Official Journal of Erciyes University Faculty of Medicine

DOI: 10.14744/cpr.2025.06572
J Clin Pract Res 2025;47(1):68–73

Brucellosis Seroprevalence and Diagnostic Challenges: A Comprehensive Review from 2017–2021

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ABSTRACT

Objective: This study aimed to evaluate the performance of the Rose Bengal test (RB), serum agglutination test (SAT), and Coombs test (CT) in samples suspected of brucellosis, analyze the seasonal distribution, examine the relationship with Crimean-Congo hemorrhagic fever (CCHF), which is frequently observed in our region, and determine the seroprevalence of brucellosis in the area.

Materials and Methods: A retrospective analysis was conducted on 12,279 brucellosis-suspected samples submitted from various clinics between 2017 and 2021. RB, SAT, and CT tests were performed, and results were analyzed concerning seasonal and yearly distributions as well as factors such as age and gender differences.

Results: Among the 12,279 RB tests conducted, 281 (2.3%) were positive, and 11,998 (97.7%) were negative. Of the positive results, 96 samples were positive for both RB and SAT, while 185 were RB positive but SAT negative. CT provided positive results in 101 out of 185 cases with discordant RB and SAT results. The seroprevalence rate was 1.5%, with higher rates observed among males and during the spring and summer seasons. A notable increase in seropositivity was observed during the pandemic.

Conclusion: This study highlights the annual and seasonal distribution of brucellosis and differences across age and gender groups. The Coombs test played a crucial role in resolving cases with discordant RB and SAT results. A rise in brucellosis cases during the pandemic was noted, with significant co-infections involving CCHF.

Keywords: Brucellosis diagnosis, coinfection, Crimean-congo hemorrhagic fever, diagnostic accuracy, serological tests.

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Cite this article as:
Fırtına Topcu K, Hasbek M. Brucellosis Seroprevalence and Diagnostic Challenges: A Comprehensive Review from 2017–2021. J Clin Pract Res 2025;47(1):68–73.

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Submitted: 24.09.2024
Revised: 19.12.2024
Accepted: 10.01.2025
Available Online: 03.02.2025

Erciyes University Faculty of Medicine Publications - Available online at www.jcpres.com

INTRODUCTION

Brucellosis is a systemic infectious disease transmitted from domestic and wild animals to humans, affecting multiple organs and systems and presenting with a wide range of clinical manifestations.[1,2] It causes economic losses in animals due to reduced reproductive efficiency and in humans due to labor loss resulting from complications associated with systemic involvement. The World Health Organization identifies brucellosis as one of the most neglected zoonotic diseases.[3] It is among the most widespread zoonotic infections, imposing a substantial global burden, with over 500,000 cases

reported annually.[4,5] The etiological agent, *Brucella spp*., is a small, nonmotile, sporeless, culture-resistant, facultative intracellular, gram-negative coccobacillus. Of its twelve recognized species, four are identified as human pathogens: *B. melitensis, B. abortus, B. suis,* and *B. canis*.[4,6] The primary transmission routes include direct or indirect contact with animal excreta and the consumption of raw meat and unpasteurized dairy products, typically through mucosal or gastrointestinal contact.[5]

The disease often manifests with nonspecific signs and symptoms, including fever, night sweats, weakness, fatigue, anorexia, weight loss, and generalized muscle and joint pain. This clinical presentation can mimic numerous other diseases, leading to frequent misdiagnosis.[1] Definitive diagnosis depends on the isolation of the bacteria from clinical samples, with blood cultures being the preferred specimens.[2] Although culture growth provides a definitive diagnosis, the sensitivity of this method depends on factors such as the timing of sample collection, the culture medium used, and prior antibiotic exposure. Failure to culture the bacteria does not exclude the diagnosis, highlighting the importance of serological tests as a critical component of the diagnostic process, which often depends heavily on these methods. The rapid agglutination test, known as the Rose Bengal test (RB), serves as a screening tool in endemic regions. The serum agglutination test (SAT) may be performed alongside RB, with titers of 1:160 or higher considered positive. In cases where a preliminary diagnosis strongly suggests brucellosis but the SAT result is negative, the Coombs test (CT) is utilized to account for the presence of blocking antibodies. The ELISA (enzyme-linked immunosorbent assay) method may also be incorporated into the diagnostic process.[7]

This study aimed to evaluate the RB, SAT, and CT results from brucellosis-suspected samples submitted to the microbiology laboratory from various clinics and polyclinics between 2017 and 2021. It sought to analyze the seasonal distribution of positive results, examine the association with Crimean-Congo hemorrhagic fever (CCHF) in differential diagnosis, and assess the seroprevalence of brucellosis within the province.

