


## Seroprevalence of human brucellosis in Travnik, Central Bosnia and Herzegovina

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### Abstract

**Objectives:** To measure and analyze the seroprevalence and risk factors of human brucellosis among clinically suspected patients in the Travnik area, Bosnia and Herzegovina in the period 2018-2023.

**Methods:** A total of 337 samples of clinically suspected brucellosis patients in Travnik, Central Bosnia and Herzegovina were collected between 2018-2023. Rose Bengal agglutination was used for sera testing. Expressive and investigative statistics were considered. Differences between the prevalence of brucellosis according to age, sex, and the seasonal incidence rate were assessed.

**Results:** The prevalence of brucellosis among the suspected patients was 23.30%. The highest prevalence rate 35.71%, was detected during year 2021. Higher rate of brucellosis was observed among males 63.86% than females 36.14%. Most cases were reported during month of June and October, 14.15% and 14.19% respectively. The highest prevalence, 56.76% rate was observed in age group 41–60 years old.

**Conclusion:** Our survey found a high prevalence of human brucellosis among clinically suspected patients in Travnik area strongly indicating that clinical suspicion is a valid criterion, which requires fast laboratory detection and patient treatment. Detecting specific anti-Brucella antibodies using agglutination Rose Bengal test is a fast a reliable diagnostic procedure for clinically suspect individuals. There is a strong association between human brucellosis and age.

**Key words:** *Brucella* spp., human brucellosis, seroprevalence, risk factors, Bosnia and Herzegovina

## Introduction

Brucellosis is a zoonotic, food-borne, endemic and re-emerging infectious disease of wild and domestic animals that represent reservoir for human infection (Pappas et al., 2005). Brucellosis is endemic in Mediterranean region (Seleem et al., 2010) and Bosnia and Herzegovina for the last thirty years (Maksimović et al., 2022). The bacterial species of *Brucella* spp. infect different hosts: *B. melitensis* infects sheep and goats, *B. abortus* cattle, *B. canis* dogs, and *B. suis* pigs (Sakran et al., 2006). Other species of this genus were isolated from wildlife animals and humans (Pappas et al., 2005).

In the Mediterranean countries and Bosnia and Herzegovina, *Brucella melitensis*, represents most frequently isolated *Brucella* species in sheep and goats (Taleski et al., 2002). The major infection route is occupational exposure to infected animals, thru inhalation of contaminated secretions of infected animals (Bosilkovski et al, 2007) and consumption of unpasteurized milk and dairy products (Volner et al., 2010).

Onset of human brucellosis cases in Bosnia and Herzegovina started with one case in 1999 but spread among the human population to reach a peak of 1000 cases in 2008 (Obradović & Velić 2010). After the mass sheep and goats vaccination program in FB&H that started in 2009 (Obradović & Velić 2010), the number of animal brucellosis cases started to decrease, but human brucellosis remained a serious threat in B&H. In the 2003-2012 period 361 infected patients was reported in Travnik, B&H (Karakaš, 2013). Serotesting of brucellosis is used routinely for animal and human screening brucellosis in many countries which detects *Brucella* antibodies in serum. Rose Bengal is a rapid, relatively low-cost and effective screening test for brucellosis.

This research studies the brucellosis seroprevalence from 2018 to 2023 in the Travnik region, Central Bosnia and Herzegovina, taking into account gender, age groups, and the seasonal distribution.

## Methods

This present research was carried out in wider area of Travnik municipality, Central Bosnia canton, which has an estimated population of 53,482 inhabitants. Travnik has typical continental to mountain climate thus its most distinguishing geographic feature is his mountain, Vlašić, one of the tallest mountains in the country. Travnik is the administrative center of the Municipality of Travnik, whose area of jurisdiction covers the town of Travnik itself and 89 other rural settlements where population is engaged in typical rural work such as farming and herding.

This study was carried out at the Laboratory for Microbiology, Polyclinic Medical Center, Faculty of Pharmacy and Health, University of Travnik, Bosnia and Herzegovina between 2018 and 2023, with the aim of determining the seroprevalence of human brucellosis and determining risk factors related to the disease. Socio-demographic data (gender, age, and occurrence season) of the patients were obtained from the clinic laboratory database. Data were collected over five years, involving the collection of samples from suspected patients following their consent. Gender is defined as female and male; age groups are identified as

<20 years old, 21–40 years old, 41–60 years old, >61 years old; and frequency of cases expressed in months.

