Journal of Animal and Veterinary Advances 10 (10): 1322-1326, 2011
ISSN: 1680-5593

First Study Prevalence of Brucellosis in Stray and Herding Dogs South of Iran

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Abstract: Brucellosis is a zoonotic infectious disease caused by *Brucella* sp. 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 brucellosis in stray and herding dogs of mix breeds from seven districts in Shiraz, capital of Fars province, located in the south of Iran. A total of 175 blood samples were obtained from dogs between October 2009 and September 2010. Sera were examined by Rose bengal and Wright test (STAT). The studied dogs were divided into three age groups (≤1, 1-4 and ≥4 years). Prevalence to brucellosis in these dogs was 29.1% (51 of 175). According to the obtained Rose bengal test results, the seroprevalence of brucellosis varied significantly ($p = 0.000$, $\chi^2 = 24.296$) from 12-60% in various districts of the state. According to the obtained results, the serum titre of tube wright test in the studied people was different from 1/5 to 1/640 and in 21.6, 25.5 and 15.7% was 1/160, 1/320 and 1/640, respectively. The STAT results in dogs 41.2% (21 0f 51) was titre ≥1/200 but the wright test was no significant difference between the different seven districts either ($p>0.05$).The infection had more prevalence in dogs <1 year old (42.9%) in comparison with dogs 1-4 years old (27.1%) and dogs ≥4 years old (22.7%), the difference between the two age groups <1 year old and ≥4 years old was statistically significant ($p = 0.056$, $\chi^2 = 3.652$). But the difference between the two age groups <1 and 1-4, 1-4 years old and ≥4 years old was not statistically significant ($p>0.05$). There was no significant difference between the different sexes either ($p>0.05$). Also There was no significant difference between the two groups stray and herding dogs either ($p>0.05$). Nevertheless, dogs had no clinical signs. This study showed that brucellosis is present among the stray and herding dogs population of the Shiraz area and preventive measures should be taken to control pathogenic bacteria.

Key words: Brucellosis, stray and herding dogs, prevalence, serology, Shiraz

INTRODUCTION

Brucellosis is still one of the world's major problems as a topical disease, both in human beings and in animals. It is caused by various species of Brucella (Boussetta, 1991). Although, brucellosis and its means of transmission were discovered over 100 years ago, the disease remains a worldwide problem, predominantly in developing countries (Mantur and Amarnath, 2008). Shiraz is located in the province of Fars in South Iran. The weather in this town is hot-arid. Dogs live in this area where cattle, sheep and goats also are intensively bred. Dogs husbandry has a vital role in the life of a number of shepherd ethnic groups in Iran. Brucellosis in animals is caused by five recognized species of the genus *Brucella*. Four species commonly infect man: *B. abortus*, *B. melitensis*, *B. suis* and *B. canis* (Al-Shamahy, 1997). This bacterium is also a cause of human brucellosis. It is believed that dogs and other canine species are the only true hosts (Carmichael and Kenney, 1970). It is important to emphasize that some infected animals may be asymptomatic, being considered important sources of infection (Johnson and Walker, 1992). When brucellosis is introduced in a confined population, the infection spreads rapidly, leading to economic losses and risks for public health (Jones and Emerson, 1984; Megid *et al*., 1999; Pickerill and Carmichael, 1972). 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. As we observed some cases of brucellosis in human beings in the area Kazerun of Fars (7.8%) (Beheshti *et al*., 2010). The aim of this study was to

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investigate the presence of brucellosis in dogs and its impact on the livestock rearing system. Several researchers have reported rates of seroprevalence ranging between 2 and 30% in dogs in various countries (Greene and Carmichael, 2006). The seroprevalence of brucellosis in various animals such as cattle, sheep, goats, camels, poultry and human were described in Iran (Boeri *et al*., 2008; Sabbaghian and Nadim, 1974). Because there is no published report about brucellosis in dogs south of Iran, this study was performed as the first crosssectional survey to uncover epidemiological patterns of brucellosis in dogs in the south part of Iran.

MATERIALS AND METHODS

Sample collection and preparation: Between October 2009 and September 2010, a total of 175 healthy dogs were randomly selected to determine the seroprevalence of brucellosis and risk factors associated with the disease from dogs. Blood samples obtained from stray (N = 105) and herding (N = 70) dogs of mix breeds from seven districts (Bidzard, Darian, Derak, Siyakhdarengun, Gharebagh, Kaftarak, Dinakan) in Shiraz, capital of Fars province, located in the south of Iran. The studied dogs were 4 months 6 years old and from mixed breeds. Blood samples were obtained by venipuncture and transferred to the laboratory under chilled conditions, as soon as possible. Serum was isolated by centrifuging the blood samples at 2000 g. All serum antibodies were tested for Brucella genus using slide agglutination by Rose bengal test at cell concentrations and Standard Tube Agglutination Test (STAT) by 2-mercaptoethanol, using whole cell antigen (Razi Vaccine and Serum Research Institute) used for the presence of antibodies against *B. abortus* strain.