KEY MESSAGES

- This study demonstrated the contribution of a stepwise approach using the Rose Bengal test (RB), serum agglutination test (SAT), and Coombs test (CT) to the diagnostic process. Approximately half of the cases were diagnosed using the final step test.
- There was an increase in brucellosis cases during the pandemic period.
- Crimean-Congo hemorrhagic fever (CCHF) is one of the critical diseases to consider in the differential diagnosis in our region.

MATERIALS AND METHODS

Ethical Approval

Approval for this study was obtained from the Cumhuriyet University Ethics Committee on November 16, 2022, with decision number 2022-11/04.

Study Design and Sample Selection

This retrospective study analyzed the results of the RB, SAT, CT, and CCHF tests from 12,279 samples submitted from various units of our hospital between January 1, 2017 and December 31, 2021. Samples were collected from patients aged 18 and over for the differential diagnosis of brucellosis. Demographic data were retrieved from the hospital information management system. Repeated results associated with treatment follow-up after an initial positive test were excluded from the analysis.

Testing Procedures

- **RB Test:** The RB test (Seromed, Türkiye) was conducted first. A total of 50 µL of patient serum and 50 µL of *B. abortus* Rose Bengal antigen were combined on a card with a white background and rotated at 100 rpm for four minutes. The presence of sizable grain clusters was deemed positive, while their absence was classified as negative.
- **SAT:** Following the RB test, SAT (Seromed, Türkiye) was performed. Patient serum was prepared in glass tubes with the relevant antigen in serial dilutions from 1:20 to 1:640. The mixture was incubated at 37°C in a vibration-free environment for 48 hours. Button-shaped sedimentations were evaluated as negative, while lace-shaped agglutinations at dilutions of 1:160 and above were considered positive.
- **CT:** The Coombs test (Diagast, France) was applied to samples with discordant RB and SAT results or when clinical suspicion of brucellosis persisted despite negative results. Tubes showing no agglutination were washed and centrifuged three times, followed by re-incubation with Coombs antiserum for 24 hours. Button-shaped sedimentations were considered negative, while lace-shaped agglutinations at dilutions of 1:160 and above were regarded as positive.

Data Analysis

The results were analyzed using SPSS (Statistical Package for the Social Sciences) 22.0 software (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as numbers and percentages. Descriptive statistical methods (mean±standard deviation, range, count, percentage) and the chi-square test (Pearson chi-square) were used for 2×2 and multi-cell designs, with a significance level set at $p<0.05$.

Table 1. Age and gender distribution of cases

	All cases (n=12279)		Seropositive cases (n=189)		p
	n	%	n	%	
Gender					
Female	7405	60.3	57	30.16	<0.001
Male	4874	39.7	132	69.84	
Age					
18–39 years	3891	31.69	81	42.86	<0.001
40–59 years	4750	38.68	70	37.04	
>59 years	3638	29.63	38	20.11	

Table 2. Distribution of seropositivity according to test combinations

Seropositive cases (n=189)	Test results
96	RB (+), SAT (+)
84	RB (+), SAT (-), CT (+)
6	RB (-), SAT (-), CT (+)
3	RB (-), SAT (+)

RB: Rose bengal test; SAT: Serum agglutination test; CT: Coombs test.

