Detection of Brucella Antibodies in Dogs From Rural Regions of Hamedan, Iran

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Abstract

Background: Dogs play an important role in the maintenance and transmission of several zoonotic pathogens. In the case of Brucella spp., the close contact of dogs with humans and livestock might cause zoonotic diseases and economic losses due to abortions and stillbirths in animals (1, 2). Brucellosis is prevalent in some regions of Iran including Hamedan where recently the first human case of infection with Brucella canis in the country was reported (3,4).

Canine brucellosis caused by B. canis, a Gram-negative facultative intracellular bacterium, is a neglected zoonosis. B. canis in dogs was firstly reported in the United States in 1966, and since then the bacterium has been detected globally, presenting itself in various forms (1.5). The predominant signs of disease in dogs, the major hosts, are abortion, infertility, stillbirth, lymphadenitis, epididymitis, orchitis, and prostatitis (2). Transmission of infection occurs via ingestion of contaminated materials or venereal routes. Diagnosis is usually based on the isolation of causative agent and/or serology techniques (6). B. canis has been reported in humans and wild canids, as well (7). Although B. canis is the important cause of brucellosis in dogs, infection with B. abortus, B. melitensis, and B. suis has also been reported (8). Considering that infected dogs can shed organisms into the environment via urine and vaginal discharges and secretions, aborted materials or feces, they play a significant role in the maintenance of Brucella spp. and its possible transmission to other dogs, cattle, and humans (8,9).

In an earlier research from Hamedan, the rate of brucellosis was detected 3% and 4.6% in sheep and goats, respectively (10). Moreover, seroprevalence rates of 8.1% in veterinarians, 15% in slaughterhouse workers, and 17% in butchers have been reported (11). The incidence rate of human brucellosis in Hamedan province is 31–41 per 100000 population, which is classified as "very high" in Iran (12). Recurrence rate of human brucellosis in this region is calculated as 6.45% (13) and direct contact of human with infected animals is the main risk factor for the disease (4,11,12,14).

In Iran, there is scanty knowledge about canine brucellosis with no information from Hamedan. Therefore, the aim of the current cross-sectional study was to determine the rate of Brucella sp. infection in dogs from Hamedan, West
part of Iran. Furthermore, a historical mini-review on the available literature on Brucella infection in dogs of Iran was presented in the discussion section.

**Methods**

**Study Region, Animals, and Serum Collection**

Between June and November 2018, Blood samples were obtained from cephalic or saphenous veins of 180 stray dogs from six rural regions of Hamedan namely Qerkhlar, Latgah, Ganj Tappeh, Simin, Cheshmeh Qassaban, and Sheverin during June and November 2018 (Figure 1). Blood samples from Ganj Tappeh and Cheshmeh Qassaban were collected for another study (15) and the rest were taken for routine surveillance program of Iranian Veterinary Organization. Sex and age of dogs were recorded in individual data forms. Dogs were categorized based on their age in three groups of less than 1-year-old, between 1 and 2 years old and more than 2 years old. The sera were separated by centrifuging the blood samples at 1000 ×g for 10 minutes and stored at –20°C until laboratory examination.

**Rose Bengal Plate Test**

Initially the sera were screened for the presence of anti-Brucella antibodies using Rose Bengal plate test (RBT), which is a routine qualitative test for brucellosis in both humans and animals. The antigens that were purchased from Razi Vaccine and Serum Research Institute, Iran, could detect B. abortus, B. melitensis, and B. suis.

For the test, 30 μL of RBT antigen (Razi Vaccine and Serum Research Institute, Iran) and 30 μL of serum sample were placed on a white ceramic tile, mixed using sterile applicator stick, rocked gently for 4 minutes, and monitored for agglutination. The formation of distinct pink granules (agglutination) was recorded as positive (6). The RBT positive samples were further evaluated using Wright serum agglutination test.

**Wright serum agglutination test (Wright SAT)**

For the first tube, 0.8 mL of physiological saline solution was dispensed while 0.5 mL of the solution was transferred to the second, third, fourth, and fifth tubes. Then, 0.2 mL of the test serum was added to the first tube and mixed properly. Serial dilution was then carried out by pipetting 0.5 mL of the mixture in the first tube to the second tube. This procedure continued until the fifth tube. The final 0.5 mL from the fifth tube was discarded. Finally, 0.5 mL of the antigens (Razi Vaccine and Serum Research Institute, Iran) was added to all the tubes. The tubes were covered, shaken, and incubated at 37°C for 20 hours. Agglutination titers were determined according to positive and negative controls (10,16).

