



Original Research

Investigation of Brucellosis Seropositivity in Hakkari Province over One Year: A Multicenter Cross-sectional Study

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Abstract

Objectives: Brucellosis is a prevalent zoonotic disease in developing countries, as well as Türkiye. The objective of this study was to investigate the prevalence of brucellosis in the Hakkari province of Türkiye over a period of 1 year.

Methods: A retrospective cross-sectional study was conducted using serum samples from 12,742 patients in Hakkari's three main hospitals from January to December 2023. Demographic data, results of serological tests, admission time and clinics were collected and recorded. To identify seropositivity for brucellosis, the Rose Bengal Test (RBT) and Standard Tube Agglutination (STA) tests were performed.

Results: Of the samples, 6.6% tested positive for RBT, and 3.2% were confirmed to be seropositive for brucellosis with an STA titer of $\geq 1/320$. The highest prevalence of brucellosis seropositivity was observed in August, with a greater prevalence among women, particularly those aged 25-44. Most patients were admitted to internal medicine clinics, and the highest seroprevalence was observed in infectious disease clinics. Seroprevalence was statistically significant across age, gender, and clinics ($p < 0.05$).

Conclusion: The present study showed that the seroprevalence of brucellosis in Hakkari province was 3.2% in 2023. Brucellosis is still endemic in our province and public health measures are needed to reduce brucellosis transmission. We believe that the results of this study will provide data for epidemiological studies in the region.

Keywords: Brucellosis, Hakkari, serological tests, seroprevalence, zoonosis

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Brucellosis is the most prevalent zoonotic disease globally, with the potential to cause significant illness in both humans and livestock. Annually, 150,000–250,000 cases of human brucellosis are documented globally.^[1] The Mediterranean region, the Middle East, and parts of Asia, Africa and Latin America have a high prevalence of brucellosis.^[2] The most recent data available in our country is

from 2019, during which 10,244 cases of brucellosis were reported.^[1] The seroprevalence of brucellosis in ours ranges from 1.3% to 26.7%.^[3] The disease is more prevalent in the southeastern and eastern regions of Anatolia, where livestock farming is highly intensive.^[1] As indicated by the Ministry of Health General Directorate of Public Health, Hakkari province has the highest incidence of brucellosis.^[4]

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Brucellosis is a disease transmitted to humans by *Brucella* bacteria. This occurs through the consumption of meat from infected animals, such as sheep, goats, and cattle, and through contact with infected body fluids, including milk and urine. Additionally, the disease can be transmitted through consumption of dairy products prepared with infected milk and through contact with the gestational material of infected animals.^[5] Brucellosis has the potential to affect any organ in the body, resulting in a variety of clinical manifestations. The most common symptoms are high fever, malaise, sweating, joint pain, and loss of appetite.^[5,6]

Although a definitive diagnosis of the disease is possible through the isolation of the causative agent from clinical samples, serological tests, including the Rose Bengal test (RBT), standard tube agglutination test (STA), Coombs test, and ELISA test, are employed in the etiological diagnosis or seroprevalence assessment because of the inherent difficulties in culturing the bacteria.^[1,7] Currently, the most employed serological screening test for the diagnosis of brucellosis is the Rose Bengal test (RBT). However, due to the potential for false positivity or false negativity, RBT and STA should be performed simultaneously.^[8]

In this study, we conducted a retrospective analysis of the RBT and STA results of samples from the microbiology laboratories of hospitals in Hakkari province in 2023. The objective of this study was to ascertain the prevalence of brucellosis in the region and to provide data for epidemiological studies in the region.

Methods

Study Location, Population and Design

The study was conducted in Hakkari province, which is in the southeast region of Türkiye. It comprises five districts, including the central district, and has a population of 287,625. Serological tests for brucellosis are performed at three centers in Hakkari province: Yüksekova Public Hospital, Semdinli Public Hospital and Hakkari Public Hospital. The study was conducted in the medical microbiology laboratory units of the three aforementioned centers between the 1st of January 2023 and December 31, 2023. In total 15,168 samples from various clinics with a preliminary diagnosis of brucellosis were retrospectively evaluated. Repeat samples from the same patient were excluded and each patient was considered a single episode. Demographic data, including age and gender, results of serological tests, admission time and clinics were obtained from the hospital data processing center. The clinical conditions of the patients were not included in the study.