RESULTS

A total of 12,279 RB tests were conducted in the laboratory for suspected brucellosis between January 2017 and December 2021. Among the patients included in the study, 4,874 (39.7%) were male, and 7,405 (60.3%) were female. All patients were aged 18 years or older, with ages ranging from 18 to 98 years, and the mean age was 49.2±16.9 years (Table 1). Of the 12,279 RB tests, 11,998 (97.7%) were negative, and 281 (2.3%) were positive. Among the positive samples, 96 (0.8%) tested positive for both RB and SAT, while 185 (1.5%) were RB positive but SAT negative. Coombs test was performed on samples with discordant RB and SAT results, showing 101 out of 185 samples as RB positive with SAT and CT negative. Additionally, 84 samples were RB positive, SAT negative, and CT positive. In the cohort with negative RB tests (n=11,998), three positive results were identified in subsequent SAT tests. Among cases with simultaneously negative RB and SAT results (n=11,995), clinical suspicion prompted CT testing in 1,910 cases, with CT yielding positive results in six instances. In total, 189 cases were determined to be seropositive for brucellosis (1.5%), while 12,090 cases were considered seronegative (98.5%) (Tables 2, 3). In the seropositive cohort, 57 (30.16%) were female, and 132 (69.84%) were male, indicating a significantly higher seropositivity rate in males ($p<0.05$). Furthermore, a significant difference in seropositivity was observed across age groups ($p<0.05$). The distributions by gender and age groups are detailed in Table 1.

Table 3. Distribution of seronegativity according to test combinations

Seronegative cases (n=12090)	Test results
12090/10085	RB (-), SAT (-)
101	RB (+), SAT (-), CT (-)
12090/1904	RB (-), SAT (-), CT (-)

RB: Rose bengal test; SAT: Serum agglutination test; CT: Coombs test.

Table 4. Distribution of seropositive cases by years and seasons

	Positive	Negative	p
Seasons			
Spring	49	2745	>0.05
Summer	60	3577	
Autumn	47	2893	
Winter	33	2875	
Years			
2017	12	2422	<0.001
2018	13	2591	
2019	33	2743	
2020	42	2260	
2021	89	2074	
Total (n=Applications/Positive)	2434/12	2604/13	

The distribution of relevant tests by years and seasons (March-April-May: spring, June-July-August: summer, September-October-November: autumn, December-January-February: winter) is presented in Table 4. A significant difference in positivity rates was observed when the results were compared across years ($p<0.05$), while no significant difference was identified between seasons in terms of positivity ($p>0.05$). When the years 2017–2019 were grouped as the pre-pandemic period and 2020–2021 as the pandemic period, a statistically significant increase in seropositivity was noted during the pandemic period ($p<0.05$).

Our region is endemic for Crimean-Congo hemorrhagic fever. CCHF is considered in the differential diagnosis of brucellosis, and cases of co-infection have been reported. Therefore, patients who underwent CCHF polymerase chain reaction (PCR) testing

Table 5. Crimean-congo hemorrhagic fever (CCHF) co-occurence in seropositive cases by years

	2017	2018	2019	2020	2021	p
CCHF positive	0	1	2	3	6	
CCHF negative	0	2	2	5	12	>0.05

were also evaluated. Among the 189 seropositive patients, CCHF PCR testing was conducted on 33 individuals, and co-positivity for *Brucella* and CCHF was identified in 12 cases. No significant difference in co-infection rates was observed between the pre-pandemic and pandemic periods (p>0.05) (Table 5).

DISCUSSION

Brucellosis is the most common bacterial zoonotic infection in our country. While it is more frequently observed in the Eastern and Southeastern Anatolia regions, it is present in all regions.[8] The annual incidence ranges from 0.3 cases per million in some developed countries to 1,000 cases per million in endemic areas.[9] The incidence of brucellosis varies regionally within our country.[10]

A meta-analysis of 51,650 participants in Türkiye (1999–2021) reported a brucellosis seroprevalence rate of 4.5%.[11] In a six-year study conducted by Çelik et al.[12] on 4,344 cases in the Istanbul region, the seropositivity rate was found to be 3%. Öner et al.[13] detected a 1.5% seropositivity rate in their study, which included 1,624 serum samples from the Tokat region. Köksal et al.[14] screened 467 samples from the Istanbul region and cases imported from neighboring provinces, identifying a seropositivity rate of 3.85%. In a four-year study by Taner et al.,[15] 107 out of 6,045 suspected brucellosis cases (1.8%) were found to be seropositive. Of the 107 *Brucella* seropositive cases, 73 (68.2%) tested positive simultaneously with the RB and SAT, while 34 (31.7%) were determined to be seropositive based on the CT results due to discordance between RB and SAT test results. In the present study, CT was also performed on patients with discordant RB and SAT results, and 47.6% of all seropositive patients were identified through this method.