Statistical analysis: Test results and potential association with age, sex, breed and districts were performed by SPSS 15.0 for windows using Fisher's exact test and Chi-square analysis. Differences were considered significant at $p \leq 0.05$.

RESULTS

About 51out of the 175 serum samples (21.9%) were found to be positive for Brucellosis. According to the obtained rosebengal test results, the seroprevalence of brucellosis varied significantly ($p = 0.000$, $\chi^2 = 24.296$) from 12-60% in various districts of the state (Table 1). In comparison between the two districts together, Bidzard with Derak ($p = 0.047$, $\chi^2 = 3.945$), Darian with Gharebagh, Dinakan and Kaftarak ($p = 0.024$, $\chi^2 = 5.094$), Derak with Gharebagh, Kaftarak and Dinakan ($p = 0.000$, $\chi^2 = 12.5$), Siyakhdarengun with Gharebagh, Dinakan and Kaftarak ($p = 0.047$, $\chi^2 = 3.947$) was statistically significant. But in comparison between other districts with was not statistically significant ($p>0.05$).

According to the obtained results, the serum titre of tube wright test in the studied people was different from 1/5 to 1/640 and in 21.6, 25.5 and 15.7% was 1/160, 1/320 and 1/640, respectively. The wright test was no significant difference between the different seven districts either ($p>0.05$). In comparison between the two districts together, Darian with Siyakhdarengun ($p = 0.003$, $\chi^2 = 8.927$) and Darian with Gharebagh ($p = 0.041$, $\chi^2 = 4.174$) was statistically significant. But in comparison between other districts with, was not statistically significant ($p>0.05$).

The Rose bengal test results, the infection had more prevalence in dogs <1 year old (42.9%) in comparison with dogs 1-4 years old (27.1%) and dogs ≥4 years old (22.7%). The difference between the two age groups <1 year old and ≥4 years old was statistically significant ($p = 0.056$, $\chi^2 = 3.652$). But the difference between the two age groups <1 and 1-4 years old also 1-4 and ≥4 years old was not statistically significant ($p>0.05$). According to the obtained STAT results in comparison between the three age groups with titre ≥1/200, the infection had more prevalence in dogs <1 year old (46.7%) in comparison with dogs ≥4 years old (41.2%) and dogs 1-4 years old (30.8%) (Table 2). However, there was no significant difference between the three age groups either ($p>0.05$).

The Rose bengal test result's prevalence rate was higher in female dogs (30.3%) than male dogs (27.6%). But according to the obtained STAT results in

Table 1: Comparative RTB and STAT results with region

	Region % (N)							
Factors	Bizard	Dariun	Derak	Siakhdarengun	Gharehbagh	Kaftarak	Dinakan	Total
RBT								
-ve	68 (17/25)	60 (15/25)	40 (10/25)	64 (16/25)	88 (22/25)	88 (22/25)	88 (22/25)	70.9(124/175)
+ve	32 (8/25)	40 (10/25)	60 (15/25)	36 (9/25)	12 (3/25)	12 (3/25)	12 (3/25)	29.1 (51/175)
STAT								
Titre <1/200	62.5 (5/8)	90 (9/10)	60 (9/15)	22.2 (2/9)	33.3 (1/3)	66.7 (2/3)	66.7 (2/3)	58.8 (30/51)
Titre ≥1/200	37.5 (3/8)	10 (1/10)	40 (6/15)	77.8 (7/9)	66.7 (2/3)	33.3 (1/3)	33.3 (1/3)	41.2 (21/51)

Table 2: Comparative RTB and STAT results with age

	Age			
Factors	≤1	1-4	≥4	Total
RBT				
-ve	57.1 (20/35)	72.9 (70/96)	77.3 (34/44)	70.9 (124/175)
+ve	42.9 (15/35)	27.1 (26/96)	22.7 (10/44)	29.1 (51/175)
STAT				
Titre <1/200	53.3 (8/15)	69.2 (18/26)	40.0 (4/10)	58.8 (30/51)
Titre ≥1/200	46.7 (7/15)	30.8 (8/26)	60.0 (6/10)	41.2 (21/51)

Table 3: Comparative RTB and STAT results between male and female

	Sex % (N)		
Factors	Male	Female	Total
RBT			
-ve	72.4 (55/76)	69.7 (69/99)	70.9 (124/175)
+ve	27.6 (21/76)	30.3 (30/99)	29.1 (51/175)
STAT			
Titre <1/200	57.1 (12/21)	60.0 (18/30)	58.8 (30/51)
Titre ≥1/200	42.9 (9/21)	40.0 (12/30)	41.2 (21/51)

Table 4: Comparative RTB and STAT results between Herding and Stray dogs

	Dog % (N)		
Factors	Herding dog	Stray dog	Total
RBT			
-ve	65.7 (46/70)	74.3 (78/105)	70.9 (124/175)
+ve	34.3 (24/70)	25.7 (27/105)	29.1 (51/175)
STAT			
Titre <1/200	50.0 (12/24)	66.7 (18/27)	58.8 (30/51)
Titre ≥1/200	60.0 (12/24)	33.3 (9/27)	41.2 (21/51)

comparison between the two sex groups with titre ≥1/200, prevalence rate was higher in male dogs (42.9%) than female dogs (40.0%) (Table 3). However, the difference was not observed among the prevalence of infection and sexes ($p>0.05$).

