

SEROPREVALENCE OF BRUCELLOSIS IN CHILDREN AND ADULTS IN THE CENTRAL BLACK SEA REGION OF TURKEY

Hacer Ozlem Kalayci, Mustafa Kerem Calgin✉

Received on January 23, 2025

Presented by B. Petrunov, Member of BAS, on February 25, 2025

Abstract

Brucellosis is a zoonotic infectious disease caused by *Brucella* species. Early diagnosis of brucellosis is important so that treatment can start early. We aimed to evaluate the seroprevalence of brucellosis among children and adult populations. Between April 1, 2021 and May 31, 2024, 3953 patients who were sent to microbiology laboratory from various outpatient clinics and wards with a preliminary diagnosis of brucellosis and tested for brucellosis were included. Serum samples of the patients were tested with Coombs Brucella Test. Patients with antibody titers of 1/160 and above were considered positive for brucellosis. Chi-square and Fisher's exact tests were used to analyze categorical variables. Seropositivity was not statistically significant between genders ($p = 0.397$), but there was a statistically significant high level of seropositivity in people under 50 years of age ($p = 0.015$). Seropositivity was observed mostly in patients from infectious diseases ($n = 52$, 40%), followed by orthopedics ($n = 47$, 36.2%) clinics. Brucellosis remains an important public health problem in endemic areas. Physicians should always consider brucellosis in the differential diagnosis.

Key words: brucellosis, seroprevalence, epidemiology

Introduction. Brucellosis is a globally important endemic zoonosis caused by gram-negative coccobacilli of the genus *Brucella*. Transmission of brucellosis to humans occurs through ingestion of contaminated animal products or direct contact with infected animals. The disease is endemic in regions such as the Middle East, Mediterranean, Central and South America. *B. melitensis* is the

most common cause of reported human brucellosis cases and is the most severe form of the disease [1–3]. Brucellosis is characterized by general symptoms such as fever, night sweats, fatigue, joint and muscle pain. For this reason, the patient may delay or neglect to consult a physician. Although morbidity due to brucellosis is quite high in Turkey, mortality is quite low as a result of early diagnosis and strict follow-up [4].

Early diagnosis of brucellosis and early initiation of treatment is important. Despite some limitations, serologic tests represent the main diagnostic modalities for the diagnosis of brucellosis in endemic and low-middle-income countries because they are low-cost, user-friendly and have a high negative predictive value. In the serologic diagnosis of brucellosis, IgG and IgM antibodies are studied with the Wright test, a Standard Tube Agglutination (SAT) test. However, some individuals develop agglutinating IgG, IgM and IgA antibodies, while others develop blocking (non-agglutinating) or missing IgG and IgA antibodies. Blocking antibodies bind to antigens, but agglutination does not occur. These incomplete antibodies are diagnosed using the Coombs test using anti-human immunoglobulin [5,6].

The seroprevalence of brucellosis varies not only between countries but also within the same country and there are no reliable data on its global prevalence. It is important to assess the seroprevalence of brucellosis in different regions and groups for disease management, prevention and control strategies [7]. The aim of this study was to determine *Brucella* seropositivity in all age groups in Ordu province in the Central Black Sea region of northern Turkey.

Material and methods. Between April 1, 2021 and May 31, 2024, 3953 patients who were sent to Ordu University Training and Research Hospital microbiology laboratory from various outpatient clinics and wards with a preliminary diagnosis of brucellosis and tested for brucellosis were included. Serum samples of the patients were tested with Metser Coombs *Brucella* Test (MetserLab, Istanbul, Turkey) on the same day according to the company recommendations. Sera were obtained by centrifugation of peripheral blood before the study. Serum samples were diluted to 1/1280 dilution in the microplate and then *brucella* test antigen was added to the wells. The microplate was then incubated at 37 °C for 24 h. Wells with agglutination in the evaluation were considered positive. Test results and patient data were recorded and analyzed through the hospital automation system. Patients with antibody titers of 1/160 and above were considered positive for brucellosis. Statistical analyses were completed using SPSS statistical software (SPSS, Chicago, IL, USA, version 20). Chi-square and Fisher's exact tests were used to analyze categorical variables. For the tests, $p < 0.05$ was considered statistically significant.