In a seroprevalence study conducted by Sümer et al.[16] in 2003 with 750 cases in our province using similar test methods, a seropositivity rate of 3.2% was reported. In another study by Sümer et al.[17] in 2000, a seropositivity rate of 2.8% was observed in a screening conducted among restaurant workers in the city center. A review of the literature reveals that no similar studies have been conducted in the region in recent years. In this study, after evaluating the results of 12,279 cases, the seropositivity rate was found to be 1.5%. CT was performed on the group of patients with discordant RB and SAT results, detecting 47.6% of all seropositive patients in this way. The seropositivity rate was consistent with the literature but lower than previous studies conducted in the region. This may suggest that control measures are proving effective. However, the larger patient cohort in this study and the limitation of one of the previous studies to the city center may have contributed to the difference in the results.

In countries with a low incidence of brucellosis, the disease is reported to be more common among men due to occupational exposure. In endemic countries, however, no significant gender difference have been observed.[18–20] Koşar et al.[21] found that 64% of 280 brucellosis cases in their study from Türkiye were women, attributing this the frequent involvement of women in animal care and dairy product preparation in rural areas. Similarly, Çelik et al.[12] reported a higher proportion of female cases (55%) compared to men in their study, though the difference was not statistically significant. Taner et al.[15] also observed a higher seropositivity rate among women (58%) compared to men (42%), but no statistically significant difference was reported. In contrast, Öner et al.[13] found a higher prevalence of brucellosis among men and noted a statistically significant difference in gender distribution. In the current study, the proportion of men among seropositive cases was significantly higher than that of women. While brucellosis does not inherently differ by age or gender, this result may reflect the greater involvement of men in agriculture and livestock farming.

When examining the seasonal distribution of brucellosis, most cases occur in the spring and summer months. Gür et al.[19] reported that 68% of cases were observed during these seasons in their study. Buzgan et al.[22] noted that the initial cases were encountered in April and May, when the consumption of fresh cheese is common. A second epidemic period was observed in September, when cheese stored during the spring months began to be consumed. Çelik et al.[12] found that brucellosis seropositivity was most common in the summer, while Altunçekiç Yıldırım et al.[23] observed the highest rates during the spring. Consistent with the literature, 82.5% of seropositive cases in this study were detected in the spring and summer months. This pattern may reflect the availability of fresh milk and dairy products in the spring and their consumption in the subsequent spring and summer.

Brucellosis, with its nonspecific symptoms, has a broad differential diagnosis. Gül et al.[24] reported a case of a patient living in a rural area who was simultaneously diagnosed with brucellosis, Coronavirus Disease 2019 (COVID-19), and Crimean-Congo hemorrhagic fever (CCHF). In this study, when the pre-pandemic and post-pandemic periods were compared, seropositivity was significantly higher after the pandemic. This increase is thought to be related to spending more time in nature due to social isolation during the pandemic and changes in dietary habits favoring more natural products.

Before the pandemic, Duygu et al.[25] detected brucellosis in 5.3% of patients hospitalized with a preliminary diagnosis of CCHF. The rate of co-infection with brucellosis and CCHF was found to be 4.16% in the same study. Karakeçili et al.[26] and Büyüktuna et al.[27] also reported cases of co-infection with brucellosis and CCHF in endemic regions before the pandemic. During the pandemic, Erdoğan et al.[28] identified both CCHF and brucellosis in a patient from a non-endemic region. In this study, 33 *Brucella* seropositive patients were screened for CCHF, and 12 were found to have co-infection. No significant difference was observed in the frequency of co-infections detected before and after the pandemic. These findings indicate that co-infections have been present both before and after the pandemic. Given the overlapping and nonspecific symptoms of these two zoonotic diseases, their co-occurrence should be considered in both endemic and non-endemic regions. We believe that raising awareness of co-infections will enhance the diagnosis and treatment processes for both diseases and improve clinical practice.

