ethics Committee (Date: 28.12.2023 and Number: 325).

RESULTS

Serum samples from 13.588 patients with suspected brucellosis were included in the study. When examined according to the departments, patients applied 53.3% internal medicine, 20.4% infectious diseases and clinical microbiology, 17.3% pediatrics, and 9.0% were patients from other outpatient clinics and services.

In a sample of 13.588 patients with suspected brucellosis, 12.3% (n: 1.676) were found to be RBT positive. STA method was applied to the sera of patients who were RBT positive. Of the 1676 RBT positive cases, 1325 were found to be seropositive with STA. Agglutination was determined in 750 (5.5%) sera positive with STA at $\geq 1/160$ titer and in 575 (4.2%) sera at $< 1/160$ titer. When the distribution of titers according to the serum tube agglutination test was examined, it was found to be positive at a rate of 9.3% at 1/20 titer, 15.6% at 1/40 titer, 18.4% at 1/80 titer, 17.2% at 1/160 titer, 16.4% at 1/320 titer, 12.6% at 1/640 titer, and 10.1% at 1/1280 titer (Figure 1).

When RBT and STA seropositivity are examined by years, in 2020, RBT and STA positivity has been determined 17.0%, 4.8% respectively. In 2021, RBT and STA positivity was found to be 18.7%, 5.8 % respectively. In 2022, RBT and STA positivity has been determined 10.5%, 7.6% respectively. In 2023, RBT and STA positivity has been determined 9.5%, 4.5% respectively (Table 1). It has been determined that the rate of seropositivity decreased in 2023 years.

The average age of patients with positive RBT was 38.7 ± 18.3 years. When the genders of the patients with RBT positive were examined, 52.7% were female and 47.3% were male. The average age of the patients who were found positive at $\geq 1/160$ titer with STA test was 37.8 ± 17.4 years. When the gender distribution of these patients was examined, 46.5% were female and 53.4% were male.

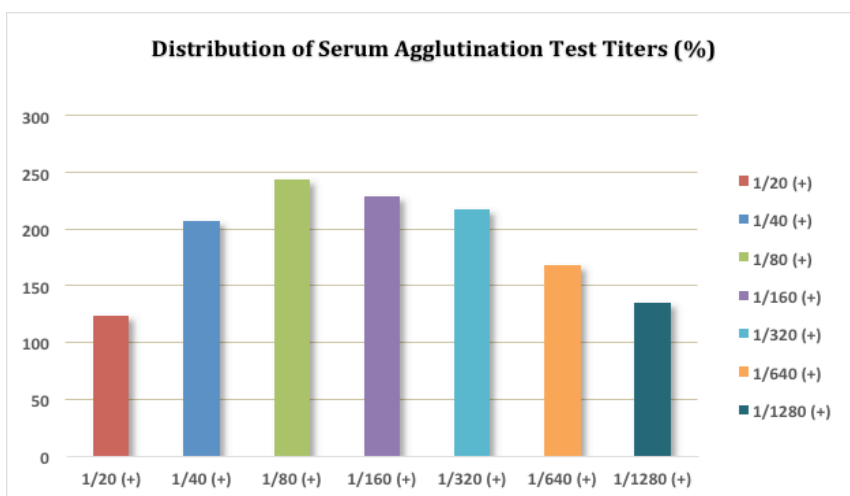


Figure 1. Distribution of serum tube agglutination test titers

Table 1. Rose Bengal test and serum tube agglutination test positivity rates (%) of the patient serums according to years

Years	Participant	Rose Bengal Test n (%)	Serum Tube Agglutination Test (1/160 and above) n (%)
2020	1729	295 (17.0)	84 (4.8)
2021	2342	438 (18.7)	138 (5.8)
2022	3146	332 (10.5)	240 (7.6)
2023	6371	611 (9.5)	288 (4.5)
Total	13.588	1676 (12.3)	750 (5.5)

DISCUSSION

Brucellosis seropositivity was determined high rate in our region. Brucellosis is still important public health problem in our country as well as all over the world. Brucellosis is observed in animals such as goats, sheep, cattle and horses. It is transmitted to humans after contact with

meat, milk or dairy products, urine and pregnancy materials of infected animals. *B. melitensis* is the most common causative agent in our country (7, 8).