Ethical approval for this study was obtained from Ordu University Non-Interventional Scientific Research Ethics Committee (Date: 08.11.2024, Decision No. 171).

Results. Of the 3953 participants, 66.5% ($n = 2628$) were female and 33.5% ($n = 1325$) were male. The mean age was 50.67 years (age range 0–99 years). Eighty-two (3.1%) women and 48 (3.6%) men had significantly high levels of specific Brucella antibodies (1/160 dilution and above) and the difference was not statistically significant ($p = 0.397$). While seroprevalence under 50 years of age was 4.1%, it was 2.6% in patients aged 50 years and over. In terms of seropositivity, significant seropositivity was found in patients under 50 years of age compared to those over 50 years of age ($p = 0.015$). Seroprevalence percentages according to gender and age in the study group are shown in Table 1.

T a b l e 1
Seroprevalence distribution by gender and age

	Cases		Seropositive	Seroprevalence	
	<i>n</i>	%	<i>n</i>	%	<i>p</i>
Sex					
Male	1325	33.5	48	3.6	0.397
Female	2628	66.5	82	3.1	
Age groups					
Under 50 y	1672	42.2	69	4.1	0.015
Over 50 y	2281	57.8	61	2.6	

In 52 patients, the titer was 1/1280 and above, in 28 patients it was 1/160, in 25 patients it was 1/320 and 1/640 and a total of 130 patients were seropositive. Accordingly, seropositivity for Brucella infection was found to be 3.3% in the study population. The distribution of positivity according to titers in patient age groups is shown in Table 2.

The highest seropositivity was observed in patients from infectious diseases ($n = 52$, 40%) and then orthopedics ($n = 47$, 36.2%) clinics. The distribution of seropositivity according to clinics is shown in Table 3.

Discussion. Brucellosis is an important endemic infectious disease and can cause a variety of symptoms including intermittent or irregular fever, headache, malaise, fatigue, excessive sweating, chills, weight loss, generalized pain and other clinical manifestations. Diagnosis is difficult due to these non-specific symptoms [2]. In developing countries, low-cost and user-friendly serologic tests are used for the diagnosis of brucellosis. These tests also have a high negative predictive value [5].

Brucella seropositivity was 15.7% in a study conducted in Afghanistan, 16% in a study conducted in Pakistan, 15.6% in a study conducted in Angola among live-stock keepers, and 3.9% in a study conducted in India among veterinarians [8–11]. A meta-analysis of 30 comprehensive studies conducted in Turkey between 1999 and 2021 revealed a brucellosis seroprevalence of 4.5% [7]. When the Brucella seroprevalence studies conducted in different regions of our country are examined, TAVŞAN et al. [12] determined Brucella seroprevalence as 0.9% in their study con-

T a b l e 2

Distribution of seropositivities in age groups according to serum dilutions

Age groups	Serum dilutions						Test	
	<i>n</i>	(%)	1/ 160	1/ 320	1/ 640	1/ 1280	Positive (<i>n</i>)	Negative (<i>n</i>)
0–9	97	(2.5)	-	-	-	-	-	97
10–19	186	(4.7)	3	-	1	3	7	179
20–29	286	(7.2)	2	2	4	5	13	273
30–39	395	(10)	4	4	4	6	18	377
40–49	708	(17.9)	6	8	4	13	31	677
50–59	913	(23)	8	8	7	12	35	878
60–69	823	(20.8)	3	3	3	8	17	806
70–79	409	(10.4)	2	-	1	4	7	402
80–89	126	(3.2)	-	-	1	1	2	124
90–99	10	(0.3)	-	-	-	-	0	10
Total	3953	(100)	28	25	25	52	130	3823

