

Four Years Brucellosis Prevalence Change of Kars/Turkey

Cigdem Eda Balkan Bozlak* and Mukremin Ozkan Arslan

*Kafkas University Medical Faculty, Medical Microbiology Department, Kars/Turkey.

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INTRODUCTION

Brucellosis is a zoonotic infection caused by microorganisms belonging to *Brucella*, a genus of gram-negative coccobacilli that behave as facultative intracellular pathogens of cows, sheep, ruminants, swine and other animals [1]. Brucellosis is a threat to public health especially in the eastern and southeastern of the Turkey, hence identifying the optimal way of preventing disease spread is important [2].

Brucellosis almost always causes fever, which may be associated with night sweats and weight loss [3-6]. Common treatment includes doxycycline and aminoglycosides, although complicated cases, such as brucellosis combined with endocarditis, neurobrucellosis, may require stronger medications administered for a longer duration and challenging treatment process [7-9]. Other than direct contact with infected animals, the disease is also foodborne; food habits are very difficult to change, in eastern of Turkey also cheese made with unboiled milk, which will assure many future cases of foodborne disease [10,11]. *Brucella melitensis*, *Brucella abortus* and *Brucella suis* are the three species generally associated with human disease, in our region *B. melitensis* common cause of the brucellosis. Rare cases of human infection with *Brucella canis* have been reported, while human cases of *Brucella ovis* and *Brucella neotomae* infection have not been reported. Little is known about the capacity of the new *Brucella* species to cause infection [10]. One possible laboratory acquired infection with a marine mammal isolate has been reported [12] and one specific sequence type (ST27) has been associated with three human infections in Peru and New Zealand [10,12]. Interestingly, the patients had had no contact with marine mammals; however contact with raw fish was a common feature of the three cases of patients. *B. melitensis* bv2 found in catfish in Egypt, suggesting that fish may constitute a novel source of infection [10,13]. There is some other types of brucella; *Brucella canis*, *Brucella suis*, *Brucella ovis*, *Brucella ceti*, *Brucella pinnipedialis*, *Brucella cetaceae*, *Brucella neotomae* [14]. In endemic areas like our region *Brucella melitensis* is the most common type [15].

The aim of this study was to investigate the seroprevalence of brucellosis titers in 5 years patients with using the rose Bengal agglutination test and coombs brucella test. Coombs brucella test is a microagglutination test as sandwich ELISA model. The sensitivity and specificity are found to be 94-95% and 98-99%. The agglutinin detect IgG, IgM and IgA. Test is effective in detecting the agglutinating and non-agglutinating immunoglobulins in acute and chronic brucellosis caused by *B. abortus*, *B. melitensis* and *B. suis*.

MATERIALS AND METHOD

A total of 761 brucellosis cases admitted to our clinic, the Department of Infectious Diseases and Clinical Microbiology of Kafkas University Hospital, over a 4 year period from 2014 to 2017, were included in the study. A retrospective study was undertaken and patient files were investigated for their gender, age, rose Bengal positivity and brucella coombs titers, as well as clinical outcomes. Firstly rose Bengal plate test, the procedure described by Foster et al. [15] was followed with little modification by Unver et al. [16]. *Brucella* Rose Bengal Plate Test antigen was prepared from *Brucella abortus* S99 strain, standardized with *Brucella* antiserum and stained with Rose-Bengal. The antigen was left at room temperature for 15 min before use and shaken well. On a clean plate, 0.05 ml milk serum was dropped. Then, 0.05 ml Rose Bengal Lam Test antigen was added to this. The antigen and milk serum were mixed and spread over an area with diameter 1.5 cm. The plate was left in the air for 2 min by turning by hand. Formation of clusters of coarse particles was assessed as positive while homogeneous

Corresponding author: Cigdem Eda Balkan Bozlak, Kafkas University Medical Faculty, Medical Microbiology Department, Kars/Turkey, E-mail: cigdemedabalkan@gmail.com

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appearance was assessed as negative. Reactions were categorized as 0, +, ++, +++, according to [17] where: 0=means no agglutination, + = barely perceptible agglutination (using magnifying glass), ++ = fine agglutination, some clearing and +++ = clumping, definite clearing. Those samples identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were regarded as positive. This test was performed following the procedure described by Unver et al. [16] mixing 1.30 µL serum mixed with equal volume of antigen. The plates were shaken for 4 min and any agglutination that appeared within this time was recorded as a positive reaction. It can detect *B. abortus*, *B. melitensis* and *Brucella suis*. In chronic infections there is 30% false negatives can be observed.

After the agglutination Brucella coombs test were performed. The procedure of test: two wells are used for each serum and well for positive control and well 1 for negative control are used for each run.

