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Serological evidences of canine brucellosis as a new emerging disease in Iran

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ABSTRACT

Objective: To detect the evidences of significant exposure of dogs with *Brucella canis* (*B. canis*) and other *Brucella* species in southeast of Iran by serological assays. **Methods:** Blood samples were prepared from 62 privately owned and 33 kenneled dogs and screened by indirect immunoflorescence assay for *B. canis*. Rose Bengal test (RBT) and standard tube test (SAT) or wright test were used for screening the other *Brucella* species. **Results:** Serological evidences of exposure to *B. canis* were found in 15.8% of the studied dogs, whereas RBT and SAT showed 29.5% and 12.6% seropositivity, respectively. **Conclusions:** This study shows that *B. canis* is endemic in southeastern of Iran and canine brucellosis can be considered as a new emerging disease. Moreover, our results demonstrate that dogs will be at high risk in brucellosis-infected farms. Therefore, further investigations must be performed to evaluate the seroprevalence and zoonotic potential of this disease in different geographical parts of Iran.

1. Introduction

Brucellosis is a zoonotic disease of worldwide distribution but it predominates in Mediterranean countries, the Middle East and Latin America[1]. Despite the strict international control and management programs, it remains endemic in many countries like Iran[2]. In many regions of Iran, *Brucella melitensis* (*B. melitensis*) biovar 1 and *Brucella abortus* (*B. abortus*) biovar 3 were reported as the most prevalent species but *Brucella suis* (*B. suis*), *Brucella neotomae* (*B. neotomae*), *Brucella ovis* (*B. ovis*) and *Brucella canis* (*B. canis*) were not isolated so far[3].

Canine brucellosis which is mainly caused by *B. canis* is a contagious zoonotic disease with venereal and oral modes of transmission that produces late abortion in females and orchitis, epididymitis and prostatitis in male dogs[4]. Limited research about canine brucellosis has been done in Asian countries, but the suspected role of dogs in the spreading of *B. abortus* and *B. melitensis* to neighboring flocks and humans was reported[5,6]. The seroprevalence of *B. canis* is not well determined in Middle East countries. Israel was reported free[7] and serological evidence of *B. canis* was just reported recently from south of Iran[8,9].

This study was planned to assess the serological evidences of *B. canis* and other *Brucella* species in dogs in Kerman city, south-east of Iran.

2. Materials and methods

2.1. Study population

During February 2009 – March 2010, 95 canine blood samples were prepared from 62 privately owned and 33 kenneled dogs (3 different kennels), respectively. The household dogs were selected from the population, which were referred to the Veterinary Hospital of the Shahid Bahonar Uniersity of Kerman. Informed consent was obtained from each dog owner prior to the study. All kenneled and owned dogs were randomly selected without limitation for age, sex or health status. A detailed questionnaire was completed for each animal to gather information about the clinical histories.

2.2. Sample collection, hematological and biochemical survey

5 mL blood samples were collected from cephalic vein, which were divided into plain and anticoagulant-containing tubes (ethylenediamine tetraacetic acid). 1 mL of blood was infused into the anticoagulant-containing tubes which were used for hematological evaluation. In plain tubes, 4 mL of the same blood was poured and allowed standing at room

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temperature for at least 1 hour before centrifugation. The sera were separated off and 1 mL aliquot was frozen at −20 ℃ for serological assay. Biochemical survey was done by remaining sera.

Complete blood counts were performed manually for all dogs and the presence of hematological disorders such as anemia (heamatocrit <37%), leucopenia or leucocytosis (less than 6 000 or more than 17 000 leukocyte/μL of blood) and changes in differential leukocyte count was recorded[10]. Total protein, albumin, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level were measured by an autoanalyser (Autolab, AMS-18A, China) using Pars Azmoon kits (Pars Azmoon, Iran) regarding to reference values[11].

