

Evaluation of the epidemiological situation *B.canis* infections in human and *B.canis* seroprevalence in Diyarbakir, Turkey

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To cite this article:

Fulya BAYINDIR BİLMAN, Sevil ERDENLİĞ GÜRBİLEK, Mine TURHANOĞLU. Evaluation of the Epidemiological Situation *B.canis* Infections in Human and *B.canis* Seroprevalence in Diyarbakir, Turkey. *Science Journal of Public Health*.

Vol. 2, No. 2, 2014, pp. 87-91. doi: 10.11648/j.sjph.20140202.17

Abstract: *Brucella canis*, which is an infectious agent of dogs, rarely causes disease in human, and displays asymptomatic or subclinical course. Carnivores are natural host for *B.canis* infection. Considering the limited number of studies investigating the seroprevalence of *B.canis* in the population in Turkey, a serologic study was planned to investigate of *B.canis* infection. For this reason, serum samples from the patients who were admitted to Diyarbakir Training and Research Hospital with various reasons other than complaints of an infectious disease were screened using *B.canis* antigen prepared in Microbiology Laboratory of Harran University, Faculty of Veterinary Medicine to identify the prevalence of this infection in the Southeastern region of Turkey. The serological tests for brucellosis caused by smooth species, (*B.abortus*, *B.suis* and *B.melitensis* etc.) are based on the reaction of antibodies against smooth-lipopolysaccharide (S-LPS) on the bacterial cell wall. But *B.canis* has a rough-lipopolysaccharide (R-LPS) in its cell wall. Therefore detection of antibodies against R-LPS requires use of specific antigen in serological diagnosis of *Brucella* infection caused by rough *Brucella* strains. Unfortunately, a standardized slide agglutination test (SAT) antigen that would serologically detect *B.canis* and other rough *Brucella* strains is not commercially available. The present study aimed to investigate seropositivity rate in the patients via SAT using *B.canis* antigen prepared in laboratory. The study comprised 2100 serum samples obtained from patients (range of age: 16-75 years; 1340 females and 760 males), who presented to various polyclinics of Diyarbakir Training and Research Hospital between 01 April 2013 and 31 June 2013. Serum samples were first examined serologically using Standard Rose Bengal test antigen (Refik Saydam Hygiene Institute, Turkey) for the infection with smooth *Brucella* species, and then negative serum samples were included in the study. Of the 2100 serum samples screened for *B.canis* using standard slide agglutination test, 33 (1.57%) gave positive result. Fourteen of these 33 patients were female and 17 were male. Seven (21%) of these subjects reported that they had dog in a period of their lives. Their personal history revealed no autoimmune, metabolic or immunosuppressive disease in the past. It is concluded that, *B.canis* should be considered in the case of fever and infection of unknown origin, particularly in those with the history of contact with dogs. It was also concluded that the development of standardized rapid screening tests is needed for routine serologic diagnosis of brucellosis caused by rough *Brucella* strains.

Keywords: *Brucella canis*, Seroprevalence, Slide Agglutination Test, Guillain-Barré Syndrome

1. Introduction

Brucella canis, which causes infections and abortion in dogs and is transferred to human by contact with secretion and extraction of aborted dogs and usually causes asymptomatic mild infection, is a Gram negative, motionless, aerobic, intracellular coccobacillus. *B.canis*,

first identified in 1966. [1] Dogs and wild Canidae are the only animal species that serve as reservoirs of *B.canis* under natural conditions. Information on the epidemiology, clinical signs, diagnosis, treatment, and prevention of canine brucellosis is readily available for animals. [2]

But, it is still unknown that the true public health significance of human *B.canis* infections.

Although the low numbers of known human cases

B.canis infection, it seems likely that a lack of clinical suspicion of the infection, its nonspecific clinical presentation, the nonavailability of approved serologic tests, and the organism's fastidiousness in culture all result in the underdiagnosis, and subsequently the underreporting, of this infection. Of particular interest is whether a significant proportion of "culture negative" conditions such as endocarditis, osteomyelitis, and septic arthritis are actually caused by *B.canis*. *Brucella* infection is already recognized as one of the causes of culture negative endocarditis and septic arthritis. [3]

Bacteremia can exist for about 2-5 years in infected dogs, and such a long time increases the risk of contamination. [4]

Studies conducted in various regions of the world reported that *B.canis* has been isolated from risky subjects such as those contacted with dogs and laboratory staff. [5-7] It may either subclinical or it is known that the agent has been isolated from severe infections such as endocarditis and pericarditis. [8, 9] Mortality is very high in such infections.