- 5 µl of serum is diluted with 195 µl saline solution in a test tube (1:40 titer). Mix well while diluting the serum sample.
- 2 wells for each serum sample and 2 wells for positive-negative control are emplaced to the microplate frame and 50 µl MCBT diluent is added to the each well.
- The diluted serum sample is added to first well, which 50 µl of (1:80 titer). It is mixed well by pipetting 3-4 times than 50 µl of 1:80 diluted sample is added to the other well (1:160 titer), 50 µl of diluted sample is discarded from last well.
- 5 µl of positive and negative control solution is added to the control wells.
- 50 µl of antigen is added to all wells (First well 1:160 titer, second well 1:320 titer).
- The plate is incubated in the special locked box with a piece of wet cotton for 24 h.
- The results are evaluated after the incubation period. Solutions are in order: 1/40, 1/80, 1/320, 1/640, 1/1280, 1/2560, 1/5120.

RESULTS

After Rose Bengal agglutination test positivity or chronic patients with symptoms these patients blood samples taken to the coombs brucella test. Results are like that as shown in the **Table 1**. Patients under the 1/160 we want second serum

sample after 20 days period and if the titers is high, treatment started in clinics. In **Table 2**; there are 761 patients when we search the ages, 419 of all patients are between 18-35 years old age, 115 patients 0-18 years old and 227 patients are more than 35 years old in four years. And 589 of the 761 patients are male, 172 of all patients are female.

Table 1. This table shows the number of patients in four years with titers.

Years/Titer	2014	2015	2016	2017
1/40	105*	22*	3*	-
1/80	80*	14*	9*	7*
1/160	84*	3*	26*	28*
1/320	68*	4*	22*	32*
1/640	67*	7*	29*	24*
1/1280	7*	4*	10*	24*
1/2560	-	-	22*	32*
1/5120	-	-	-	28*

*: number of patients according to titers

Table 2. This table shows the number of patients, age and gender.

Age/Gender	0-18	18-35	35- ...
Male	78*	318*	193*
Famale	37*	101*	34*

*: number of patients with these age and gender

Figure 1 shows that four year brucellosis change of our hospital. It seems in 2014 numbers are very high when we look the other 3 years period numbers are decrease visibly.

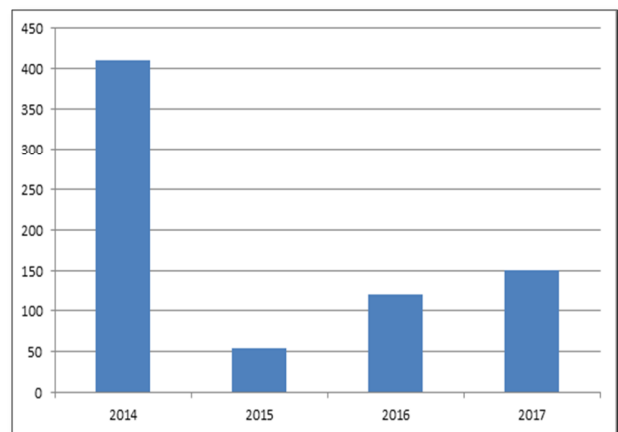


Figure 1. Graphics of the brucella prevalence change in four year.

DISCUSSION AND CONCLUSION

Brucellosis is the most frequent zoonotic infectious disease in the world, affecting more than 500 000 people every year [16]. In the eastern part of Turkey, seropositivity has been reported to be as high as 27.2% [17]. In endemic countries, brucellosis is more prevalent age group is 15-35 [18,19]. In our study most of cases are between 18-35 years old age too but more than 35 years old patient's rates are high to undeniable. In our geographic region Kafkas basin the most common route of spread is the consumption of regional milk products produced from raw milk like Van lake basin [20]. In a study from Spain which is epidemic region for brucellosis, the sensitivity value for SAT was found as 65.8%, for Coombs test as 91.5% and for BrucellaCapt as 95.1% [16]. In another study from same region the sensitivity and specificity values for Rose Bengal, SAT, Coombs Test and Brucellacapt was found over 90% whereas IgM and IgG ELISAs have the lowest sensitivity (60% and 84%, respectively), gold standart is culture of bacteria of course [21,22]. In our study Brucella Rose Bengal test is used first and then BrucellaCapt used for titers 761 patient found positive in four year period. Most of the patients coming from our region because of the location of our city. In the similar study in Erzurum they found a significant difference between the two regions among the Hınıs and Oltu individuals aged 10-20 and 20-60 provinces which people use boiling dairy products and no boiling products [11].

The high individual prevalence of brucella seropositivity in people in the intensive dairy farm owners around Kars agree with the characteristics of these husbandry systems, the levels of brucella infections tend to be relatively high on intensive farms, whether these have indigenous cattle or introduced sheep. The first stage in controlling zoonotic diseases is periodic observation of the prevalence and distribution of the diseases in animals, making timely intervention against epidemics, vaccination, and prevention of the disease in animals possible for these zoonotic diseases.

In multiple analyses, the relations disappeared regarding to close contact with animals and occupation while the other relations remained. Data of 761 patients were completed and analyzed in the study. Of them, 77% were males and 22% were females. Most of them are farmers and animal owners.

Because no available or convenient data are published in the Kars brucellosis prevalence, our data cannot be compared easily with previous studies. In 2014 there are 411 patients above the 1/40 titer, 2015; 54 patient, 2016; 121 patient, 2017; 151 patient above the 1/40 titer. The numbers are decrease devastatingly after 2014 because of the decrease of the number of animals and animal health policies we think.

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