Microbiological Methods

Patient samples were centrifuged at 4000 rpm for 10 min, and serum was separated. RBT (ADR - Advanced Diagnostics & Research, Türkiye) was used as the screening test. In this test, the RBT antigen (standardized suspension produced from *B. abortus* S99 strain) was mixed with patient serum. The mixture was rotated by hand for four minutes at room temperature to assess any signs of agglutination. The test was considered positive in the presence of visible coarse particulate precipitates and negative in the presence of a homogenous appearance.

A quantitative STA (ADR - Advanced Diagnostics & Research, Türkiye) test was conducted on samples with positive RBT results. In accordance with the manufacturer's instructions, the samples were diluted in saline solution at a ratio of 1:10. After serial dilutions of serum samples in a total of seven wells in microplates, the *Brucella* antigen was added to each test well and the control well. The samples were incubated at 37°C for 24 h. The results were analyzed visually on dilution plates up to a dilution of 1/1280, with the first well of microplate being 1/20. A net-shaped image covering the entire bottom of the plate was considered positive, whereas the observation of a button-shaped depression in the center of the plate was considered negative. Since the study was conducted in a region endemic to brucellosis, STA test titers of 1/320 and above were considered seropositive.

Statistical Analysis

A descriptive analysis was conducted, in which the number and percentage values of the count type variables and the median and minimum-maximum values of the measurement type variables were presented. The chi-square test was employed to evaluate the relationships between categorical variables. The IBM SPSS Statistics 29.0 (Armonk, New York: IBM Corp.) software package was used for data entry and analysis. The significance level was set at $p < 0.05$.

Ethical Approval

The research was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Scientific Research and Publication Ethics Committee of the Hakkari University, decision date 28/03/2024 and numbered 1.

Results

A total of 15,168 samples were retrospectively analyzed during the study period. Repeat samples from the same patient were excluded. In total, 12,742 patient samples that met the study criteria were evaluated. The RBT was positive in 6.6% (845/12,742) of the patients and in 3.2% (405/12,742) of the patients, and the STA test titer was 1/320 and above (Fig. 1).

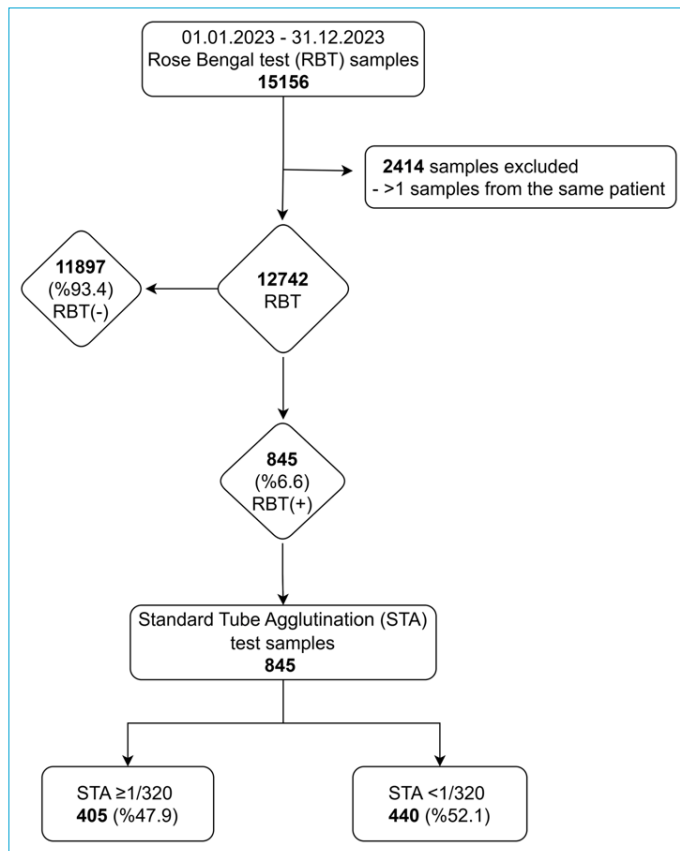


Figure 1. Flowchart.