T a b l e 3

Distribution of seropositivity according to clinics

Clinics	Seropositivity	%
Infectious diseases	52	40
Orthopedics	47	36.2
Internal medicine	12	9.2
Neurosurgery	12	9.2
Physical medicine and rehabilitation	7	5.4
Total	195	127

ducted in Şanlıurfa city centre. In Samsun, VURAL et al. [12] found seropositivity as 3.4% in their retrospective study of 5-year brucellosis suspected patients. While brucellosis seroprevalence was found to be 5% in the study by TEMİZ et al. [14], it was found to be 0.9% in Amasya, a region where low endemicity is expected [15]. Between 2005–2011, the seroprevalence of brucellosis in Istanbul and neighbouring cities was found to be 3% [16]. Brucella seroprevalence may vary depending on the difference in serologic tests used in diagnosis. In our study, Brucella seropositivity was found to be 3.3% and is compatible with the data of our country. The actual magnitude of human brucellosis is thought to be much higher than the reported figures. This is due to factors such as dietary habits, processing methods of milk and dairy products, animal husbandry practices and environmental hygiene. Public education and awareness can contribute to reducing disease transmission [1].

Studies have reported different results regarding the relationship between gender and brucellosis prevalence. In some studies, no significant relationship was

observed in terms of gender [4,17]. In our study, we did not detect a statistically significant difference between genders. Although brucellosis is an age- and gender-neutral disease, it is reported to be more common in men due to occupational risk in regions where the incidence is low. The researchers emphasized that the seropositivity observed in the male gender may be due to the fact that more men are engaged in animal husbandry [18]. Temiz et al. [14] found that *Brucella* seropositivity was higher in women.

Brucellosis is a zoonosis that can be seen at any age, mostly in young and middle-aged adults, and can be easily confused with many diseases. Although some studies have not found a significant correlation between age and seropositivity, many studies have reported that most cases are seen between the ages of 20–40, which is the productive age group, depending on the frequency of contact with animals [13–15,18]. In our study, a statistically significant high seropositivity was found below the age of 50 years and it was observed that the patients were mostly in the 20–49 age group ($p = 0.015$).

Since brucellosis causes various clinical pictures, patients may present to different outpatient clinics. In our study, the highest seropositivity rate was found in serum samples sent from the infectious diseases outpatient clinic. This was followed by the orthopedics clinic and this was found to be directly proportional to the clinical compliance of patient complaints. Clinicians working in these branches should definitely consider brucellosis in the differential diagnosis of patients presenting with non-specific symptoms such as fever, sweating, fatigue, anorexia, headache and muscle pain.

In conclusion, brucellosis is still an important public health problem in endemic regions such as Turkey. Therefore, the diagnosis, treatment and follow-up of brucellosis are very important and surveillance studies ensure the follow-up and control of the infection. Due to the impact of brucellosis on public health, the public should be informed about the disease and its transmission routes and necessary trainings should be provided. In addition, physicians should always consider brucellosis in differential diagnosis. This is the first study evaluating *brucella* seropositivity in the Central Black Sea region.

REFERENCES

- [1] NEJAD R. B., R. C. KRECEK, O. H. KHALAF et al. (2020) Brucellosis in the Middle East: Current situation and a pathway forward, *PLoS Negl. Trop. Dis.*, **14**(5), e0008071.
- [2] LAI S., Q. CHEN, Z. LI (2021) Human brucellosis: An ongoing global health challenge, *China CDC Wkly*, **3**(6), 120–123.
- [3] QURESHI K. A., A. PARVEZ, N. A. FAHMY et al. (2023) Brucellosis: epidemiology, pathogenesis, diagnosis and treatment – a comprehensive review, *Ann. Med.*, **55**(2), 2295398.

