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A New Perspective to the Brucellosis From East of Turkey; Does the Infections Really Decrease Over the Years?

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Abstract: Brucella infection is a zoonotic disease caused by gram-negative bacteria in the structure of coccobacillus. The most frequently isolated species in our region are B. abortus, transmitted from, cows and B. melitensis, transmitted from sheep. The microorganism infects humans by direct contact with animal tissues or blood, and often via unpasteurized animal products. This study aimed to determine the prevalence of brucellosis in our region, its distribution according to years, seasons, age, and sex, and to observe the progress of brucellosis cases by years. Materials and Methods: The Rose Bengal agglutination test was used for screening. The BrucellaCapt test was applied to patients who were positive for Brucella screening test or who had clinically suspected brucellosis. We considered patients with 1/160 and above titers as positive. Results: The total number of samples that came to the laboratory from 2016 to 2019 was 20. 330, from which 19.595 were Brucella-negative, while 735 were Brucella-positive. The number of Brucella strains grown in the blood culture was determined as 12, and the Public Health laboratory identified all strains as Brucella melitensis. The distribution of patients according to age, sex, season, and branches were examined by the years. Conclusion: In conclusion, the incidence of Brucella cases in our region is a known fact. While it was expected that the cases would tend to decrease in recent years, it has been observed that it has increased slightly due to reasons such as imported livestock, insufficient sanitation conditions, and lack of awareness of the farmers.© 2021 NTMS.

Keywords: Brucella, Prevalance, Distrubution.

1. Introduction

Brucella infection is azoonotic disease caused by Brucella bacteria in the structure of gram-negative coccobacillus (1). From the bacteria in the group, *B. abortus* is transmitted from cows, *B. melitensis* and *B. ovis* from sheep, *B. suis* from pigs, *B. canis* from dogs, and *B. neotomae* from rats. Approximately 300 million of the 1.4 billion sheep in the world are thought to be infected with Brucella. While these bacteria cause abortions by attaching to a carbohydrate substance called erythritol found in the uterus of animals, it is believed that it has no such effect in humans (1-3). Nevertheless, abortions in animals cause severe damage to the economy (4).

Brucella can be transmitted to humans by direct tissue contact with animals or by blood, as well as often by non-pasteurized products (cheese, milk, poorly boiled yogurt, etc.). Brucella dies when exposed to 60 °C for 10-15 minutes. The recommended boiling method is to keep the milk product at the same temperature for about 15 minutes at the boiling temperature. Microorganisms

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can live for 6 weeks in animal-habited barn dust, 10 weeks in water, 30 days in ice cream made from raw milk, 4-5 months in salt-free cream oil made from raw milk in a refrigerator, and 45 days in brined cheese containing 10% salt (5).

The main symptom Brucella produces in humans is joint pain and suddenly rising fever (undulant fever). Chronic brucella can progress to arthritis, hepatitis, encephalitis, endocarditis, and orchitis, causing infertility in men. It has been shown in some studies that chronic cases may cause even depression (6).

Also, Brucella is among the category B bioterrorism agents. It is not seen in developed countries because of the industrial processing of dairy products and attention to the vaccination of animals. As in Turkey, it continues to exist frequently in developing countries such as the Middle East, Asia, Africa, and South America due to the insufficient sanitation conditions and failure to take precautions (7, 8).

This study aimed to determine the Brucella prevalence in our region and to determine the distribution according to years, seasons, age, and sex. It was intended to demonstrate the Brucella infection progress, which is thought to decrease, with the data of our region.