Among the STA test results of RBT-positive patients, 27 (3.2%) were negative, and 143 (16.9%) were positive at titers 1/40, 125 (14.8%) 1/80, 145 (17.2%) 1/160, 123 (14.5%) 1/320, 94 (11.1%) 1/640, and 188 (22.3%) 1/1280. Consequently, 405 (47.9%) patients had STA test results at or above the 1/320 titer, while 440 (52.1%) patients had STA test results below the 1/320 titer. In 2023, the incidence rate in Hakkari province was 140.8 per 100,000 population. Figure 2 illustrates the distribution of the number of screening tests and seropositive patients evaluated during the

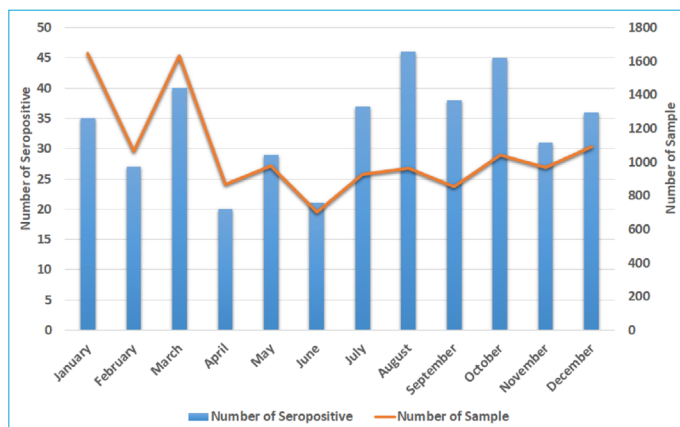


Figure 2. Distribution of brucellosis seropositivity by months.

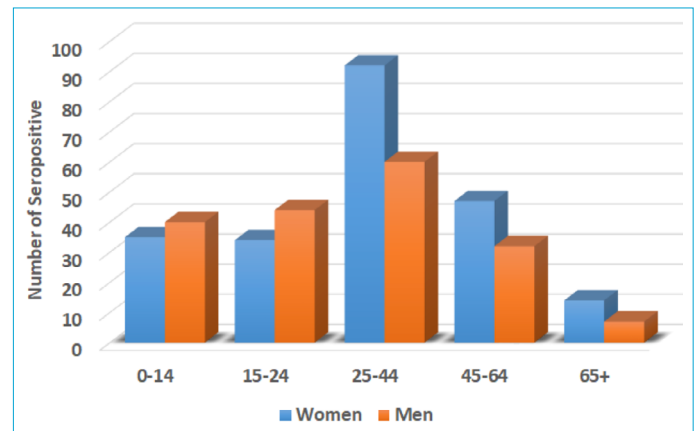


Figure 3. Distribution of brucellosis seropositivity by sex.

study period according to months. Accordingly, the highest prevalence of brucellosis seropositivity was observed in August, with the lowest prevalence observed in April ($n=46$, 11.4%; $n=20$, 4.9%, respectively).

Women constituted 67.4% (8,582/12,742) of the patients included in the study and 54.8% (222/405) of the seropositive patients. The median age of the brucellosis seropositive patients was 31 years, and 37.5% (152/405) were between 25 and 44 years. Only 5.2% (21/405) of the patients were 65 years of age or older. The distribution of seropositive patients according to sex and age is presented in Figure 3. RBT was most frequently requested from the internal medicine clinics; seropositivity was most frequently detected in infectious disease clinics.

