

Research Article

Seroprevalence of Brucellosis in Aksaray Region

Aksaray Bölgesinde Bruselloz Seroprevalansı

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Abstract

Purpose: Brucellosis is the most common bacterial zoonosis in the world caused by brucella genus bacteria and is a significant cause of morbidity and mortality in humans. The definitive diagnosis of the disease is the isolation of the agent in culture, but serological tests are often used in diagnosis. This study aimed to determine the seroprevalence of brucellosis in our region.

Material and Method: The study included 7087 serum samples sent to the medical microbiology laboratory with suspicion of brucellosis from clinics between January 2023 and December 2024 to Aksaray education and research hospital. The Rose-Bengal (RB) and Coombs agglutination test results of sera were retrospectively examined.

Results: Out of the 7087 patients included in the study, 622 (8.8%) had a positive RB test, and 579 patients (8.2%) had a Coombs agglutination test titer of 1/160 and above. The median age of patients with a positive Coombs agglutination test was detected as 41.57 ± 3.9 (min 1 - max 80) and 56.8% (n=329) of these patients were male and 43.2% (n=250) were female. Brucellosis seroprevalence was 47.6% in the 20-50 age range and 69.4% in the 20-60 age range. We also determined the seroprevalence of brucellosis as 17.6% in the 0-20 age range and 9.2% in those over 65 years of age.

Conclusion: Preventing the transmission of brucellosis to humans primarily depends on controlling and eradicating disease in animals. In this context, we believe that success will be achieved through promotional controls, especially through effective policies, measures and projects that will be jointly developed by the Ministry of Agriculture and universities.

Keywords: Brucellosis, Coombs agglutination test, Seroprevalence

Öz

Amaç: Bruselloz, brucella cinsi bakterilerin neden olduğu dünyada en sık görülen bakteriyel zoonozdur ve insanlarda önemli bir morbidite ve mortalite nedenidir. Hastalığın kesin tanısı etkenin kültürde izolasyonudur ancak tanıda sıklıkla serolojik testler kullanılır. Bu çalışmada bölgemizdeki bruselloz seroprevalansının belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya, Ocak 2023-Aralık 2024 tarihleri arasında Aksaray Eğitim ve Araştırma Hastanesi kliniklerinden bruselloz şüphesi ile tıbbi mikrobiyoloji laboratuvarına gönderilen 7087 serum örneği dahil edildi. Serumların Rose-Bengal (RB) ve Coombs aglütinasyon test sonuçları retrospektif olarak incelendi.

Bulgular: Çalışmaya dahil edilen 7087 hastanın 622'sinde (%8,8) RB testi pozitif iken 579 hastada (%8,2) Coombs aglütinasyon test titresi 1/160 ve üzeri idi. Coombs aglütinasyon testi pozitif olan hastaların ortalama yaşı $41,57 \pm 3,9$ (min 1 - maks 80) olarak belirlendi ve bu hastaların %56,8'i (n=329) erkek, %43,2'si (n=250) kadındı. Bruselloz seroprevalansı 20-50 yaş aralığında %47,6 iken 20-60 yaş aralığında ise %69,4 olarak idi. Ayrıca Bruselloz seroprevalansını 0-20 yaş aralığında %17,6, 65 yaş üstünde ise %9,2 olarak belirledik.

Sonuç: Brusellozun insanlara bulaşmasının önlenmesi öncelikle hastalığın hayvanlarda kontrol altına alınmasına ve eradikasyonuna bağlıdır. Bu bağlamda özellikle Tarım Bakanlığı ve üniversitelerin ortaklaşa geliştireceği etkili politikalar, önlemler ve projelerle tanıtımsal kontroller temelinde başarı sağlanacağına inanıyoruz.

Anahtar Kelimeler: Bruselloz, Coombs aglütinasyon testi, Seroprevalans

INTRODUCTION

Zoonotic pathogens are becoming increasingly important worldwide. *Brucella* spp., one of the most important zoonotic pathogens, causes brucellosis and poses significant public health concerns. Brucellosis is an acute or insidious disease, and patients must have at least one of the following symptoms along with fever: fatigue, loss of appetite, weight loss, headache, intense sweating, especially at night, and widespread muscle and joint pain throughout the body (1).