2. Material and Methods

Our study includes the results of patients admitted to our hospital between 2016 and 2019, 20330 patients sample was examined. Ethical clearance was obtained for study. The Rose Bengal test is a screening test for B rucella with a sensitivity of 96-100% and a specificity of 91-100% in acute cases (9). Rose Bengal lam agglutination test (Seromed, Turkey) was used as the screening test. In this test, the Rose Bengal test antigen (*B. abortus* S99 strain) was mixed with patient serum and stirred by hand with rotation movements for 4 minutes to see if there were any signs of agglutination. Samples with agglutination were considered positive, while those without agglutination were accepted as negative. The Brucellacapt test (*B. abortus* S99 strain and *B. melitensis* biotype-3) was applied to the serums of the patients with positive screening tests for *B. abortus, B. melitensis*, and *B. suis*, as well as patients with clinically suspected brucellosis despite a negative Rose Bengal test (10). The specificity and sensitivity of the Brucellacapt test is 98-99% and 94-95%, respectively. The IgG, IgM, IgA, and non-agglutinin, which are produced in brucellosis, reveal IgG and IgA's. The test results were assessed between 1/20-1/5120 titers. Although some sources accept Brucellacapt sample positivity as 1/160, while others suggest 1/320, we classified patients with 1/160 and above as positive (11, 12). If symptoms were present, confirmatory blood was drawn from the relatively low-titered patients. Blood cultures were incubated for 1 week and bone marrow cultures for 21 days using the BD BACTEC 9120 system (Becton Dickinson-Spain, Madrid, Spain). Blood and bone marrow cultures were taken into blood and chocolate agars and kept in aerobic and waxed jars. The culture-positive samples demonstrated gram-negative coccobacillus with gram staining; agglutination was performed with positive patient sera, and the samples were sent to the reference laboratory of the Turkish General Directorate of Public Health.

2.4. Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc, Chicago, IL, USA). Numbers (n), percentage (%), mean, and standard deviation (SD) were given for the descriptive statistics. The independent samples t-test or Mann-Whitney U test were used to compare numerical variables. Pearson Chi-Square or Fisher's exact tests were used to analyzing categorical data. Pearson correlation was used to search for relationships between scale variables. Agreements of the two laboratory tests to identify Brucella positivity were calculated by Cohen's Kappa Coefficient. The level of significance, p, was set at 0.05.

3. Results

The total number of samples coming to the laboratory between 2016-2019 was 20330. While the number of Brucella negative patients was 19.595, the number of Brucella positive patients was 735. The number of Brucella strains grown in the blood cultures was determined as 12, and all isolates were identified as *Brucella melitensis* by the Turkish General Directorate of Public Health laboratory.

According to Cohen's Kappa test the Rose Bengal and Brucella tube agglutination test was almost equally accurate. Kappa=0.987, p=0.000. According to the Chi-Square test there is no statistically significant difference between Brucella positivity according to the seasons. x^2:2.834, p=0.116. There is a statistically significant difference between the units and Brucella positivity. Infectious diseases, Internal medicine, Pediatrics are most common units. x^2:44,652, p=0.007. There is no statistically significant difference between gender and brucella positivity. x^2:0,797, p=0.213.

Table 1 shows the distributions of sex, mean age, Rose-Bengal positivity, and the number of positive cases as to the 1/160 threshold. The number of positive patients by years is given in Figure 1.
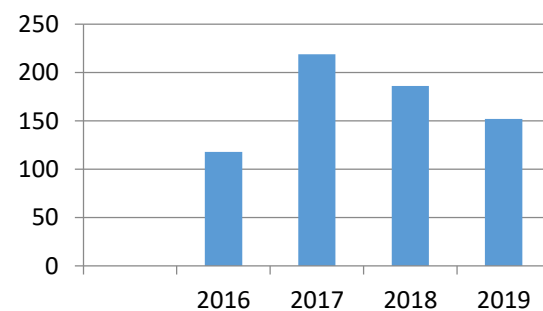

Figure 1: Number of positive patients through the years 2016-2019.

Figure 2 shows the positive patient distribution and percentage slices according to the seasons, while Table 2 demonstrates the number of positive cases according to the departments.
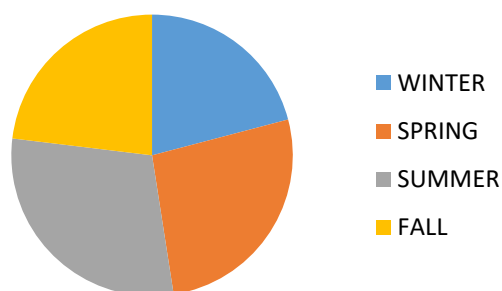

Figure 2: Distribution of positive patients by seasons (2016-2019).

According to Mann Whitney U test there is a statistically significant difference between age and Brucella positivity average age is between 37.42±19.62. z:-3,115, p=0.002. There is a statistically significant difference between Brucella positivity by years in there is a peak in 2017. z:-2,147, p=0.032.

4. Discussion

In our study, serum MPO levels were significantly lower in MS patients than in the healthy control group. There was no significant correlation between serum MPO levels and age, disease duration, ARR, EDSS scores in MS patients. Considering disease progression, there was no significant difference between RRMS patients and SPMS patients in terms of MPO levels.