Data were collected from 339 patients referred to Clinic. Rose Bengal Brucellosis Antigen test was initially used to screen brucellosis. Serum samples were screened for antibodies against *Brucella* spp. (*B. melitensis*, *B. abortus* and *B. suis*) using the slide agglutination test obtained from IDEXX Laboratories, Inc, Maine, USA. According to the manufacturer instructions, samples showing even slight presence of agglutination indicate infection. The suspension of *Brucella* antigen is calibrated to give a positive reaction at a dilution 1:45 and a negative reaction at a dilution 1:55. A total of 30  $\mu$ l of each serum sample was placed on a plate and an equal volume of antigen was placed next to the sample to be tested. A sterile plastic stirrer was then used to mix the serum and the antigen thoroughly before slowly rocking the sample for 4 min to observe the agglutination. The result was appreciated by examining the degree of agglutination. Slightly notice able clumping was reported as positive; however, an absence of agglutination was considered as negative. Positive and negative controls (obtained by IDEXX) were introduced for each series of analysis included to confirm the performance of this serological test. Additionally standardized positive patient's samples were used as a control.

Data collected were transferred into a Microsoft Excel 10 worksheet (Microsoft Corporation, Redmond, WA, USA). Expressive and investigative statistics were used to describe the data. Differences between the seroprevalence of brucellosis according to age, sex, and the seasonal incidence rate were assessed. In all analyses, the confidence level was set at 95% and a  $P$  value < 0.05 was considered significant.

## Results

### Rose Bengal test for brucellosis antibodies

A total of 339 sera samples were included in this study, and 79 (23.3%) were found to be positive for brucellosis when tested with Rose Bengal test. The prevalence of seropositive ranged from 18.03 to 35.71 %, which was the highest in 2021, and high prevalence of human brucellosis was statistically significant when compared to the prevalence in the other years ( $p > 0.05$ ) (Fig. 2).

The results of seropositive brucellosis cases in relation to different epidemiological criteria such as gender, age and a season are summarized in (Table 1).

### Association of positive brucellosis according to gender

The prevalence of the infection was  $63.86 \pm 9.40$  of the studied cases in males and  $24.54 \pm 6.35$  in females. This frequency was constant during all the years of survey resulting in statistically significant association ( $p > 0.05$ ) between gender and the presence of brucellosis in the Travnik region of Bosnia and Herzegovina (Figure 1).

### Association of positive brucellosis according to age

Regarding the age, the percentage of positive cases for brucellosis was highest in the age group 41 - 60 years old over the period of this study (50.00–72.73%), followed by the age group 21 - 40 years (14.29–50.00%) and the age group >60 years old (10.00–28.77%) (Table 1 and Figure 2). The lowest rate was found in the age group of younger than 21 years resulting in statistically significant association ( $p > 0.05$ ). There is a strong association

between human brucellosis and age, as shown in Figure 2. Significant differences between the prevalence of infection and age group of between 41 and 60 years among all years were noticed.

#### Association of positive brucellosis according to season

According to this study the highest incidences of human brucellosis were reported in summer, month of June (7.69–50.00%) and October (11.54–42,86%) and the lowest in winter, February (1.27 -5.26%) (Figure 3).

### Discussion

Brucellosis is an endemic zoonotic disease in developing world that causes devastating losses to the livestock industry and livestock holders (Franc et al., 2018). It is both emergent and endemic disease in Bosnia and Herzegovina and responsible for more than 3,000 patients recorded till now in the Federation of Bosnia and Herzegovina (13). Brucellosis is a zoonosis of the world-wide importance, especially in developing countries and countries of Mediterranean (Wareth et al., 2019). Empirically based estimation of human brucellosis incidence is suggesting at least 1.6–2.1 million new cases of human brucellosis are likely to occur every year (Laine et al, 2023). Our study was conducted to determine the seroprevalence of human brucellosis among clinically suspected patients in the Travnik region, Central Bosnia Canton, between 2018 and 2023.