**Statistical Analysis**

Statistical analysis was performed using Chi-square test (χ²) with a confidence interval (CI) of 95% (SPSS 16.0, SPSS Inc., Chicago, IL, USA). P value less than 0.05 was considered significant.

**Results**

Based on the screening results by RBT, the rate of Brucella infection was found in 3.3% (6/180; 95% CI: 0.7%–5.9%) of animals. Six seropositive dogs were from Qerkhlar (n=1), Ganj Tappeh (n=2), Simin (n=3) regions (Figure 1). All of the positive dogs had a titer of 1:80 antibodies according to Wright SAT. No statistically significant difference was observed between infection rate and gender (P=0.907) or age groups (P=0.772) (Table 1).

**Discussion**

In this study, sera of 180 dogs from Hamedan province were tested for brucellosis using RBT and Wright SAT assays. Six (3.3%) dogs reacted positive with titers of 1:80. Tadjebakhche and Gatel (17) were the first who tested canine blood sera for brucellosis in Iran in 1972. Since then, several serological studies were performed in various regions, employing different diagnostic techniques (Table 1) (17-29). Seroprevalence of brucellosis in the present study (3.3%) was in the range of that previously reported from Iran (Table 2). Differences in the incidence of canine brucellosis in Hamedan compared to other regions of Iran could be attributed to climatic differences. Furthermore, farmers’ knowledge about brucellosis has significantly increased in recent years; this has led to less exposure of stray dogs to livestock and their aborted foetuses. The role that dogs play in the incidence of human brucellosis

<table>
<thead>
<tr>
<th>No. of Dogs (%)</th>
<th>No. of Seropositive Dogs (%)</th>
<th>Statistical Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td>χ²=0.013, P=0.907</td>
</tr>
<tr>
<td>Male</td>
<td>64 (35.6)</td>
<td>2 (3.1)</td>
</tr>
<tr>
<td>Female</td>
<td>116 (64.4)</td>
<td>4 (3.4)</td>
</tr>
<tr>
<td>Age groups (y)</td>
<td></td>
<td>χ²=0.516, P=0.772</td>
</tr>
<tr>
<td>&lt;1</td>
<td>63 (35)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>1-2</td>
<td>56 (31.1)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>61 (33.9)</td>
<td>3 (4.9)</td>
</tr>
</tbody>
</table>

Table 1. Seroprevalence of Brucella sp. Infection in Dogs From Hamedan According to Different Sexes and Age Groups.
is unclear in Iran due to lack of comprehensive reports in this field. However, seropositivity of dogs with zoonotic Brucella species indicate the possibility of transmission of these bacteria from dogs to humans, as well as farm animals in the region.

In this study, specific B. canis antibodies could not be investigated; however, in Ahvaz city, 102 blood samples from companion dogs were examined using a commercial Rapid Canine Brucella Ab Test Kit (Bionote, South Korea), from which 4.9% were found infected (25). In a study conducted in Fars province using the same kit, 10.6% of examined dogs reacted positive (30). Moreover, in Kerman province, seropositivity to B. canis was detected 15.8% using an immunofluorescence antibody (IFA) test kit (MegaFLUO® BRUCELLA canis, Megakor, Austria) (16). This rate was 20.9% in São Paulo, Brazil (using blood culture method), 4.9% in Mississippi, USA (using rapid serology method), and 4.4% in South Africa (using 2-mercaptoethanol-tube agglutination test) (5,6,31). Regarding the fact that rapid diagnostic kits and IFA slides for B. canis are not imported to Iran regularly, it is suggested that Iranian researchers focus on the domestic production of such diagnostic kits.

In the only PCR-based study in Iran, 14 out of 94 (14.9%) tested blood samples from companion dogs of Isfahan and Shahrekord cities were reported to be positive by conventional-PCR (32). As the PCR products in the latter study were not confirmed by nucleotide sequencing and the dogs did not show any sign of brucellosis, these results should be taken with caution. More recently, DNA of Brucella sp. was detected in vaginal swabs of 3 out of 70 (4.3%) dogs referred to a teaching hospital in Kerman (33).