2.3. Serological survey

All sera were examined for the presence of the antibody against *B. canis* by indirect immunofluorescence antibody (IFA) test kit MegaScreen® FLUOBRUCELLA (MegaCor, Horbranz, Austria) in accordance with the manufacture's recommendations. The stained slides were read under 400 magnification fluorescence microscopes (Olympus, BH-2). Evaluation was carried out with green and red filter. According to the recommendation of the kit, IgG titers of 1:50 and greater was considered to reflect *B. canis* infection at an undetermined time and the sera were only tested at a dilution of 1:50 in the present study.

In the next step, all sera were further analyzed by two serological screening test, Rose Bengal test (RBT) and standard tube test (SAT) or wright test to estimate the seropositivity to other *Brucella* species (*B. abortus*, *B. melitensis* and *B. suis*) which all have seroreaction to *B. abortus* S99 antigen obtained from Bacterial Vaccines and Antigens Production Department of Razi Institute, Iran.

RBT was done by adding 30 μL of animal sera and 30 μL of rose Bengal antigen which were mixed together on a slide and gently shook for four minutes at room temperature. Simultaneously, a similar process was done for standard antiserum (Razi Institute, Iran) and finally positive serum samples (containing anti-*Brucella* antibodies) were detected through observation of agglutination within 4 minutes.

In wright test, for detection of agglutinable IgM and IgG antibodies, serial dilution of animal sera (0.5 mL) was made from 1/20-1/1 280 in 7 different tubes. Then 0.5 mL of standard antigen added to each sample. Also 0.5 mL of the standard antigen was added to 0.5 mL of normal saline as the negative control. Samples were kept at 37 ℃ for 24 hours and finally analyzed to detect the positive samples and the titer of sera in comparison with the negative control. The highest serum dilution showing agglutination was considered as the titer of serum. The SAT was positive if agglutination appears at 1/80 titer or higher in this study.

2.4. Statistical analysis

The SPSS software package version 15 (Chicago, IL, USA) was used for the statistical analysis. Seropositivity in IFA, SAT and RBT test was set as an outcome variable, and the independent variables were sex, age (>3 years, 3-6 years, <6 years), animal source (client-owned, kenneled), clinical findings (symptomatic, asymptomatic) and hematological and biochemical alterations. Pearson's *Chi* square (χ^2) test was performed by using two × *K* contingency tables of exposure variables. $P \leqslant 0.05$ was considered significant.

3. Results

Seropositivity to *B. canis* was 15.8% in this study by IFA method whereas RBT and SAT showed 29.5% and 12.6% seropositivity, respectively. No significant difference was observed in *B. canis* seroprevalence concerning gender, age and animal source ($P>0.05$). Significant association was seen between seropositivity in IFA test and the presence of specific clinical signs ($P<0.001$) (Table 1). Three female dogs (20%) had abortion in their past clinical history. Three male seropositive dogs (20%) with average age of 6.5 years had several unsuccessful mating and infertility in their past histories (Table 1). Castration was done in the Saluki (No.13) and mix dog (No.73) by their owner consent and reduced viability, head-to-head sperm agglutination and morphological abnormalities of sperms in the mix dog and azoospermia in the Saluki dog was noted. Besides, scrotum dermatitis and orchitis was observed in the Saluki dog. Severe balonopostitis which has not been reported later as a specific brucellosis sign was noticed synchronously in this case (Figure 1).

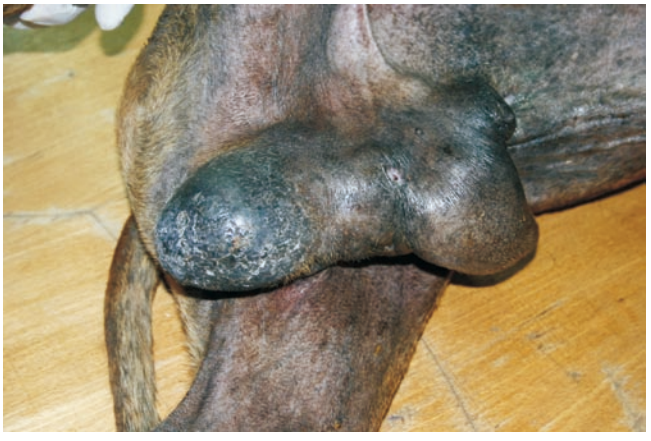

Figure 1. Severe balanopostitis, orcititis, and scrotum dermatitis in dog No.13.