MS is a neuroinflammatory autoimmune disease. In MS, inflammation, demyelination, and axonal damage of both the brain and spinal cord impair physical and cognitive abilities (16)**.** In MS, some pathophysiological processes, including chronic inflammation of the CNS, oxidative stress, blood-brain barrier disruption, demyelination, axonal and neuronal damage, and remyelination, are observed (17).

When the Public Health data were examined, it was evident that, although at a low level, there was an increase in the number of Brucella cases since 2015 (Figure 4). Additionally, our region is also within the area marked dark, where Brucella cases are prevalent (Figure 3) (13, 14). Brucella is a serious infection, especially in less developed or developing countries such as our area, where animal farming is highly prevalent but not industrialized. The lack of vaccination, disadvantaged animal care conditions, as well as the presence of Brucella bacteria, appears as the main reasons for not being able to cope with the disease. In this context, the general aspect of the Brucella cases in recent years in our study area is presented. According to the 2017 data of the Turkish General Directorate of Public Health, the incidence of Brucellosis cases in our city was detected as 82.4%. The 2018 and 2019 data are not announced yet (13). The outcome of our study is parallel to Turkey's data.

After 2016, Brucella analysis and detections were clearly increased in the area. Although verbal communications with local animal owners indicated that the situation might be caused by the altering of the domestic animal race and the absence of vaccination, no supporting scientific data could be found. The surveys on the people who engage in husbandry showed that 66% of them had knowledge about Brucella, whereas 84.5% used unpasteurized dairy (15). As the surveys indicated, the fundamental reason for the high prevalence is that even though the disease is known, there isn't adequate prevention.

In our research, a significant difference was found in the patients submitted from especially infectious diseases, internal medicine, and pediatrics policlinics and clinics compared to other services (p=0.007, Chi-Square) (16). Again, as it is stated in our research, people admitted to the hospital with any symptom might be diagnosed with Brucella. Thus, it might be indicated to execute a routine screening for brucella in our area.

A significant difference was observed when data were analyzed per the years (p=0.032, Mann Whitney U). Brucella cases increase gradually throughout the years. Particularly after 2015, an apparent rise was also observed in our country (17). According to the data for 2015 from the Turkish General Directorate of Public Health, 4173 positive patients were diagnosed, whereas, in 2016, this number increased to 5148 (18). After 2016, these numbers show a tendency to grow more. Considering that 2019 is not yet passed, it should be recognized that the numbers in our research will continue increasing further.

Again between Brucella positivity and age, there was a substantial difference. The mean age of all patients was 37.42±19.62, which indicates that middle-aged people engage in husbandry. Engagement and contact with animals and the consumption of dairy are less common among the young generations. Despite the frequency of positive patients in pediatric clinics, when the entire sample is considered, there is no correlation between age and the number of Brucella patients; however, there is an increase in the prevalence with rising age. Also, a thesis study conducted in Erzincan shows that there is no statistical difference in the infection according to the age groups. However, the prevalence is higher in the group of people who are older than 45 (19). When seasonal factors were considered, besides the fact that more Brucella positive patients were diagnosed during the summer, a significant difference was not found. In other studies conducted in our country, parallel to ours, Gültepe and his friends discovered no distinct difference between gender and age groups in their research in Van (20). When positivity ratios are analyzed per the monthly distributions, they appear to be increasing as from March, reaching the highest level in August, and as from October, they are observed to decrease to the prior ratios.