Based on the fact that there is limited number of studies about the prevalence of *B.canis* in the population, this study aimed to determine the prevalence of this infection in the Southeastern Anatolia region of Turkey.

2. Methods

Serum samples of 2100 patients aged between 16 and 75 years, who admitted to the policlinics of Diyarbakır Training and Research Hospital for any complaint other than infectious disease between 01 April 2013 and 31 June 2013, were analyzed by slide agglutination method using *B.canis* antigen.

First the serum samples were serologically evaluated for infection due to smooth *Brucella* species using Standard Rose Bengal test antigen (Refik Saydam Hygiene Institute, Turkey), and then negative serums were included in the study.

3. Preparation of *B.canis* SAT Antigen

B.canis (RM 6/66) reference strain for preparing SAT antigen was obtained from Pendik Veterinary Control Institute, Brucellosis National Reference Laboratory. The method defined by Alton et al. was used to prepare the antigen. [10] The organism was mixed in the *Brucella* broth agar at 37°C and at 200-600 rpm and incubated for 96 hours. At the end of this period, the culture was collected and inactivated at 80°C for an hour. Inactivated cultures were then centrifuged. The pellet, which was formed after centrifugation procedure, was washed with phosphate buffered saline (PBS, pH 7.4) solution for three times. Thereafter, cell suspensions were stained by adding 5 ml of 2% Rose Bengal dye into each 100 ml of cell suspension. Stained cell suspension was stirred and kept at 4°C for one night and then the concentration of stained cell suspension was adjusted to 6% using tris maleate buffer (TMB, pH

9.0). Prepared antigen was tested in terms of sensitivity and specificity using negative and positive serum panels, which were obtained from OIE International Reference Laboratory.

4. Application and Evaluation of Slide Agglutination Test

All serum samples included in the study were analyzed by SAT without diluting. For this purpose, one drop (0.05 ml) of serum sample was put onto the surface and mixed with the same amount of *B.canis* antigen. The mixture was gently shaken for three minutes and then analyzed in terms of agglutination. The results were considered as either negative or complete agglutination. Each time, positive and negative control serums were included in the test.

5. Results

Serum samples were first examined serologically using Standard Rose Bengal test antigen (Refik Saydam Hygiene Institute, Turkey) for the infection with smooth *Brucella* species, and then negative serum samples were included in the study.

Of the 2100 serum samples were collected from people who were admitted to Diyarbakır Training and Research Hospital with various reasons other than complaints of an infectious disease and then serum samples screened for *B.canis* using standard slide agglutination test.

At the end of the study, complete agglutination was detected in 33 (1.57%) of 2100 serum samples. Of these 33 subjects, 14 were female and 17 were male with 7 (21%) reported having a dog in any time of their lives.

Personal histories of these subjects revealed no autoimmune, metabolic or immunosuppressive disease.

Results of the study were found consistent with the data from similar studies performed in different regions in Turkey. [11, 12]

Serum samples are still being kept at -80°C to be analyzed again using I-ELISA (Indirect Enzyme-Linked Immuno Sorbent Assay), which is planned to be prepared later.

6. Discussion

There is little reliable information on the prevalence and severity of *B.canis* infection in humans. Because of this, the optimal public health response for a recognized human exposure to an infected dog is unknown. Nevertheless, health staff do receive reports of infected dogs from veterinary diagnostic laboratories and should have a response plan. [13]

Because of the lack of reliable data on the severity and incidence of human *B.canis* infections, and incomplete surveillance data. However, there is no doubt that *B.canis* is pathogenic for humans and can, on occasion, cause

significant illness. So, it seems most prudent to recommend some form of public health follow up on human exposures to *B.canis* until the virulence and the epidemiology of this disease is further defined. [3]

Serological diagnostic methods for *B.canis*, which can cause both subclinical and severe infections in human, are not routinely used in hospitals since they have not been standardized yet.

The diagnosis of the disease is based on mostly serology since bacterial isolation is time consuming and requires to have BSL3 facilities. The infection in humans was reported serologically in Turkey although there are very few. The most widely used serological tests are slide agglutination test, tube agglutination test with and without 2-mercaptoethanol (2ME-SAT and SAT; 2ME-TAT and TAT, respectively) and agar gel immunodiffusion test (AGID). SAT is very sensitive, practical and easily interpreted widely used screening test. [14]

Production of rough brucellae antigen requires a meticulous work because *B. canis* shows great tendency to become sticky rope formation after long incubation period in relatively acidic pH. The sticky formations are largely prevented by using alkaline resuspending buffers [15, 16]

It is necessary to highlight the importance what is known about *B.canis* from the public health perspective, point out gaps in knowledge, and develop recommendations for managing human exposures to this bacterium.

