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Short Paper

A serological survey on *Brucella canis* in companion dogs in Ahvaz

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Summary

Canine brucellosis is a zoonotic infectious disease caused by *Brucella canis*. This bacterium can be transmitted to humans as well as other dogs. It is a significant cause of reproductive failure, predominantly in kennels. The aim of this study was to determine the seroprevalence of *Brucella canis* infection in companion dogs referred to the Veterinary Hospital of Shahid Chamran University of Ahvaz in the southwest of Iran. A total of 102 blood samples were obtained from dogs between 2006 and 2008. Sera were examined by Immunochromatography assay. The studied dogs were divided into two age groups (1-5 years and >5 years) and based on clinical signs (related signs to brucellosis such as scrotum dermatitis, diskospondylitis, lymphadenitis, abortion and infertility) into two groups also. Prevalence to *Brucella canis* antibodies in these dogs was 4.90% (5 of 102). The infection had more prevalence in dogs above 5 years (9.3%; 4 of 43) in comparison with dogs less than 5 years (1.69%; 1 of 59), but the difference between the two age groups was not statistically significant (P>0.05). There was no significant difference between the different sexes either (P>0.05). Nevertheless, the difference for related signs to brucellosis was significant between the groups (P=0.018). Three out of 14 cases (21.4%) which had clinical signs and two out of 88 cases (2.3%) which had no clinical signs were seropositive. This study showed that antibody against *Brucella canis* is present among the companion dog population of the Ahvaz area and preventive measures should be taken to control pathogenic bacteria.

Key words: *Brucella canis*, Companion dogs, Prevalence, Serology, Ahvaz

Introduction

Canine brucellosis is caused by *Brucella canis* (*B. canis*), a rough, small, gram-negative, intracellular bacterium. *Brucella canis* was first recognized in 1966 as a cause of abortion in females and testicular atrophy, epididymitis and infertility in males and generalized lymphadenitis in both sexes (Tilley and Smith, 2000; Hollett, 2006).

This bacterium is also a cause of human brucellosis. It is believed that dogs and other canine species are the only true hosts (Greene and Carmichael, 2006). Natural infections occur most commonly after ingestion of contaminated placental materials or aborted fetuses, vaginal discharges from infected bitches that are in heat or who abort, and during breeding. Following an abortion, organisms may be shed for several weeks or, intermittently, for months. Males may also shed organisms in urine. Several researchers have reported rates of seroprevalence ranging between 2 and 30% in dogs in various countries (Greene and Carmichael, 2006).

Definitive diagnosis of *B. canis* infection in dogs depends on both bacteriological examination and serological methods. Following the development of serological

methods, the diagnosis of infection has become available in routine diagnostic laboratories. The exact diagnosis of the disease requires isolation of the causative agent. Serological monitoring or blood cultures are required before a dog can be declared negative (Greene and Carmichael, 2006).

Rapid diagnosis of brucellosis is especially important in order to isolate infected dogs and prevent secondary infections of susceptible animals. Rapid slide agglutination test (RSAT), 2-mercaptoethanol (2-ME), microplate agglutination test (MAT), Rosebengal, ELISA and PCR are commonly used serological methods in the diagnosis of brucellosis (Hinić *et al*., 2008; Kimura *et al*., 2008). Though these tests are more sensitive, specific and more reproducible, they can be carried out only in specialized laboratories.

Immunochromatography assay is the most common rapid field diagnostic method used in clinical practice because the test procedure is simple and rapid, and can be performed by veterinarians. The result of the evaluation showed an overall relative sensitivity and specifity of 95.8 and 99.7%, respectively (Boebel *et al*., 1979; Esfandiari and Klingeborn, 2000). The purpose of this study was to determine the seroprevalence of *Brucella canis* infection in companion dogs in the Ahvaz area, southwestern Iran.

Materials and Methods

Sample collection and preparation

A total of 102 blood samples obtained from companion dogs referred to the Veterinary Hospital of Shahid Chamran University, Ahvaz, capital of Khoozestan province, located in the southwest of Iran, 2006-2008. The studied dogs were 1-14–year-old and from three breeds: German shepherds, Doberman pinschers, and Mixed. The reason for referring to the hospital was sequel of vaccination or different forms of diseases. The sampling was carried out only once. After obtaining the sera, they were separated in new tubes. The serum samples in vacuum blood tubes were separated by centrifugation at 1000 rpm for 5 min. The studied dogs were divided into two age groups (1-5 years and >5 years) and based on clinical signs (related signs to brucellosis such as scrotum dermatitis, diskospondylitis, abortion and infertility) into two groups also.

Immunochromatography assay

Sera were examined with a commercial Rapid *B. canis* Ab Test kit (Cat No: RB21-03) (Manufactured by Anigen, Animal genetics, Inc., Korea), according to the manufacturer's instructions. This kit is a chromatographic immunoassay for the qualitative detection of *Brucella canis* antibody in whole blood, plasma or serum. Sensitivity and specifity of these kits were 95.8 and 99.7%, respectively (Esfandiari and Klingeborn, 2000).

