



CASE REPORT

Outbreak Investigation of Brucellosis at a Kennel in Iran

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ABSTRACT

This study was undertaken to assess the seroprevalence of *Brucella canis* at a kennel in Iran. Serum samples (n=21) were examined for the presence of *B. canis* antibodies with immunochromatography assay. Out of these, 57.2% were positive for *B. canis* antibodies. All sero positive dogs were more than 2 years old. Of these, majority (75%) was female dogs and 66.6% had the history of late abortion between 40 and 55 days of gestational period accompanied with brownish or gray-green vaginal discharge. The aborted puppies usually appeared partially autolysed. Scrotal dermatitis and orchitis were the only signs in male dogs. Prevention measures were also performed to control the infection in this kennel.

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INTRODUCTION

Four out of six species of *Brucella* (*Brucella canis*, *B. abortus*, *B. melitensis* and *B. suis*) are known to infect dog (Hollett, 2006). *Brucella canis* was identified as the causative agent of canine brucellosis (Carmichael, 1966). The disease has been reported in many geographical regions of the world. The infection is endemic in the South and Central America; but it is sporadic in Europe & Asia (Mosallanejad *et al.*, 2009; Corrente *et al.*, 2010).

Brucella canis is a rough or moccoid, small, Gram-negative intracellular bacterium (Hollett, 2006). It affects all breeds of dogs and can occasionally infect human beings (Wanke, 2004). Infected dogs shed organism in vaginal secretions, semen, urine, milk, saliva, nasal and ocular secretions, and feces. In addition it may transmit transplacentally (Johnson and Walker, 1992). The *B. canis* does not threaten the dog's life, but it causes great economic loss and reproductive failure due to abortion; thus the breeding potential in professional kennels is decreased.

Definite diagnosis of *B. canis* depends on bacteriological isolation (Wanke, 2004), however, PCR offers an alternative. Furthermore, serological evaluations such as tube agglutination test (TAT), agar gel immunodiffusion (AGID), rapid slide agglutination test (RSAT), rapid screening agglutination with 2-mercaptoethanol (2ME-RSAT) are often considered as effective diagnostic tools (Keid *et al.*, 2007). Rapid detection kits & dipsticks are common, convenient, fast, and reliable methods for

diagnosis of brucellosis including *B. canis*. These tests can easily be performed by both veterinarians and kennel owners. Detection of *Brucella* bacteria in animals' fluids, regardless of its viability of the organism, is epidemiologically important. The *B. canis* is a common cause of chronic or asymptomatic infections; therefore any animal shedding *Brucella* organism in the body fluids should be considered as a potential infection source.

Studies on canine brucellosis in Iran are sparse. Hence, there was a dire need to carry out such study which can point out prevalence of this disease in canines. Thus, aim of this study was to report the seroprevalence of *B. canis* infection in a kennel in Shiraz, Fars province, southern Iran.

Case history and sampling

A 5-year-old female German shepherd dog was referred to the Small Animal Clinic of the School of Veterinary Medicine, Shiraz University, Iran. The bitch had a history of poor reproductive performance and aborted several times. Repeated antibiotic therapy with amoxicillin (22 mg/kg BID for 10 days) was ineffective. Whole blood and serum sample were collected aseptically, and submitted for serological and bacterial evaluation. *Brucella* spp. was isolated in the blood culture and the serological test revealed presence of *B. canis* antibodies in the serum by immunochromatography assay. The dog was treated with oral tetracycline (30 mg/kg) twice daily for 28 days as well as intravenous

streptomycin (20 mg/kg) once daily, for 14 consecutive days (Nicoletti, 1991).

The kennel to which the above female dog belonged was situated in Shiraz, southern Iran (29°50'N, 52°46'E) and had 21 dogs (6 males and 15 females). In the female dogs, history of abortion and stillbirth prevailed and one of the male dogs had the signs of scrotal dermatitis and orchitis. Most abortions occurred lately, particularly during the 7th to 9th weeks of gestation. Abortions were usually followed by a mucoid, serosanguinous or gray-green vaginal discharge that persisted for up to six weeks. The dogs under study were 6 months to 5 years old and from 5 breeds, i.e., Shih Tzu, German shepherd, Boxer, Gray din and Terrier, kept all in one open box and had direct contacts with each other. Animals' blood samples were collected with and without anticoagulant agent. The whole blood and sera were evaluated.

Rapid test kit detection

Blood samples were examined with a commercial rapid *B. canis* Ab test kit (Cat No: RB21- 03; M/S Anigen, Animal Genetics, Inc., Korea). This kit was a chromatographic immunoassay for the qualitative detection of *B. canis* antibodies in canine whole blood, plasma or serum. As reported by the manufacturer, its sensitivity and specificity of the kits vs blood culture were 93% and 100%, respectively.

RESULTS AND DISCUSSION

To our knowledge, similar studies on canine brucellosis in Iran are rare. In a previous serological survey in Ahvaz, west southern Iran, 4.9% dogs were found positive (Mosallanejad *et al.*, 2009). In the present study, specific *B. canis* antibodies were identified in 57.2% dog's serum samples, out of these, 75% of positive cases were from females.

General symptoms and clinical signs of brucellosis in canine are not very evident and they may vary depending on affected organ and the infected dogs may show mild or no clinical signs of infection. Clinical signs are inadequate to diagnose canine brucellosis because many infected males are clinically normal. However, when there is a history of abortion, diskospondylitis or poor reproductive performance, the infection should be suspected (Kim *et al.*, 2007). However, in some studies, the main clinical signs mentioned in males are orchitis, testicular atrophy, epididymitis and prostatitis (Wanke, 2004). The only sign in seropositive dogs in our investigation were scrotal dermatitis and orchitis in one case that developed due to the constant licking. Other males were clinically asymptomatic.

The classical symptom in females is late abortion between 30 and 57 days of gestational period with a higher frequency noted between days 45 and 55 (Wanke, 2004). In our study, 66.6% of the female seropositive dogs had the history of late abortion between 40 and 55 days of their gestational period accompanied with prolonged brownish or gray-green vaginal discharge. The aborted puppies usually appeared partially autolyzed.

The common routes of *B. canis* transmission are genital, oronasal or conjunctivae mucosa, therefore, dogs cohabitation with the infected cases especially males may

result in spreading of infection (Carmichael and Joubert, 1988). In this evaluated kennel, the dogs were divided into 2 or 3 member groups and each group was kept in a separate cage. The cages were close together and had a common sewage duct; thus, the animals may contact to sewage especially urine and contaminated water used for washing the cages. This was probably a predisposing factor for transmission of infection. In the present study, all the infected animals were more than 2 years indicating that older dogs may be more exposed to *B. canis* infection.

The *B. canis* bacteria are sensitive to a variety of antibiotics, but they are intracellular organisms and many drugs are ineffective and accordingly, no treatment is recommended (Wanke, 2004). In the present study, the infected dogs were not treated, but prevention strategies were carried out to control the infection. The preventive program included isolation of the suspected dogs and identification and elimination of the infection sources. The cages were also cleaned well with quaternary ammonia and iodides. Quarantining of new added dogs to the kennel for at least one month was practiced. Accurate and consecutive serologic testing programs for new added animals and female breeders were done. The results of this study showed that serological screening tests are vital for diagnosis of *B. canis* infection in kennels. The numbers of cases in this survey were limited and additional investigations are needed to further evaluate the seroprevalence of infection in this region.

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