Measured seroprevalence of 23,3% of human brucellosis in the Travnik region is likely to be caused by the lifestyle in rural areas, where traditional farming and close contact with ruminants is still present. Demographic profile of patients with brucellosis in terms of gender, age, and monthly incidence every year, were reported in this study. In this study, around 65,82% of studied cases were males and 34,17% were females. The percentage of positive cases for brucellosis was highest in the age group of between 41 and 60 years old over the period of this study 56.76%. According to this study the highest incidences of human brucellosis were reported in summer, month of June (14,15%) and October (14,49%) and the lowest in winter, February (0,88%)

This results can be compared with a recent study conducted in neighbouring Zenica-Doboj Canton between 2008 and 2018 were 38.8% prevalence was measured, males were predominant within 77.9% cases with most affected age was the 25-49 years group 44.5% cases, respectively (Uzunović et al, 2022). The main epidemiological characteristic of human brucellosis found in our study are in accordance with previously published data from B&H: male predominance over female (Obradović & Velić, 2010; Ahmetagić et al., 2015; Krkić-Dautović et al., 2006), 25-49 age group was the most affected (Obradović & Velić, 2010), patients were mostly from rural areas (Ahmetagić et al., 2015), March-June was mostly the peak period (Uzunović et al, 2022).

High brucellosis prevalence in males is mainly caused by the fact brucellosis is a work-related infection, in rural Bosnian areas man predominantly have direct contact with animals, while farming and taking care of the animals. Similar to our findings, other studies have shown a male predominance as well in the ratio of 2:1 and 3:1 (Rahamathulla, 2019). High prevalence of brucellosis was observed in adults age group. This is very likely because people are coming into contact with infected animals more often when they become adults. The month of June was reported as significant moth of brucellosis appearance witch coincide with March-June

peak period of lambing season which is quite extended in BiH due to climate variations (Šerić-Haračić et al, 2008.), which can be applied to the Travnik area, which has a moderate mountain climate in rural parts of the municipality.

Through specific diagnostic of the cases suspected for human brucellosis, as well as through serological testing, we tried to reveal a level of general exposition to *Brucella* spp.

## Conclusion

Our survey found a high prevalence of human brucellosis among clinical suspected patients in Travnik area strongly indicating that clinical suspicion is a valid criterion, which requires fast laboratory detection and patient treatment. Detecting specific anti-*Brucella* antibodies using agglutination test Rose Bengal is a fast and reliable diagnosing procedure for clinically suspect individuals.

Based on the reported results of this and other available studies concern should be given to a combined human and animal brucellosis control program based on eradication program in ruminants and prioritization of preventive strategy programs thru One Health multidiscipline concept.

Spreading the awareness about brucellosis and risk factors of infection should be developed in community especially rural ones where people have close contact with host animals. Consumption of raw and unpasteurized milk and milk products or undercooked meat should be eradicated.

## Authors Declaration Statements

### Ethics approval and consent to participate

The present study has been approved by the Medical Director of Polyclinic Medical Center, Travnik, Bosnia and Herzegovina. All participants signed questionnaire and they were maintained.

Before starting data collection, ethical approval was obtained from the Medical Director of Polyclinic Medical Center, Travnik, Bosnia and Herzegovina to access the raw data. During the data collection and processing stage, the information was anonymous, and confidentiality of data was assured.

### Declaration of Competing Interest

Declarations of interest: none

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### Authorship Statement

AB contributed to the data collection and conducted processing of results, writing and editing of the manuscript; NP was responsible for the organization and coordination of this idea; ET and JA were responsible for analysis, data collection and processing of results. All authors have approved the final manuscript.

Table 1. Data recorded from patients diagnosed with brucellosis in Travnik, Central Bosnia region between 2014 and 2018

