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RESEARCH ARTICLE

Epidemiological Survey of *Brucella canis* Infection in Different Breeds of Dogs in Fars Province, Iran

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ABSTRACT

This study was conducted to determine the prevalence of *Brucella canis* antibodies in different breeds, sex and ages of dogs in southern of Iran. A total of 113 whole blood samples were taken from different breeds based on exotic or native sources. The samples were examined with immunochromatography assay for detection of *B. canis* antibodies. Twelve dogs were serologically positive (10.62%). There was significant differences in ratio of infected dogs between breeds (exotic or native), ages (less, equal or more than 2 years old) and the history of vaccination (against rabies, leptospirosis, parvovirus, adenovirus type 2, canine distemper, parainfluenza) (P<0.001). However, the results were not significant statistically, among both sex (P=0.058) and the history of clinical signs (P=0.456) in seropositive dogs. Based on this study and the other investigation in companion dogs from southwest of Iran, it seems that the mixed and spray (native) breeds are not infected with *B. canis*, yet. Conversely, the exotic breeds would be the source of bacterium in Iran. Therefore, preventive and control measures are strongly recommended.

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INTRODUCTION

Seroepidemiological surveys are undertaken when existing information relating to interpretation between animal and disease agent is inadequate. These types of surveys are also basic to the study of infectious diseases (Monroe *et al*., 1975). Canine brucellosis may occur with four species of Brucella (*Brucella canis, B. abortus, B. melitensis* and *B. suis*). *B. canis* is a rough or mocoid, small, Gram-negative intracellular bacterium that can affects all breeds of dogs (Wanke, 2004; Hollett, 2006). *B. canis* infection in human is uncommon but is possible (Lucero *et al*., 2010). The infection in dog may display very few clinical symptoms other than late abortion in the female and orchitis in the male (Kim *et al*., 2007). The common routes of *B. canis* transmission are genital, oronasal or conjunctivae mucosa (Carmichael and Joubert, 1988). Bacteriologic culture, Polymerase chain reaction (PCR) and serologic tests 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 used for identifying the infection in suspect animals (Keid *et al*., 2007). Currently, rapid detection kits and dipsticks are available for diagnosis of *B. canis* infection.

Canine brucellosis has been reported in many countries. The infection is endemic in the South and Central America; but it is sporadic in Europe and Asia (Mosallanejad *et al*., 2009; Corrente *et al*., 2010). In Asia, the disease has been identified in India (Srinivasan *et al.*, 1992), Pakistan (Gul and Khan, 2007), Philippines (Baluyut and Duguies, 1997), Korea (Park and Oh, 2001; Kang *et al*., 2009; Bae and Lee, 2009), Japan (Katami *et al.*, 1991; Kim *et al*., 2006), China (Jiang, 1989), Turkey (Diker *et al.*, 1987; Öncel, 2005), Malaysia (Joseph *et al.*, 1983), Argentina (López *et al.*, 2009) and Taiwan (Tsai *et al.*, 1983). There is only one seroprevalence survey on *B. canis* in companion dogs in southwest of Iran (Mosallanejad *et al.*, 2009). Hence, epidemiological studies on canine brucellosis are vital to advance our understandings of disease incidence, progression, and outcome in this region. It can also help scientists to find effective prevention and treatment strategies for this disease. The aim of this study was to demonstrate the seroepidemiology of *B. canis* antibodies in different populations of dogs in Fars province, southern Iran.

MATERIALS AND METHODS

Blood sampling: Whole blood samples in EDTA tubes were taken from different breeds, exotic (62) and native (51) in different areas of Fars province, Iran. In this study, the pure breeds of dogs that imported from abroad such as Doberman, German shepherd, Rottweiler, Boxer, Bulldog, Terriers and so on, were considered as exotic breeds; and the mixed or spray dogs were considered as native breeds. The clinical signs related to *B. canis* were recorded in this sampling, includes the history of scrotum dermatitis, diskospondylitis, abortion, long-term vulvar discharge and infertility.

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, sensitivity and specificity of the kits vs blood culture were 93 and 100%, respectively.

Statistical analysis: Test results and potential association with age, sex, breed, history of vaccination (against rabies, leptospirosis, parvovirus, adenovirus type 2, canine distemper, parainfluenza) and clinical signs were performed by SPSS 18.0 for windows using Fisher's exact test and Chi-square analysis. Differences were considered significant at $P \leq 0.05$.

RESULTS

All data with assigned groups and the differences between them have been shown in Table 1. Analysis of 113 sera samples collected from dogs revealed 12 (10.61%) dogs seropositive for *B. canis*. All *B. canis* seropositive dogs (19.35%) belonged to exotic breeds (Table 1). Among the gender, female dogs showed the highest (16.07%) seroprevalence of *B. canis* as compared to male dogs ($P<0.058$). Similarly, aged dogs suffered more from *B. canis* ($P<0.001$). Dogs with the history of vaccination showed the highest seroprevalence of brucellosis than non-vaccinated ($P<0.001$). The statistical analysis indicated not significant differences between both sex ($P=0.058$) and the history of clinical signs ($P=0.456$) in seropositive dogs.