The Rosbangal test results, the infection was more prevalent in herding dogs (34.3%) compared with stray dogs (25.7%). According to the obtained STAT results, in comparison between the two dogs groups with titre ≥1/200 the infection was more prevalent in herding dogs (50%) compared with stray dogs (33.3%) (Table 4). But there was no significant difference between the two groups stray and herding dogs either ($p>0.05$). Nevertheless, dogs had no clinical signs.

DISCUSSION

An unusually high prevalence of brucellosis in the Shiraz city area is indicated in stray and herding dogs. The present study is the first report on the prevalence of brucellosis in dogs in south of Iran using Rose bengal and STAT. It was revealed that 29.1% (51 out of 175) of stray and herding dogs in the Shiraz area were affected with brucellosis.

The STAT is a presumptive (screening) test (Charisis, 1999) having the advantages of speed and sensitivity however, a recent study (House *et al*., 1975). The seroprevalence rate in this study was found to be 29.1%. Dogs showed a wide variation of brucellosis occurrence, from 12% in low prevalence regions to 60% in high prevalence regions. The disease in high endemic regions such as Africa, Mediterranean, Middle East, parts of Asia and Latin America remains an uncontrolled problem (Godfroid *et al*., 2005; Refai, 2002). Several researchers have reported rates of seroprevalence ranging between 2 and 30% in dogs in various countries (Greene and Carmichael, 2006). Obtained findings from the study were nearly consistent with other studies in different regions of the world. Knowledge of the prevalence of brucellosis in affected dogs in the Shiraz area is important because brucellosis is contagious and there are many stray and herding dogs that are not examined. These animals can be a concern in transmission of the disease to other dogs and humans. Most human infections result from physical contact with infected animals (Gul and Khan, 2007). Transmission typically occurs through contact with infected animals or materials with skin abrasions (Palmer *et al*., 1998).

The study showed that the prevalence of infection was more in ages of <1 years old (42.9%) in comparison with dogs 1-4 years old (27.1%) and dogs ≥4 years old (22.7%), the difference between the two age groups <1 and ≥4 years old was statistically significant ($p = 0.056$, $\chi^2 = 3.652$). This shows that lower age may increase exposing probability to infection.

Brucellosis is probably found throughout most of the world however, in 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 Ontari were tested for antibodies to Brucella canis by a rapid slide agglutination test. About 31 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). The study indicated high prevalence of brucellosis. In all parts of Iran, human brucellosis is endemic and still remains a healthcare problem (Refai, 2002). In serological survey another seroprevalence of brucellosis of iran, in human kazerun 7.8% (Beheshti *et al*., 2010), stray dogs mashhad 5.35% (Sardari *et al*., 2003), camels kerman 10.5% (Rafieipour and

Ziaei, 2007), dogs ahvaz 4.9% (Mosallanejad *et al*., 2009) and seroprevalence 2.5% in mashhad horses (Tahamtan *et al*., 2010). According to the obtained present study results, the infection was more prevalent in Shiraz compared with other regions iran. In serological survey another, 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 (Oncel *et al*., 2005). In another study for evaluation of the clinical utility of the immuno-chromatography 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).

According to the obtained present study results the infection was more prevalent in herding dogs (34.3%; 24 of 70) compared with stray dogs (25.7%; 27 of 105) and agreement with STAT results but there was no significant difference between the two groups stray and herding dogs ($p>0.05$). Results indicate further the need for more specific test antigens and their extensive field evaluation. The Shiraz city dog population would seem a model one for this purpose, since dogs are abundant and brucellosis appears to have the opportunity for natural herd spread because of limited control measures.

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 seropositive (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 the survey, 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.

CONCLUSION

The findings described in this study emphasize the importance to develop a national program and response protocol for prevention of brucellosis in Iran. This is crucial, especially considering the fact that there is no human vaccine available. There is no vaccine approved for use in dogs in Iran. There is also no effective treatment for brucellosis in infected animals (Charisis, 1999). Therefore, brucellosis continues to be a major problem in Iran despite the existence of a test and slaughter strategy program for eradication.

ACKNOWLEDGEMENTS

This research project was supported by the Bacteriology Department, Razi Vaccine and Serum Research Institute-Shiraz, Shiraz, Iran.

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