<b>Data Year</b>	<b>2018</b>	<b>2019</b>	<b>2020</b>	<b>2021</b>	<b>2022</b>	<b>2023</b>
<b>Total number of samples tested</b>	125	63	61	28	36	26
<b>Number of seropositive samples</b>	26	19	11	10	7	6
<b>% of brucellosis seropositive</b>	20.80	30.15	18.03	35.71	19.44	23.08
<b>Brucellosis in males (%)</b>	69.23	63.16	63.64	80.00	57.14	50.00
<b>Brucellosis in females (%)</b>	30.77	36.84	36.36	20.00	42.86	50.00
<b>Brucellosis in age groups (%)</b>						
<b>&lt;20 yr.</b>	3.85	5.26	9.09	0.00	0.00	0.00
<b>21–40 yr.</b>	15.38	36.84	0.00	20.00	14.29	50.00
<b>41–60 yr.</b>	53.85	36.84	72.73	70.00	57.14	50.00
<b>&gt;60 yr.</b>	26.92	21.05	18.18	10.00	28.57	0.00
<b>Brucellosis in months (%)</b>						
<b>January</b>	0.00	0.00	18.18	10.00	42.86	0.00
<b>February</b>	0.00	5.26	0.00	0.00	0.00	0.00
<b>March</b>	0.00	5.26	36.36	0.00	0.00	0.00
<b>April</b>	34.62	15.79	9.09	0.00	0.00	16.67
<b>May</b>	30.77	0.00	0.00	10.00	0.00	33.33
<b>June</b>	7.69	10.53	0.00	50.00	0.00	16.67
<b>July</b>	0.00	5.26	0.00	0.00	14.29	33.33
<b>August</b>	0.00	26.32	0.00	20.00	0.00	0.00
<b>September</b>	3.85	5.26	9.09	0.00	0.00	0.00
<b>October</b>	11.54	5.26	27.27	0.00	42.86	0.00
<b>November</b>	11.54	0.00	0.00	0.00	0.00	0.00
<b>December</b>	0.00	21.05	0.00	10.00	0.00	0.00

Figure 1. Seroprevalence of human brucellosis in males and females in Travnik, Central Bosnia region for six years. SD are shown (I) and the linear trend line

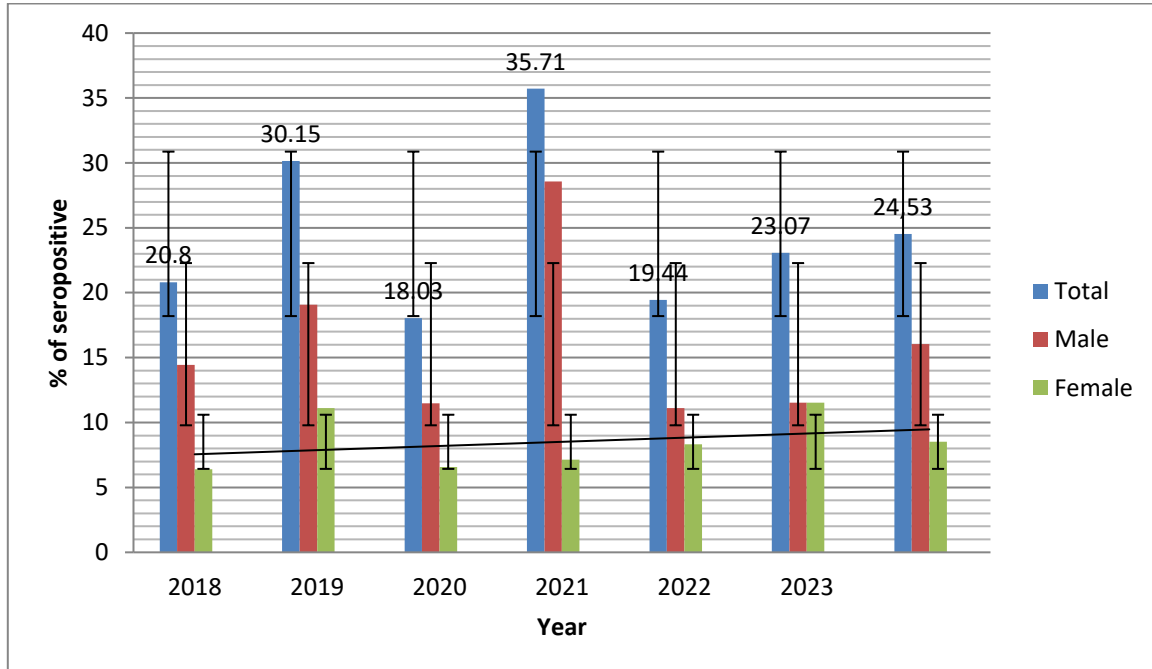


Figure 2. Distribution of positive human brucellosis among different age groups in the Travnik, Central BiH region for 6 years (2018–2023)