The seroprevalence rate was 2.6% (222/8582) in women who were seropositive for brucellosis and 4.4% (183/4160) in men. The highest seroprevalence rate was observed in the 15-24 age range, at 4% (78/1957). Among the clinics, the highest seroprevalence was observed in infectious diseases with 9.5% (152/1603). Statistically significant correlations were identified between seroprevalence rates according to age, gender and clinics ($p=0.03$, $p<0.001$, $p<0.001$, respectively). The prevalence rates of seropositive and seronegative patients according to age, sex and clinic are presented in Table 1.

Discussion

The present study determined that the seroprevalence of brucellosis in Hakkari province was 3.2% in 2023. It was observed that cases of brucellosis were most frequently admitted to internal medicine clinics, in young adult women, with a higher incidence during the summer and autumn months.

The meta-analysis conducted by Kiran and Uçku^[9] the seroprevalence of brucellosis in Türkiye was estimated as 4.5%. The analysis also showed that the prevalence of brucellosis

Table 1. Characteristics of patients

	Seropositive n=405 (%)	Seronegative n=12337 (%)	Total n=12742 (%)	p
Age, Median.[min,max]	31.[2 – 87]	35.[0 – 123]	35.[0 – 123]	0.03
0-14	75 (3.8)	1883 (96.2)	1958 (100)	
15-24	78 (4.0)	1879 (96.0)	1957 (100)	
25-44	152 (3.2)	4590 (96.8)	4742 (100)	
45-64	79 (2.7)	2851 (97.3)	2930 (100)	
65+	21 (1.8)	1134 (98.2)	1155 (100)	
Sex				0.001
Female	222 (2.6)	8360 (97.4)	8582 (100)	
Male	183 (4.4)	3977 (95.6)	4160 (100)	
Clinics				0.001
Infectious Diseases	152 (9.5)	1451 (90.5)	1603 (100)	
Internal Medicine	98 (1.7)	5601 (98.3)	5699 (100)	
Pediatric	93 (4.2)	2129 (95.8)	2222 (100)	
Physiotherapy Rehabilitation	33 (1.7)	1921 (98.3)	1954 (100)	
Orthopedic	20 (4.0)	484 (96.0)	504 (100)	
Others	9 (1.2)	751 (98.8)	760 (100)	

varied between regions. The seroprevalence of brucellosis was found to be 0.9% in a study conducted between January 2010 and April 2010 in 1050 people over 18 years of age in Şanlıurfa province.^[10] A study conducted in Samsun between 2014 and 2019 found that 7.5% of 13,796 patient samples with suspected brucellosis were RBT positive, with a seropositivity rate of 3.4%.^[11] A study conducted in Istanbul between 2013 and 2017 revealed that 107 cases (1.8%) of brucellosis, out of a total of 6,045 cases, were found to be seropositive.^[12] A total of 2,500 serum samples were evaluated in the Ordu province between 2014 and 2018, with seropositivity determined to be 12.2%.^[13] Between August 2018 and October 2019, a total of 2630 serum samples were analyzed with the Brucellacapt test in the province of Tokat, with a seropositivity rate of 0.9%.^[14] A total of 1096 serum samples were analyzed in Van province between January and August 2021, with a seropositivity rate of 2.2%.^[15] A recent study conducted in Diyarbakır evaluated 5,196 samples from patients suspected of having brucellosis over the course of a year. Of these samples, 336 (6.5%) were RBT-positive. Of the RBT-positive patient samples, 257 (76.5%) were found to have a positive Brucella-Coombs test titer of 1/320 or higher, and the brucellosis seroprevalence was determined to be 5%.^[16]