CONCLUSION

In conclusion, brucellosis continues to be a significant disease of concern. This study evaluated the distribution of brucellosis by gender, age group, years, and seasons and examined the effects of key factors such as the pandemic period and co-infections. Our findings indicate that brucellosis is an infection influenced by various demographic and seasonal factors, requiring comprehensive evaluation to enhance the accuracy of test results. The study highlights the limitations of relying solely on RB to evaluate seropositivity and emphasizes the importance of CT as an additional confirmatory tool in such cases. These findings provide a valuable foundation for future research and clinical practice. As no similar study has been conducted recently in our region, this research also contributes valuable insights into regional data.

Ethics Committee Approval: The Cumhuriyet University Non-interventional Clinical Research Ethics Committee granted approval for this study (date: 16.11.2022, number: 11/04).

Author Contributions: Concept – KFT; Design – KFT, MH; Supervision – KFT, MH; Resource – KFT, MH; Materials – KFT, MH; Data Collection and/or Processing – KFT, MH; Analysis and/or Interpretation – KFT, MH; Literature Search – KFT, MH; Writing – KFT, MH; Critical Reviews – KFT, MH.

Conflict of Interest: The authors have no conflict of interest to declare.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

REFERENCES

1. Karahocagil MK. Clinical manifestations. Turk Klin J Inf Dis Spec Top 2012; 5(1): 24-33.
2. Kılıç S. Microbiological diagnosis. Turk Klin J Inf Dis Spec Top 2012; 5(1): 46-66.
3. Kurmanov B, Zincke D, Su W, Hadfield TL, Aikimbayev A, Karibayev T, et al. Assays for identification and differentiation of brucella species: A review. Microorganisms 2022; 10(8): 1584. [CrossRef]
4. Akinyemi KO, Fakorede CO, Amisu KO, Wareth G. Human and animal brucellosis in Nigeria: A systemic review and meta-analysis in the last twenty-one years (2001-2021). Vet Sci 2022; 9(8): 384. [CrossRef]
5. Zheng R, Xie S, Lu X, Sun L, Zhou Y, Zhang Y, et al. A systematic review and meta-analysis of epidemiology and clinical manifestations of human brucellosis in China. Biomed Res Int 2018; 2018: 5712920. [CrossRef]
6. Di Bonaventura G, Angeletti S, Ianni A, Petitti T, Gherardi G. Microbiological laboratory diagnosis of human brucellosis: An overview. Pathogens 2021; 10(12): 1623. [CrossRef]
7. Clinical Bacteriology Diagnostic Standards Working Group. Microbiological Diagnosis of Brucellosis. In: National Microbiology Standards, Infectious Diseases Laboratory Diagnostic Guide. 2015. p. B-MT-19.
8. Directorate General of Public Health, Department of Zoonotic and Vector-Borne Diseases. Bruselloz. Available from: URL: https://hsgm.saglik.gov.tr/tr/zoonotik-ve-vektorel-hastaliklar/bruselloz.html. Accessed Jan 14, 2025.
9. Tuon FF, Gondolfo RB, Cerchiari N. Human-to-human transmission of brucella - A systematic review. Trop Med Int Health 2017; 22(5): 539-46. [CrossRef]
10. Directorate General of Public Health, Department of Zoonotic and Vector-Borne Diseases. 2017 Brucellosis Incidence Map. Available from: URL: https://hsgm.saglik.gov.tr/depo/birimler/zoonotik-ve-vektorel-hastaliklar-db/Dokumanlar/Istatistikler/Web_Bruselloz_haritasi.pdf. Accessed Jan 14, 2025.
11. Kıran P, Uçku R. Seroprevalence of human brucellosis in Turkey: A comprehensive meta-analysis. Zoonoses Public Health 2024; 71(7): 844-54. [CrossRef]
12. Çelik DG, Sirekbasan S, Abdelkareem A, Yüksel P, Aslan M, Sarıbaş S, et al. The results of serum agglutination (wright) and rose-bengal tests in the diagnosis of brucellosis: 6 years retrospective seroepidemiological evaluation of Cerrahpasa Medical Faculty serology laboratory datas. Cerrahpasa Med J 2018; 42(1): 80-5.
13. Öner SZ, Türkoğlu E. Seroprevalence of brucellosis from a region where low endemicity is expected. Med J West Black Sea 2020; 4(1): 18-23. [CrossRef]