- [4] ARVAS G., Y. AKKOYUNLU, M. BERKTAS et al. (2013) The prevalence of brucellosis in adults in northeastern region of Turkey, *Jundishapur J. Microbiol.*, **6**(3), 262–264.
- [5] DI BONAVENTURA G., S. ANGELETTI, A. IANNI et al. (2021) Microbiological laboratory diagnosis of human brucellosis: An overview, *Pathogens*, **10**(12), 1623.
- [6] TURAN O., T. KARSLIGIL (2023) Importance of the Coombs test in diagnosing the Brucella, *Ann. Clin. Anal. Med.*, **14**(8), 668–671.
- [7] KIRAN P., R. UCKU (2024) Seroprevalence of human brucellosis in Turkey: A comprehensive meta-analysis, *Zoonoses and Public Health*, **71**(7), 844–854.
- [8] AKBARIAN Z., G. ZIAY, W. SCHAUWERS et al. (2015) Brucellosis and *Coxiella burnetii* infection in householders and their animals in secure villages in Herat Province, Afghanistan: A cross-sectional study, *PLoS Negl. Trop. Dis.*, **9**(10), e0004112.
- [9] ALI S., Z. NAWAZ, A. AKHTAR et al. (2018) Epidemiological investigation of human brucellosis in Pakistan, *Jundishapur J. Microbiol.*, **11**(7), e61764.
- [10] MUFINDA F. C., F. BOINAS, C. NUNES (2017) Prevalence and factors associated with human brucellosis in livestock professionals, *Rev. Saude Publica*, **51**, 57.
- [11] SHOME R., T. KALLESHAMURTHY, P. B. SHANKARANARAYANA et al. (2017) Prevalence and risk factors of brucellosis among veterinary health care professionals, *Pathog. Glob. Health*, **111**(5), 234–239.
- [12] TAVŞAN Ö., S. TEKIN-KORUK, İ. KORUK (2015) The prevalence of brucellosis and associated risk factors in Şanhurfa City Center, *Klinik Dergisi*, **28**(1), 11–17.
- [13] VURAL D. G., C. ÇINAR, Ç. ÇOBAN et al. (2021) Retrospective evaluation of 5-year brucella outcomes in a university hospital, *J. Biotechnol. Strategic Health Res.*, **5**(2), 132–136, (in Turkish).
- [14] TEMİZ H., N. ÖZCAN, E. ARAÇ, E. ÖZBEK (2022) Brucellosis Seroprevalence in Dicle University Medical Faculty Hospital: RoseBengal test and Brucella Coombs test results, *Dicle Med. J.*, **49**(4), 660–666, (in Turkish).
- [15] ÖNER S. Z., E. TÜRKOĞLU (2020) Brucellosis seroprevalence in a region expected to have low endemicity, *Med. J. West Black Sea*, **4**(1), 18–23, (in Turkish).
- [16] ÇELİK D. G., S. SIREKBASAN, A. ABDELKAREEM et al. (2018) Serum agglutination (Wright) and Rose-Bengal test results in the diagnosis of brucellosis: A 6-year retrospective seroepidemiological evaluation from Cerrahpaşa Medical Faculty Serology Laboratory, *Cerrahpaşa Tıp Dergisi*, **42**(1), 80–85, (in Turkish).
- [17] UCAR M., A. YILMAZ, S. ÇELEBİ, S. YILMAZ (2023) Seroprevalence of brucellosis in individuals engaged in animal husbandry in Erzurum region, *Genel Tıp Derg.*, **33**(6), 768–774.
- [18] KHALILIAN M. S., J. RAMAZANPOUR, S. M. HOSSEINI et al. (2021) Trends of human brucellosis in Central Iran (2010–2018), *J. Res. Med. Sci.*, **26**, 55.

*Medical Microbiology Department, Faculty of Medicine, Ordu University,
52200 Ordu, Türkiye*
e-mails: kalayciozlem55@gmail.com, mkcalgin@gmail.com