Brucellosis could be seen all over the world, but it still causes one of the most important health problems in developing countries and is endemic especially in the mediterranean region, the middle east, central Asia and Latin America (1,2). Also, in our country due to factors such as widespread animal husbandry and the use of dairy products, zoonotic pathogens and *brucella* spp. are becoming increasingly important. In Türkiye, cases are mostly reported from the eastern and southeastern Anatolia regions where animal husbandry is widespread, and it can be seen in other regions, albeit rarely (1).

Brucellosis, which is mainly a disease of large and small cattle, also threatens human health and causes economic and labor losses. There are four types of *brucella* that cause disease in humans, however, *B. abortus* and *B. melitensis* are more frequently detected as the cause of brucellosis (3). Brucellosis is an important occupational disease for veterinarians, butchers, animal husbandry workers and laboratory workers. It is also an important bioterrorism agent due to its low infectious dose, persistence in the environment and host, ability to spread by aerosol and difficulty in treatment (1,4).

Serological and bacteriological methods are usually used to diagnose the disease. The gold standard for diagnosis is the production of the agent in blood, bone marrow or other tissue cultures, but it is difficult to grow this agent in culture. Therefore, serological tests are used in diagnosis. The applicability and evaluation of these tests are easy, cheap and do not require experienced personnel. The most used serological tests in diagnosis today are the Rose Bengal test (RB), Wright test, Coombs test, complement fixation test or immunocapture tests (5,6)

In Türkiye, various control and eradication programs have been implemented for brucellosis since 1930, and it is a notifiable infectious disease (1). According to the data of our country, the incidence of the disease increased again between 2015 and 2019, exceeding 10 per hundred thousand. This

makes it more valuable to determine brucellosis seroprevalence on a regional basis (7).

In this study, we aimed to retrospectively examine the RB test and Coombs agglutination test titers in patient serum samples sent to Aksaray training and research hospital medical microbiology laboratory from various services and polyclinics with suspicion of brucellosis between 2023-2024 to determine the seroprevalence of brucellosis in Aksaray.

MATERIALS AND METHODS

The study included 7087 serum samples sent to the medical microbiology laboratory with suspicion of brucellosis from various polyclinics or services affiliated to Aksaray education and research hospital between January 2023 and December 2024. The RB and Coombs agglutination test results of sera were retrospectively examined. This study was approved by the Aksaray University Faculty of Medicine, Clinical Research Ethics Committee (03.10.2024-2024/95).

Rose Bengal Test

After all reagents were brought to room temperature, 50 microliters of patient serum and 50 microliters of saline, one drop of BRUCEL-RB (Tulip Diagnostics, India) antigen suspension was pipetted onto the circle on the slide and rotated for four minutes. During the evaluation phase, the presence of agglutination observed macroscopically indicates the presence of 25 IU/mL *brucella* antibodies (IgM or IgG) in the patient serum.

Coombs Agglutination Test

After the patient serum and test reagents detected positive in the RB test were brought to room temperature, 50 microliters of patient serum prepared in the 1/40 - 1/1280 dilution range was pipetted into the wells in the REDCELL *brucella* gel test (Red Cell Biotechnology, Türkiye) plate. The gel matrix was centrifuged for 20 minutes. If there are no *brucella* antibodies in the serum during the evaluation phase, *brucella* antigens settle to the bottom of the tube, if there are, the antigen-antibody complex remains on the gel in the form of a pink line. According to coombs agglutination method, titers of 1/160 and above are considered as positive.

Statistical Analysis

After the tests in the study were completed, the data obtained were evaluated in a computer environment using the IBM-SPSS (Version 24.0) statistical package program. For descriptive statistics, number, percentage, mean \pm standard deviation (SD), minimum (min), maximum (max) values were used.

RESULTS

Of the 7087 patients included in the study, 622 (8.8%) had a positive RB test, and 579 patients (8.2%) had a Coombs agglutination test titer of 1/160 and above. The median age of patients with a positive Coombs agglutination test was detected as 41.57 ± 3.9 (min 1 - max 80) and 56.8% (n=329) of these patients were male and 43.2% (n=250) were female. Figure 1 demonstrated the distribution of brucella antibody titers according to the number of patients. Also, Figure 2 demonstrated the distribution of Coombs agglutination test positivity according to age ranges.