Recently, limited number of case reports are trying to attract attention to the fact that dog brucellosis can cause severe clinical pictures in human in the presence of specific diseases, such as metabolic diseases and immune deficiency syndrome. [17] Besides the fact that exact incidence in human is indefinite, it is believed that contact with dogs substantially enhances the risk in the event of immune deficiency. [18] It is thought that exacerbation of an underlying disease in target organs might be associated with the appearance of infection. Interestingly, in different studies in the last decade, *B.canis* infection was detected in the patients, in whom the etiology of fever has been investigated on the basis of Gaucher disease, a lysosomal storage disease, and the general status was improved in such patients along with the positive response to antibiotherapy. These results raised the thought that there might be a relation between Gaucher disease and brucellosis, which share the clinical and physical symptoms. [19] Likewise, various publications report concurrent Brucellosis in the patients with Guillain-Barré Syndrome. [20-23]

Diagnosis of brucellosis is made by isolation of the agent, serological tests and molecular methods. Tests used for serological diagnosis include rapid slide agglutination test (SAT) prepared for *B.canis*, micro agglutination test (MAT), tube agglutination test (TAT), 2-mercaptoethanol tube agglutination test (2ME-TAT), agar gel immunodiffusion (AGID) test and ELISA test. [24, 25] In human, antibodies against *B.canis* do not react with *B.abortus* S99 strain, which carries smooth-LPS. Thus, it should be considered

that *B.canis* might be the agent in the patients that have brucellosis-like symptoms with negative reaction in Rose Bengal test. Analyzing serum samples of such patients by SAT prepared with the strains including R-LPS may be important in terms of not missing out *B.canis* brucellosis for the diagnosis in human. [26]

Whilst close results are observed in seroprevalence studies, the highest result was from Mexico with 13%. [27] Seropositivity was found to be 0.3% in Germany, 0.4% in the military population of the United States of America and 0.6% in Florida people. [28-30]

The first study in Turkey about *B.canis* infection in human was conducted by Diker et al. [31] and seroprevalence of the disease was reported to be 1.6% in the suspected brucellosis patients in Bursa province. Köksal et al. [32] conducted a study in the patients having brucellosis-like symptoms in Adana and reported the prevalence of infection to be 8.3%.

In 2011, Sayan et al. [11] detected positive *B.canis* antibody in the serum samples of 1746 patients with clinical symptoms of brucellosis but without negative Rose Bengal test from 6 different regions of Turkey. Data of this study report the rates of *B.canis* positivity 8.9%, 3.8%, and 3.7% for rapid slide agglutination test, microagglutination test and 2-mercaptoethanol rapid slide agglutination test respectively. The sensitivity, specificity, positive predictive value and negative predictive value of rapid slide agglutination test were in turn 100%, 94.6%, 42% and 100%. In another study, Sayan et al. [12] reported the seropositivity with SAT to be 1.6% (31/1930) in healthy donors in Kocaeli.

These data are consistent with the results of present study, which evaluated the serum samples of various patients without infectious symptoms.

In another study which determining the seropositivity rate of infection *B.canis* seroprevalance of the occupational risk group, *B.canis* and S-typed *Brucellae* antibodies were found %9.2 and %0, respectively. Seventy-six serum samples were investigated by Rapid Slide Agglutination Test (RSAT) and Modified Plate Agglutination Test (MPAT) for R-typed agent and Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) for S-typed agents. In this report, serum samples were collected from people working in dog shelters and veterinary medicine and thus, seropositivity rate was higher than the general population. [33]

Vaccination procedure has recently become a current issue, and rBLSOmp31-IFA was reported by Clausse et al. [34] as the first recombinant vaccine with successful outcomes in mice.

In conclusion, it is thought that extensively performing SAT for *B.canis* and including in the routine serological analyses of *B.canis*. Particularly, on the basis of metabolic disease and immune system-related diseases in the patients with brucellosis-like clinical symptoms would provide healthier evaluation of the disease in human.

However, larger scale, multicenter studies with different

patient and risk groups should be conducted to further evaluate the epidemiology of *B.canis* infections in Turkey.

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