14. Köksal MO, Durmuş MA, Beka H, Ağaçfidan A. Investigation of brucella seroprevalence and risk factors in patients admitting to a university hospital. ANKEM J 2020; 34(2): 65-70.
15. Taner Z, Dinç HÖ, Demirci M, Gareayaghi N, Kurt A, Özbey D, et al. Investigation of brucella seropositivity in patients with suspected brucellosis: A 4-year retrospective evaluation. Turk Mikrobiyol Cem Derg 2019; 49(4): 212-8. [CrossRef]
16. Sümer H, Sümer Z, Alim A, Nur N, Ozdemir L. Seroprevalence of brucella in an elderly population in mid-Anatolia, Turkey. J Health Popul Nutr 2003; 21(2): 158-61.
17. Sümer Z, Alim A, Sümer H, Özdemir L. Brucella seropositivity in the restaurant workers, Sivas city center. Turkish J Infect 2000; 14(1): 69-70.
18. Taşova Y, Saltoğlu N, Yılmaz G, İnal S, Aksu HSZ. Brucellosis: An evaluation of clinical, laboratory and treatment features of 238 adult cases. Turkish J Infect 1998; 12(3): 307-12.
19. Gür A, Geyik MF, Dikici B, Nas K, Cevik R, Sarac J, et al. Complications of brucellosis in different age groups: A study of 283 cases in southeastern anatolia of Turkey. Yonsei Med J 2003; 44(1): 33-44. [CrossRef]
20. Tansel Ö, Yavuz M, Kuloğlu F, Akata F. Evaluation of 40 brucellosis cases admitted to the Trakya University Hospital. Turkish J Infect 2003: 17: 1-4.
21. Koşar A, Aygündüz M, Yaylı G. Comparison of two different treatment methods in 280 cases of brucellosis. Turkish J Infect 2001; 15(4): 433-7.
22. Buzgan T, Karahocagil MK, Irmak H, Baran AI, Karsen H, Evirgen O, et al. Clinical manifestations and complications in 1028 cases of brucellosis: A retrospective evaluation and review of the literature. Int J Infect Dis 2010; 14(6): e469-78. [CrossRef]
23. Altunçekiç Yıldırım A, Çetinkol Y. Investigation of brucellosis seroprevalence in Ordu city: Five-years review. J Biotechnol Strat Health Res 2020; 4(2): 129-33. [CrossRef]
24. Gül Ö, Binay UD, Barkay O, Karakeçili F. The importance of differential diagnosis during pandemic: A case report with coexistence of COVID-19, brucellosis and crimean-congo hemorrhagic fever. Bull Microbiol 2022; 56(2): 365-70. [CrossRef]
25. Duygu F, Sari T, Kaya T, Bulut N. Brucellosis in patients with crimean-congo hemorrhagic fever. J Arthropod Borne Dis 2017; 11(4): 463-8.
26. Karakeçili F, Çıkman A, Akın H, Gülhan B, Özçiçek A. A case of brucellosis and crimean-congo hemorrhagic fever coinfection in an endemic area. Bull Microbiol 2016; 50(2): 322-7. [CrossRef]
27. Büyüktuna SA, Öksüz C, Hasbek M. Concurrent brucellosis and crimean-congo haemorrhagic fever: A case report. Klimik J 2019; 32: 338-40. [CrossRef]
28. Erdoğan A, Erdoğan H. Crimean-congo hemorrhagic fever (CCHF) and brucellosis coinfection in Alanya: A case report. Klimik J 2023; 36: 82-5. [CrossRef]