Clinical and radiological findings confirmed discospondylitis in L1-L2 and L7-S1 vertebra in a 10 year old Great Dane dog (No. 92) which suffered from rare limb paresis (Table 1).

Although hyperglobinemia was very common (40%) in IFA positive animals, the association of this laboratory finding and seropositivity was not significant ($P>0.05$).

RBT and SAT positive animals were almost asymptomatic. However, generalized lymphadenomegaly was noted in 14.3% and a multiparous dog (No.23) had still birth history in her last partution. Infected dogs showed no predominant alteration in haematological findings, and there was no significant difference in the haematological and biochemical values between seropositive and seronegative animals ($P>0.05$).

4. Discussion

Brucellosis continues to be a major problem in Iran despite the existence of a 'test and slaughter' strategy program for eradication. While epidemiology of canine brucellosis is

Table 1
Source, signalment, health and *Brucella* spp. infection status of studied dogs.

No. of dog	Clinical finding and history	IFA	SAT	RBT	Laboratory findings	Source	Signalement
1	Clinically healthy	+	−	−	Leukocytosis	O	German shepherd, 2Y, F
3	Clinically healthy	−	1/80	+	−	O	German shepherd, 9Y, F
4	Clinically healthy	−	1/80	+	Hyperglobulinemia	O	Doberman, 2.5Y, F
9	Clinically healthy	+	−	−	−	K	Mix, 3Y, F
10	Infertility	+	1/80	−	Hyperglobulinemia	O	German shepherd, 3Y, M
11	Generalized lymphadenomegaly	+	−	+	Hyperglobulinemia	O	Mix, 7Y, F
13	Infertility, orcititis, epididymitis scrotum dermatitis, balanopostitis	+	−	−	Hyperglobulinemia, leukocytosis, elevated ALP, BUN, ceratinine	K	Saluki, 7Y, M
18	Clinically healthy	−	1/160	+	−	K	Mix, 9Y, M
23	Generalized lymphadenomegaly, still birth in 2 pregnancy	−	−	+	Hyperglobulinemia	O	German shepherd, 9Y, F
25	Clinically healthy	+	−	−	−	O	Pointer, M, 4Y
27	Clinically healthy	−	−	+	−	O	German shepherd, 2Y, M
28	Clinically healthy	−	−	+	−	O	German shepherd, 3Y, M
30	Clinically healthy	−	−	+	−	O	German shepherd, 5Y, M
31	Generalized lymphadenomegaly	−	1/160	+	Hyperglobulinemia	O	Doberman, 4Y, M
37	Clinically healthy	+	−	−	−	O	German shepherd, 5Y, M
38	Clinically healthy	−	−	+	−	K	Sharpie, 6Y, F
39	Clinically healthy	−	−	+	−	K	Doberman, 2Y, F
43	Abortion	+	−	−	Leukocytosis	O	Great dane, 2Y, F
47	Clinically healthy	+	−	−	Anemia	K	German shepherd, 2Y, M
48	Clinically healthy	+	1/160	+	Leukocytosis Hyperglobulinemia −	K	German shepherd, 1Y, M
49	Clinically healthy	−	−	+	Anemia	K	Mix, 1Y, F
50	Generalized lymphadenomegaly, Weight loss	−	−	+	−	K	Mix, 1Y, F
53	Clinically healthy	−	1/80	+	−	K	Mix, 2Y, F
55	Clinically healthy	−	1/320	+	Leukocytosis	O	Mix, 2Y, F
61	Abortion	+	−	−	Leukocytosis, anemia	O	Mix, 5Y, F
65	Generalized lymphadenomegaly	−	−	+	Leukocytosis, anemia, hyperglobulinemia	K	German shepherd, 9Y, M
66	Clinically healthy	−	−	+	Leukocytosis, anemia	O	German shepherd, 10Y, M
67	Clinically healthy	−	1/160	+	−	K	Mix, 11Y, M
69	Clinically healthy	−	1/80	−	Leukocytosis	O	Mix, 1Y, M
71	Clinically healthy	−	−	+	Leukocytosis	O	Mix, 1Y, F
72	Clinically healthy	−	1/80	+	Leukocytosis, anemia	O	Boxer, 5Y, F
73	Infertility, generalized lymphadenomegaly	+	−	−	Hyperglobulinemia, elevated ALT and AST	O	Mix, 10Y, M
75	Clinically healthy	−	−	+	−	K	Doberman, 8Y, F
76	Abortion	+	−	−	Hyperglobulinemia	O	German shepherd, 10Y, M
83	Clinically healthy	−	−	+	−	O	Boxer, 2.5Y, F
84	Clinically healthy	−	−	+	−	O	Great Dane, 2Y, F
86	Clinically healthy	+	−	+	−	O	German shepherd, 2Y, M
88	Clinically healthy	−	−	+	−	O	Doberman, 1Y, M
92	Diskospondilitys	+	−	+	−	O	Great Dane, 10Y, F
95	Clinically healthy	−	1/80	+	−	O	German shepherd, 3Y, M
Total	Clinically healthy: 28	15	12	28	20	O: 27	40
	Symptomatic: 12	−	−	−	−	K: 13	