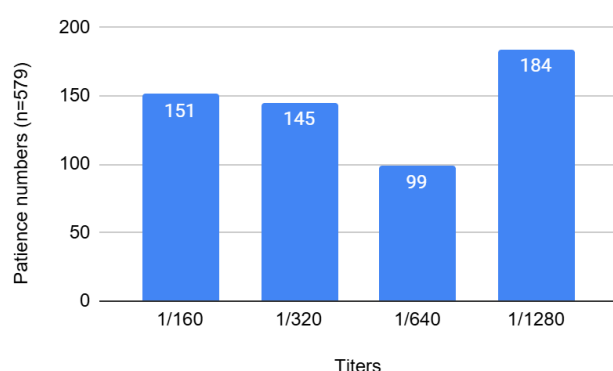


Figure1. Distribution of Brucella antibody titers according to the number of patients

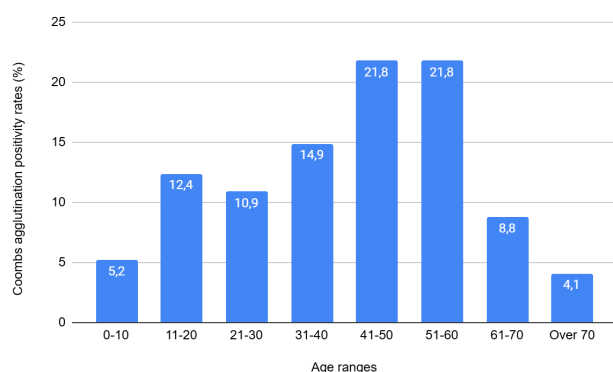


Figure 2. Distribution of Coombs Agglutination Test positivity according to age ranges

DISCUSSION

Brucellosis, first described by Marston in 1859 as "Mediterranean Gastric Fever", is considered the most common zoonotic disease in the world. Although brucellosis has been eliminated in developed countries, it continues to be a significant public health problem in developing countries. According to the World Health Organization (WHO), 500,000 new cases are reported each year in the

world. WHO also considers brucellosis to be one of the seven most neglected diseases (8,9).

Brucellosis occurs in humans through the consumption of unpasteurized milk and its products, inhalation of infected aerosols, and direct contact of animal secretions with damaged tissues (10). In this regard, cases in Türkiye are generally reported in regions where animal husbandry is common. Although it is a disease that must be reported in Türkiye, it is estimated that the number of notifications in both animal and human cases is well below the expected number. Despite this, brucellosis is still endemic in Türkiye according to the number of reported cases (1,7).

In seroepidemiological studies, seropositivity rates of 9-25% have been reported in occupational groups at risk for brucellosis, such as butchers, livestock breeders, slaughterhouse and dairy workers, and 1-8 % in those not in the risk group. Brucellosis is more common among young and middle-aged adults. According to Türkiye datas, 50-60% of cases are between the ages of 20-50, 10-15% in children, and 10% in those over the age of 65 (1). In the current study, similar to studies conducted in our country, we observed that the seroprevalence of brucellosis was 47.6% in the 20-50 age range, and 69.4% when we evaluated the age range as 20-60. We detected the seroprevalence of brucellosis to be 17.6% in the 0-20 age range and 12.3% in those over 60 years of age (Figure 2).

The clinical picture of brucellosis in humans is generally non-specific. Not all people who encounter brucella develop active brucellosis. Although most people living in endemic areas have positive brucellosis serological markers, there is no clinical history of infection. The mortality rate due to brucellosis is very low (0.1%). Death is mostly due to brucella endocarditis, meningitis or brain abscess due to late diagnosis and treatment (1,7).

Bacteriological and serological methods are used primarily in the diagnosis of brucellosis. Since clinical symptoms are misleading in the diagnosis of brucellosis, laboratory results play an important role in the diagnosis of the disease. The gold standard for laboratory diagnosis is the growth of the agent in blood or bone marrow or other tissue cultures. However, the growth of the agent in culture takes a long time and the detection rate of the agent is between 40% and 70%. Serological diagnosis is made with tests such as the RB test, wright test, Coombs test, complement fixation test and immunocapture test. Agglutination tests are based on the reactivity of antibodies formed against the smooth lipopolysaccharide of brucella. Rose bengal agglutination test has high sensitivity (>99%); it is a low specificity test (8,11). Serological methods are easier, faster and safer than growing the agent in culture, so they are often preferred in