In this study, no statistical correlation was found between the age of dogs and seropositivity. Conversely, in previous studies (25,30,31), higher seroprevalences were detected in older dogs which could be due to the fact that an increase in age of dogs has a direct relationship with the probability of acquiring the disease.

### Table 2. Serological Studies on Canine Brucellosis in Iran From 1972 Onward

<table>
<thead>
<tr>
<th>Area</th>
<th>Year</th>
<th>No. of Tested Dogs</th>
<th>Method(s): No. of Positive Cases (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tehran</td>
<td>1972</td>
<td>41</td>
<td>Wright + CFT: 2 (4.9%)</td>
<td>(17)</td>
</tr>
<tr>
<td>Tehran and Karaj</td>
<td>1975</td>
<td>225</td>
<td>Card test: 6 (2.7%)</td>
<td>(18)</td>
</tr>
<tr>
<td>Shiraz</td>
<td>1996</td>
<td>228</td>
<td>RBT + Wright + 2-ME: 2 (0.88%)</td>
<td>(19)</td>
</tr>
<tr>
<td>Tabriz</td>
<td>1996</td>
<td>112</td>
<td>RBT: 23 (20.5%)</td>
<td>(20)</td>
</tr>
<tr>
<td>Mashhad</td>
<td>1997</td>
<td>100</td>
<td>RBT: 38 (38%)</td>
<td>(21)</td>
</tr>
<tr>
<td>Mashhad</td>
<td>2003</td>
<td>280</td>
<td>RBT: 15 (5.35%)</td>
<td>(22)</td>
</tr>
<tr>
<td>Neyshabur</td>
<td>2007</td>
<td>50</td>
<td>RBT: 9 (18%)</td>
<td>(23)</td>
</tr>
<tr>
<td>Ahvaz</td>
<td>2009</td>
<td>102</td>
<td>Rapid B. canis Ab test kit: 5 (4.9%)</td>
<td>(24)</td>
</tr>
<tr>
<td>Ahvaz</td>
<td>2010</td>
<td>116</td>
<td>Rapid B. canis Ab test kit: 12 (10.1%)</td>
<td>(25)</td>
</tr>
<tr>
<td>Markazi</td>
<td>2011</td>
<td>110</td>
<td>RBT: 6 (5.4%)</td>
<td>(26)</td>
</tr>
<tr>
<td>Shiraz</td>
<td>2011</td>
<td>175</td>
<td>RBT: 51 (29.1%)</td>
<td>(27)</td>
</tr>
<tr>
<td>Urmia</td>
<td>2017</td>
<td>256</td>
<td>NS: 28 (10.9%)</td>
<td>(28)</td>
</tr>
<tr>
<td>Mashhad</td>
<td>2019</td>
<td>173</td>
<td>ELISA IgG: 34 (19.6%)</td>
<td>(29)</td>
</tr>
<tr>
<td>Hamedan</td>
<td>2019</td>
<td>180</td>
<td>RBT: 6 (3.3%)</td>
<td>This study</td>
</tr>
</tbody>
</table>

*Year of publication; Wright’s serum agglutination test; Complement fixation test; Rose Bengal test; 2-mercaptoethanol Brucella agglutination test; Not stated.
of infection via mating and coming into contact with infectious materials (31).

Generally female dogs pose a greater risk to humans as Brucella organisms are shed in the birth fluids and vaginal discharges (31). However, similar to previous findings, no significant correlation was observed between the development of infection and gender of dogs (16,25,30), showing that both sexes appear to be equally susceptible (31,34).

Conclusions
Although the seroprevalence of Brucella sp. was not high in Hamedan, further screening programs on dog population and designing a plan for control of infection is highly recommended in different regions of Iran. The presence of infected dogs in rural regions is an important risk factor for the transmission of disease to livestock causing economic losses due to abortions and stillbirths. It is suggested that villagers, shepherds, and their families especially children should be provided with the information about risks of getting infection when handling an infected dog.

Ethical Approval
Blood samples were taken from dogs after getting official permission and under supervision of Institutional Animal Ethics and Research Committee of Iranian Veterinary Organization (IVO, Iran), Hamedan Office (Certificate No. 32/1397.4.1).

Conflict of Interest Disclosures
None.

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