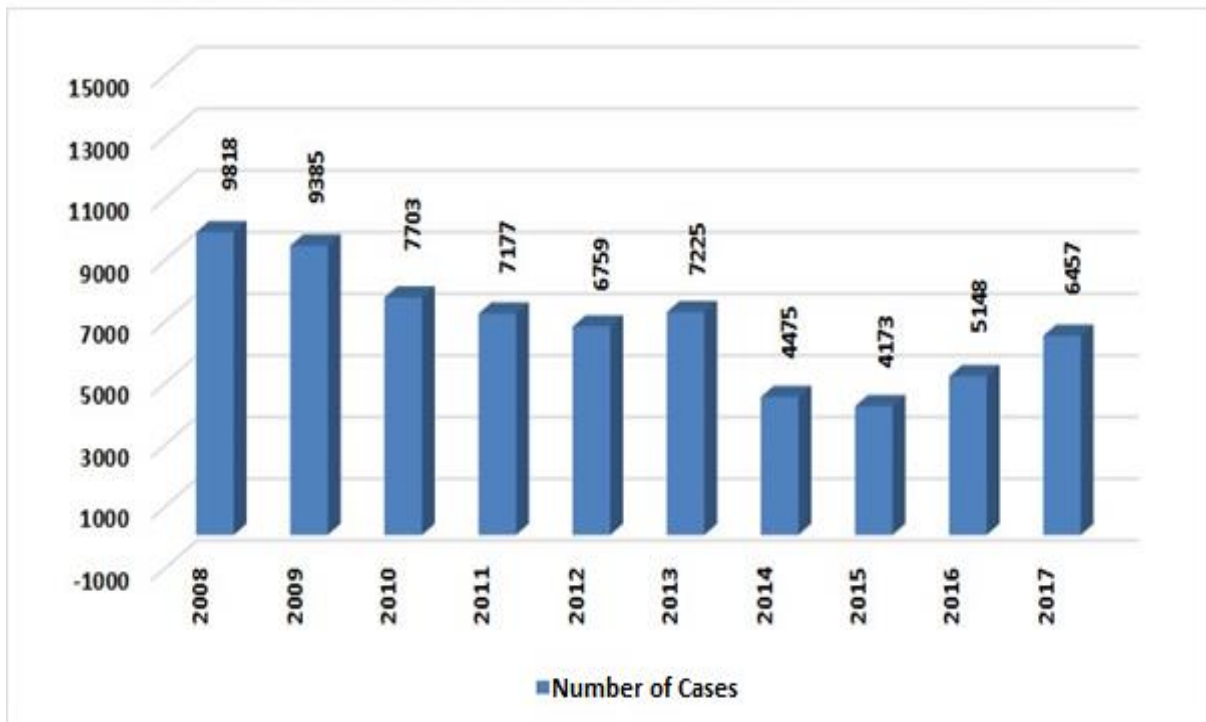**Figure 3:** Brucella distrubution (map by 2017 Public Health Data) (13).

Figure 4: Distribution of Brucella-positive cases by year (Turkish Public Health Data) (14).

Table 1: Demographic Data of the Positive Patients in Table.

Sex	Female		Male
n:735	n:299 (%40.7)		n:436 (%59.3)
Age average		37.42±19.62	
Rose	**Negative**		**Positive**
n:20330	n:19 595		n:735
1/160 limit	**Under 1/160**		**Over 1/160**
	n: 129 (17.6%)		n: 606 (82.4%)

This outcome brings the rise of cheese consumption in summer months and thecommonly consumed herby cheese to mind, which is made in Van without boiling the milk. Whereas in our area, the reason for not having a seasonal difference might be related to the consumption of foods that were not cooked well, bacteria which stay alive almost 4 months in butter or 2 months in brine cheese, and the time for fresh animal diary being roughly equal throughout the year. Besides, the different milking periods for sheep and cows, which are the hosts for Brucella abortus and Brucella melitensis, may have contributed to the lack of seasonal difference. The milking period for cattle is more extended than that for the sheep. Additionally, a few months of milking time difference exists between warm cities like Iğdır and colder districts like Selim and Sarıkamış.

In our research, no difference was found between gender and Brucella positivity. This outcome was attributed to the almost equal contributions of the middle-aged women and men in milking and nurturing processes; they have nearly the same contact with animals.

Table 2.: Distribution of positive patients by services (in decreasing order).

	n/%
Infectious diseases	267/36
Internal medicine	264/36
Pediatrics	131/17
Orthopedics	15/2
Family Medicine	9/1
Urology	8/1
Physical Medicine and Rehabilitation	8/1
General Surgery	7/1
Neurosurgery	6/1
Neurology	5/1
Biochemistry	5/1
Emergency medicine	4/0.5
ENT	2/0.3
Anesthesia	2/0.3
Cardiology	1/0.1
Dermatology	1/0.1

5. Conclusions

Consequently, the frequency of Brucella cases in our region is a well-known fact. Despite the expected decrease in the cases during the recent years, with the lack of sanitation and the inadequate awareness for the people working in husbandry and animal import business, even if small, an increase was observed. We consider that raising the awareness primarily among the people working in animal farming, educating the consumers to not consume the dairies unless they are sure of its pasteurization, and generalized application of dairy food processing rules are some of the fundamental preventive measures for the decrease in the prevalence of this disease.

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