the diagnosis of brucellosis. Also, they are important in the diagnosis and follow-up of the disease (6,8,12). In the routine serological diagnosis of brucellosis, screening is first performed with the RB test and in this study the RB test positivity rate was 8.8%. However, the Coombs agglutination test positivity rate was 8.2% (Figure 1). This may be because the RB test is a screening test and was performed at lower sera dilutions. In addition, the specificity of the Coombs Agglutination test is higher than the RB test and is more valuable in excluding brucella infection. However, in endemic areas, positivity can be detected at a titer of 1:160 after previous infection. Therefore, the results should be evaluated with clinical findings and a 4-fold increase in titers should be demonstrated in sera taken after 2-4 weeks (6).

In Türkiye, 10,244 cases of brucellosis were reported in 2019, and the incidence of the disease increased from 5.8 to 12.3 per 100,000 between 2014 and 2019. When the distribution of cases is considered, the increase in the Central Anatolia region, as well as the Eastern and Southeastern Anatolia regions is remarkable (1,7). In the current study, the seroprevalence of brucellosis was 8.2% in the patient population admitted to the hospital in Aksaray. In other recent brucellosis seroprevalence studies conducted in Türkiye, seropositivity rates were determined as 6.5% in Diyarbakır, 1.3% in Rize, 3.4% in Samsun, and 3.8% in a university hospital in Istanbul (11,12,13,14). We believe that the reason for the high seropositivity rate in the current study compared to other recent studies may be due to the widespread animal husbandry in Aksaray and the high consumption of dairy products of unknown origin. However, there is a need for current and comprehensive studies conducted in Türkiye, especially in geographical regions where the incidence is high.

Türkiye has a unique geographical, cultural and economic location between Europe and Asia. This situation is a risk factor for the spread of brucellosis, especially from its eastern and southeastern neighbors where it is endemic. The reported incidence of brucellosis in endemic regions of the world varies between <0.01 and >200 per hundred thousand. The incidence of brucellosis in our country is 26 per hundred thousand, in Iran 24, in Iraq 28 and in Syria, where the incidence is highest in the world, it is reported as 161 per hundred thousand, and it is a serious public health problem (8). It is estimated that 57.5% of the population in Africa is at risk of brucellosis, a disease that the whole world overlooks and has difficulty accessing data on. However, brucellosis is historically endemic to Africa but, it is estimated that most cases of brucellosis in Africa go unreported. It has been determined that the countries with the highest population at risk after Africa are in Asia (47.7%), Europe (24.3%) and America (19.4%) continents, respectively (9,15).

Despite advances in diagnostic and therapeutic opportunities, brucellosis remains a serious global public health burden. The epidemiology of brucellosis changes with changing lifestyles and evolving human-animal interactions. "One Health" is a vital concept for effective investigation of the human-animal-ecosystem interface to prevent and control the reemergence of brucellosis (15). The epidemiological features of brucellosis observed in humans vary depending on many social factors. Human brucellosis is continuously increasing in traditionally endemic regions such as the Mediterranean and surrounding countries, and its re-emergence is frequently reported in fully controlled countries in North America and Southern Europe (9,15). Therefore, implementation of surveillance studies and screening tests is crucial to respond promptly to changes in seroprevalence. Reducing human brucellosis requires control of animal brucellosis. For the success of brucellosis programs, surveillance, eradication, vaccination and control programs require strong sectors and international technical and scientific cooperation. In this context, we believe that success will be achieved through promotional controls, especially through effective policies, measures and projects that will be jointly developed by the Ministry of Agriculture and universities.

Declarations

Ethics Committee Approval: Ethics committee approval was obtained from the Aksaray University, Health Sciences Scientific Research Ethics Committee (Date: February 13, 2025, Decision No: 2025/33). This study was conducted according to the principles of the Declaration of Helsinki.

Authorship Contributions: Concept: FE, DA, OS, HO. Design: FE, DA, OS, HO. Data Collection or Processing: DA, OS, HO. Analysis or Interpretation: FE, DA. And Literature Search: DA, OS, HO. Writing: FE. All authors approved the final version of the manuscript.

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Authorship Contributions: Concept: FG, YD, ST. Design: FG, ST, AA. Data Collection or Processing: ST, AA. Analysis or Interpretation: FG, YD. Literature Search: ST,

AA. Writing: FG and YD. All authors approved the final version of the manuscript.

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