The sensitivity of the Rose Bengal test is high in the diagnosis of brucellosis. It is recommended to be used as a rapid diagnostic test in patients with a history of contact and compatible symptoms. In meta-analyses, the specificity of the test was also

found to be high, but it is not appropriate to use it alone in the diagnosis of brucellosis. Since it is an antibody screening test, false-negative results may occur in the early stages of the disease (9). The sensitivity and specificity of STA test have been found to be high for the diagnosis of brucellosis. In cases where early treatment is required or in cases where culture cannot be performed, STA test can be used in rapid diagnosis. In cases where RBT is positive in patients with suspected brucellosis, it should be confirmed by STA test because it is more specific (9).

When brucellosis seropositivity was examined in studies conducted in Eastern Anatolia and Southeastern Anatolia regions, it ranged between 3.1% and 17.0% (8, 10-12). According to the results of our study, the brucellosis seropositivity was found to be 12.3% according to the RBT test in Ağrı. Of the 1676 cases with RBT positive, 1325 were positive by STA. Agglutination was detected in 750 of the sera that were positive with STA at titers of 1/160 and above. According to the STA test, a seropositivity rate of 5.5% was found.

A total of 2942 serum samples taken from patients with suspected brucellosis in Malatya were retrospectively examined for brucellosis by Rose Bengal, STA and Coombs agglutination methods. Rose Bengal test was positive in 8.5% of serum samples. The STA method was applied to those who tested positive for Rose Bengal. Agglutination was detected in 4.5% of serum samples at titers of 1/160 and above by STA. Seropositivity was detected in 5.5% of serum samples by Coombs agglutination test (8).

The brucellosis seroprevalence of 271 participants engaged in animal husbandry in Erzurum was investigated by Rose-Bengal and STA methods. Rose-Bengal slide agglutination test found 17.0% seropositive. STA method was applied to 46 serum samples with positive Rose Bengal test and seropositivity was detected in 10.7% (10).

Between June 2019 and 2021, Rose Bengal and Brucellacapt (Vircell, Spain) test (BCT) was performed for the diagnosis of brucellosis in Van. The Brucellacapt test is a *Brucella* agglutination method with Coombs

based on agglutination that detects all antibodies for the diagnosis of brucellosis. Rose Bengal tested positive in 4.6%. Brucellacapt test revealed that 3.1% were positive (11). In the study in which brucellosis seroprevalence was investigated in Diyarbakır, Rose-Bengal slide agglutination test was applied as a screening test. BCT test was applied to the sera of patients with a positive screening test. A seropositivity rate of 6.5% was detected by RBT in a sample of 5,196 patients with suspected brucellosis evaluated in the study. BCT titer was found to be positive at 1/320 or more in 76.5% of the sera that were RBT positive (12)

In studies conducted in Marmara, Central Anatolia and Black Sea regions, seroprevalence varies between 0.8% and 3.8% (13-15). The seroprevalence of brucellosis in Amasya was examined by Brucellacapt test. As a result of the study, brucellosis seroprevalence was found to be 0.8% (15). A total of 2646 patients were included in the study in which the seroprevalence of brucellosis cases in Samsun was examined. Serum samples of the patients were evaluated with Brucellacapt test. BCT was positive in 3.0% of patients (14). Brucellacapt test was used in the diagnosis of brucellosis infection at Istanbul University Medical Faculty Hospital. Brucellosis seroprevalence was found to be 3.8% (13).

Effective control strategies of brucellosis include different methods such as surveillance, prevention of transmission, and culling of the infection reservoir (16). Vaccination of animals is recommended in endemic areas with high levels of brucellosis. Studies show that when animal brucellosis is controlled, there will be a significant decrease in human brucellosis cases (17).

In conclusion; Brucellosis is common in the Eastern Anatolia region. Therefore, brucellosis continues to be an important public health problem in our region. Serological test results could contribute to the establishment of regional epidemiological data and to increase measures. It is important to inform the people about the brucellosis infection and its transmission. Clinicians should consider brucellosis in the differential diagnosis in our hospital.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Ağrı İbrahim Çeçen University Scientific Research Ethics Committee (Date: 28.12.2023 and Number: 325).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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