DISCUSSION

B. canis is a potential zoonotic pathogen that infects almost exclusively dogs and wild *Canidae*. Canine brucellosis has been diagnosed in many geographical areas. It occurs in wild dog packs, new untested animals, kennels, puppy mills and even backyard mistakes (Hollett, 2006).

There is no comprehensive epidemiological study on canine brucellosis in Iran. There is only one serological survey on *B. canis* with prevalence of 4.9% in companion dogs (German shepherd, Doberman pinscher, and Mixed breeds) in Ahvaz, Iran (Mosallanejad *et al.*, 2009). Current study showed that the prevalence of *B. canis* antibodies was 10.62% in dogs in Fars province, Iran. The bacterium is probably found throughout in many geographical areas of the world; however, New Zealand and Australia appear free of this organism. The prevalence of infection varies in different countries. Reports document worldwide outbreaks from Alabama, Mexico, Britain, Europe, Brazil, Texas, Colorado, Illinois, Wisconsin, Michigan, Ontario, Japan, China, and Georgia (Hollett, 2006). Also there are detection and isolation reports from other countries such as Italy (Corrente *et al.*, 2010) and Canada (Forbes and Pantekoek, 1988). In similar study in Turkey, 7.45% of dog serum samples were positive for *B. canis* antibodies by ELISA (Taner *et al.*, 2005).

The results of present study showed that all the infected dogs belonged to exotic breeds as compared with non infected mixed or stray dogs ($P<0.001$). Conversely, a study of stray dogs in Tennessee demonstrated a greater than three-fold rate of infection versus non-stray dogs (Hollett, 2006). In the previous study in Iran, the prevalence *of B. canis* antibodies was not evaluated in stray or mixed dogs as compared with pure breeds (Mosallanejad *et al.*, 2009). In our study, all of stray and mixed breed were seronegative for *B. canis* antibodies. These differences may indicate that in endemic area, stray dogs are the source of infection, because of controlling and preventive measures has been taken in companion dogs.

There is no report of *B. canis* infection in both human and stray dogs in some countries. Detection of canine brucellosis in exotic dogs in these regions may indicate the new source of infection from abroad. To our knowledge, there is no documented report on seroprevalence or seroepidemiology of *B. canis* in human

Table 1: Different groups of dogs with seropositive and seronegative test results for *Brucella canis*

Groups		Total Samples	Serology			Pearson Chi-square value	Fisher's Exact Test P value
			Negative	Positive	Positive %		
Sex	Male	57	54	3	5.26	3.477	0.058
	Female	56	47	9	16.07		
Breed	Exotic	62	50	12*	19.35	11.044	0.001
	Native	51	51	0*	0		
Age	<24 Months	86	84	2*	2.32	26.085	0.001
	≥24 Months	27	17	10*	37.03		
Clinical Signs	Negative	99	89	10	10.10	0.226	0.456
	Positive	14	12	2	14.28		
Vaccination	Negative	52	50	2*	3.84	11.445	0.001
	Positive	61	51	10*	16.39		

Asterisk indicate significant differences ($P<0.05$).

in Iran. The only study on frequency of *B. melitensis* in which *B. canis* antigens used for screening in card and tube agglutination tests conducted in 1982 (Makarem *et al*., 1982). However, controlling programs for *B. melitensis* and *B. abortus* infection are performed routinely in Iran; there is no such plan for this purpose in *B. canis.*

Recently, the contact of human and companion dogs has increased in Iran and there were some referral patients with the apparent clinical signs of brucellosis but serologically negative for smooth species of *Brucella* antibodies (*B. melitensis* and *B. abortus*). Thus, the authors suggest exclusive serological surveys for detection of *B. canis* antibodies in human and dogs' population. Such unusual clinical presentation of brucellosis caused by *B. canis* has been reported (Lucero *et al.*, 2005a,b).

The differences between seropositive dogs with and without history of vaccination ($P<0.001$), equal, more or less than 2 years old ($P<0.001$) were significant, statistically. But there were no significant differences between dogs with and without clinical signs ($P=0.456$), male and female dogs ($P=0.058$). Asymptomatic dogs harbor *B. canis* organisms for prolonged intervals. From initial exposure time to bacteremia, it longs approximately 3 weeks. After this period, the organism localize in targeted genital tissues to seed a continuous or recurrent release that can last for months to years. In a kennel environment, the aborting bitch is one of the high risk potential sources in spreading of *B. canis* infection (Hollett, 2006).

Conclusions: There were seropositive dogs for *B.canis* in some areas of Iran (4.9-10.6%). Because of zoonotic potential of canine brucellosis and economic loss as result of canine reproduction failure, exclusive surveys for detection of canine brucellosis should be assessed in Iran. In addition, the results of the present study indicated the presence of *B. canis* antibodies only in exotic dogs in Iran. The transportation and purchasing of exotic breeds without any controls and quarantine rules in the country border entrance allowed the infection to spread. To our knowledge, serological screening tests for detection of *B. canis* antibodies are not performing in Iran routinely. Therefore, preventive and control measures are strongly recommended.

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