According to the data of Ministry of Health General Directorate of Public Health, the incidence rate of brucellosis in Hakkari province in 2019 was 53.4 per 100,000 population.^[4] In our study, the incidence rate in Hakkari province was 140.8 per 100,000 population in 2023. Under-reporting of brucellosis to the surveillance system may explain this. Al-

though the seroprevalence of brucellosis was determined to be 3.2% in our study, the detection of 405 seropositive patients in one year is noteworthy when compared with both the number of reported cases and the findings of other studies. Furthermore, the relatively low seroprevalence may be attributed to the fact that 12,742 patients underwent screening for brucellosis. This may have been due to the fact that brucellosis is endemic to the region, its symptoms and signs are similar to those of various diseases, and clinicians ordered tests without an adequate differential diagnosis. The elevated amount of screening tests conducted in internal medicine and pediatric clinics, despite relatively low seropositivity rates, supports this hypothesis. Culture is considered the gold standard for the laboratory diagnosis of brucellosis, it is not always possible. Therefore, various low-cost, simple and rapid serological methods are used in the diagnosis. RBT and STA tests are the most widely preferred methods all over the world. However, both tests have a chance of resulting in false negatives.^[17] While RBT is particularly successful in the diagnosis of acute brucellosis, it can give high rates of false negative results in chronic and complicated cases. In the STA test, false negative or low titer positive results may be detected due to the presence of Prozone phenomenon and blocking antibodies in the early and late stages of brucellosis.^[18] Therefore, it is recommended that STA results should be confirmed by tests with high sensitivity and specificity such as Brucellacapt or ELISA, especially in endemic areas.^[17,18] However, these tests were not available in the centers where this study was conducted. In the present study, the STA test titer was found

to be 1/160 or below in approximately 50% of RBT-positive patients, who were consequently evaluated as seronegative. Given that the study was conducted in a region where brucellosis is endemic and the prevalence of prior infection is high, the seropositivity rate may have been comparatively low due to the absence of BrucellaCapt or ELISA.

In its 2022 report, the European Centre for Disease Prevention and Control (ECDC) indicated that the highest incidence of brucellosis was observed in July and September.^[19] Although the disease can be observed throughout the year in our country, its frequency increases in the spring and summer months, coinciding with the breeding periods of sheep and the production of cheese.^[12,13,20] Gursoy et al.^[21] reported that cases were most frequently observed in March, August, and November. Mert et al.^[22] similarly reported that 74% of cases occurred during the summer and autumn months. Similarly, in our study, the highest incidences were observed during the summer and autumn months. These months correspond to the calving period of animals in the Hakkari province. It can be argued that the increase in the number of cases was due to intensive contact with animals and excretion during the study period. Furthermore, the consumption of fresh cheese made from raw milk during these months may have contributed to the observed increase in the number of cases.

Human brucellosis is an infectious disease that presents with non-specific signs, including fever, sweating, malaise, anorexia, headache, and muscle pain.^[6] As a result, patients most commonly present to internal medicine, infectious diseases, and physiotherapy and rehabilitation clinics. Similarly, in our study, most patients presented to clinics with internal medicine clinics.

In developed countries, most cases of brucellosis are observed in males, because of the increased occupational risk.^[6] As documented in the ECDC 2022 report, the incidence of brucellosis is higher in males aged >45 years.^[19] In our country, brucellosis is observed in all age groups; however, it is particularly prevalent among young women because of their involvement in animal husbandry and exposure to milk and milk products.^[12,16,20] In our study, a similar distribution was observed, with more than half of the seropositive individuals being women aged 15–45. The observed differences in seropositivity, age, and sex were statistically significant.

The cross-sectional design of the study provides a snapshot of the prevalence of brucellosis during the study period; however, it does not establish causal relationships. Furthermore, the current study was limited by a lack of knowledge regarding the clinical characteristics of the patients, previous history of brucellosis, and use of more sensitive tests

in the evaluation of samples. Despite these limitations, our study offers valuable insights into the prevalence of brucellosis among patients admitted to hospitals in the Hakkari province.

Conclusion

Brucellosis remains a significant public health problem in our province. Public health measures are needed to reduce the seroprevalence of brucellosis.

Disclosures

Ethics Committee Approval: The study was approved by the Hakkari University Scientific Research and Publication Ethics Committee (date: 28.03.2024, no: 1).

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