Procedure and interpretation of the test

The Anigen Rapid C. Brucella Ab test kits were used in our study. Interpret test results were at 5-10 min. If a color band appeared in the left section of the result window, it was shown that the test was working properly. This band was the control band. If another color band appeared in the right section of the result window, this band was the test band. The presence of two color bands (T and C) within the result window, indicated a positive result (Esfandiari and Klingeborn, 2000).

Statistical analysis

Test results and potential association with age, sex, breed and clinical signs were performed by SPSS 10.0 for windows using Fisher's exact test and Chi-square analysis. Differences were considered significant at $P \leq 0.05$.

Results

Five out of the 102 serum samples (4.90%) were found to be positive for *B. canis*. The infection was more prevalent in dogs above 5 years (9.3%; 4 of 43) compared with dogs less than 5 years (1.69%; 1 of 59), but the difference between the two age groups was not statistically significant ($P>0.05$). There was no

significant difference between different sexes and breeds, although the seroprevalence rate was higher in females than in males (P>0.05). Nevertheless, the difference for related signs to brucellosis was significant between the two groups (P<0.05). The most common signs were scrotom dermatitis (2 cases), lymphadenitis (3 cases) and infertility (1 case) in the dogs that had positive serum. Some cases had more than one sign. Three out of 14 cases (21.43%) which had clinical signs and two of 88 cases (2.27%) which had no clinical signs were seropositive. In relation to sex, 4.65% (2 of 43) of male and 5.1% of (3 of 59) female dogs were seropositive.

Discussion

The present study is the first report on the prevalence of *Brucella canis* infection in dogs in Iran using immunochromatography. It was revealed that 4.90% (5 out of 102) of companion dogs in the Ahvaz area were affected with *B. canis*. The results indicated that not all cases that had related signs with brucellosis are caused by *B. canis*. Yet the only way to know if a dog has *B. canis* infection is through a positive diagnostic test (Serological tests or blood cultures). Obtained findings from our study were nearly consistent with other studies in different regions of the world.

Knowledge of the prevalence of *B. canis* in affected dogs in the Ahvaz area is important because *B. canis* is contagious and there are many stray dogs that are not examined. These animals can be a concern in transmission of the disease to other dogs and humans.

Our study showed that the prevalence of infection was more in ages of above 5 years (9.3%) compared with those less than 5 years (1.69%), although the difference was not significant (P>0.05). This shows that higher age may increase exposing probability to infection.

Brucella canis is probably found throughout most of the world; however, New Zealand and Australia appear to be free of this organism. The prevalence of infection varies in different countries (Greene and Carmichael, 2006). In a survey in Turkey, a total of 362 serum samples in Istanbul and Ezmir provinces, 27 cases, (7.45%) were found to be positive for *B. canis* by ELISA (Taner *et al*., 2005). In another study for evaluation of the clinical utility of the immunochromatography assay for serodiagnosis of dogs suspected of having brucellosis, the results were compared with those obtained for hemoculture and the rapid screening agglutination with 2-mercaptoethanol. All of the experimentally infected dogs were positive in ICA, HC and 2-ME RSAT from 3-7 weeks after infection, respectively (Kim *et al*., 2007). It is reported that vaginal swab PCR can be a good candidate as a confirmatory test for brucellosis diagnosis in bitches suspected of being infected (Keid *et al*., 2007). Other surveys on 12949 dogs showed 0.3-42.7% infection in 23 provinces and cities in China (Shang, 1989).

In another serological survey for canine brucellosis that had been conducted on 341 dogs from different regions of the province of Quebec, a significant titer was found in six sera (1.6%) with the 2-mercaptoethanol tube agglutination test (Higgins *et al*., 1979). Also, sera from 2000 dogs from southwestern Ontario were tested for antibodies to *Brucella canis* by a rapid slide agglutination test. Thirty-one of these sera gave suspicious titres and one showed positive titer (Bosu and Prescott, 1980). A retrospective study of 135 dogs with diskospondylitis revealed 14 dogs with concurrent *Brucella canis* infection (Kerwin *et al*., 1992). Although canine brucellosis is rare in Canada, an outbreak of *Brucella canis* infection is reported within a kennel (Brennan *et al*., 2008).

In one patient that had symptoms compatible with brucellosis the routine tests using *Brucella abortus* antigen were negative, however, it was positive for *B. canis* (Lucero *et al*., 2005). A total number of 1549 dogs from Miyagi Prefecture were surveyed during a year for *B. canis*, 173 of 1549 dogs (11.2%) were sero-positive (Kikuchi *et al*., 1979). Anti *B. canis* antibodies were detected in 16 of 219 dogs (7.3%) in Buenos Aires (Boeri *et al*., 2008).

In our survey, the difference for the related signs to brucellosis were significant between the groups (P<0.05). We emphasis that signs such as scrotum dermatitis,

diskospondylitis, lymphadenitis, abortion and infertility are very important in the diagnosis of brucellosis. Also, isolation of the affected dogs is important for prevention of disease transmission to healthy dogs and humans. Any animal with brucellosis should be removed from the kennel or other breeding stock before infecting the entire colony. Before breeding the dog, both the male and female dog should be examined by testing for the disease.

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