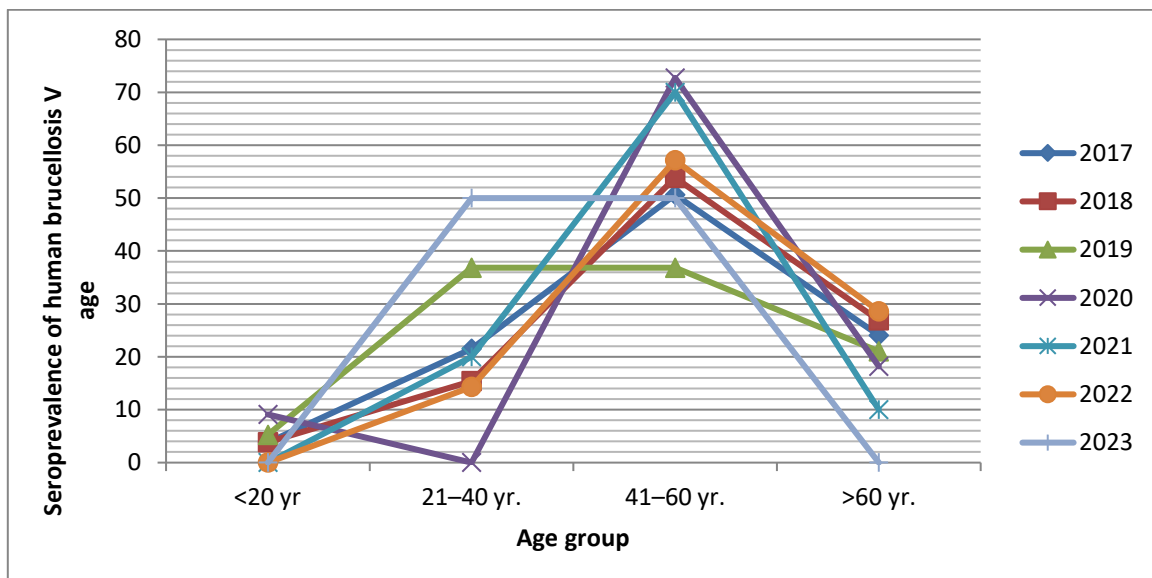
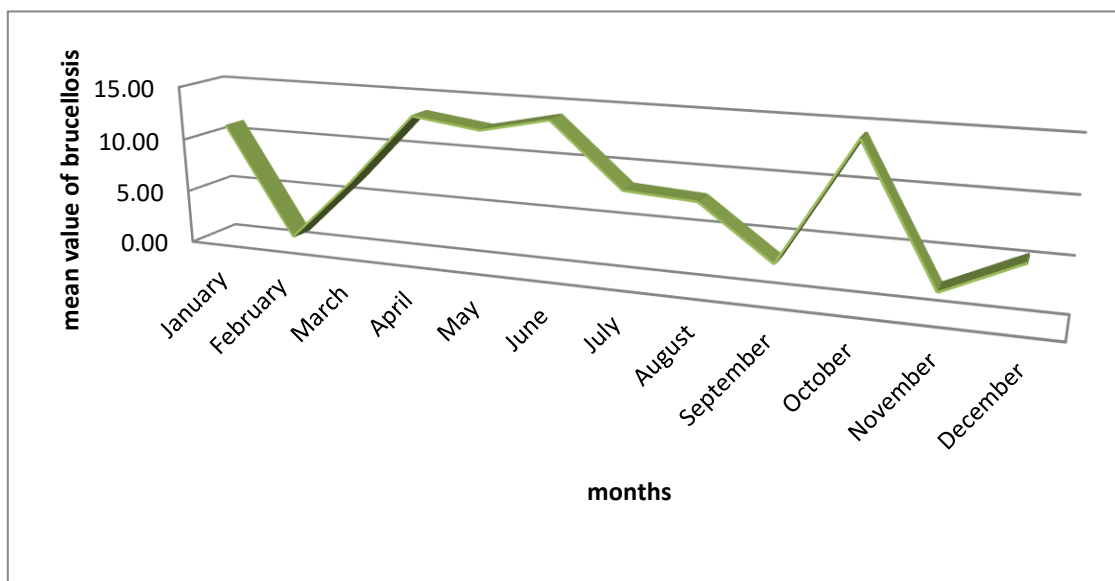


Figure 3. Reported cases of human brucellosis, by month of the year, in Travnik, Central BiH region for 6 years (2018–2023)



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