K: Kenneled; O: Owned; M: Male; F: Female; Y: Year.

undetermined in Iran, several researchers have reported rates of *B. canis* seroprevalence ranging from 2% to 30% in dogs in various countries[12] and there are few evidences about naturally acquired *B. abortus*, *B. melitensis* and *B. suis* infection in dogs[6,13,14].

The diagnosis of *B. canis* infection in dogs is primarily based on serological methods. However, false-positive results are common due to cross-reactions of antigens from several bacteria, *e.g.* from mucoid strains of *Pseudomonas*, *Bordetella bronchiseptica*, *Streptococcus*, and *Staphylococcus*[15]. The definitive diagnosis could be achieved by bacterial isolation but negative blood culture should not set as criteria for excluding canine brucellosis[16,17] because of intermittent periods of

bacteremia[4,18].

Based on IFA results, rate of seropositivy to *B. canis* (15.8%) in southeastern of Iran was higher compared with pervious local records which were 4.9% and 10.34% in urban and rural dogs by immuno chromatogeraphy method in the southern part[8,9].

Infection with *B. abortus* was reported in dogs that ingest aborted fetal tissue from infected livestock[6,19,20] whereas in our study just four dogs (No. 23, 55, 66 and 95) were shepherds and had direct contact with farm animals and in others, seropositivity may gained via contact to infected dogs or consumption of un-pasteurized dairy products. Currently, Iran is considered free of *B. suis*, because swine industry is not on-stream due to religiosity. So positive SAT and RBT results presumably related to *B. abortus* and melitensis infection in the current study.

It has been confirmed that canine brucellosis rarely caused serious illness in dogs. However, therapeutic programs are not scheduled in infected cases due to public health aspects and chronic nature of the disease[12]. In our study, as most of the infected dogs were asympotomatic, dog owners did not accept euthanasia. Kennel owners were informed about the importance of disease and all IFA, RBT and SAT seropositve dogs were advised to be neutered and quarantined, but as same as owned dogs, euthanasia was not elected by breeders in the present investigation. This could be a major problem in other countries where there is no strict legislation in control and prevention of canine brucellosis.

Dahlbom *et al* reported that all dogs with reproductive problems were infected with canine herpesvirus-1 (CHV1) in Finland whereas there was no serological evidence of *B. canis* infection in finish dogs with reproductive signs[21]. The seroprevalence of CHV1 was estimated 20.7% by Babaei *et al* in southeastern of Iran. So both of these infective agents could create reproductive disorders in dogs and must be differentiated carefully by clinicians in Iran[22].

In conclusion, this study showed that *B. canis* is endemic in southeastern of Iran and canine brucellosis could be considered as a new emerging disease. Moreover, our results demonstrated that dogs could be at high risk in brucellosis-infected farms.

Further investigations must be performed to evaluate the seroprevalence and zoonotic potential of this disease in different geographical parts of Iran.

Conflict of interest statement

We declare that we have no conflict of interest.

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