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Brucellosis in dogs: A comparison of *Brucella* infection in herding and stray dogs in Iran (Tehran and West Azerbaijan)

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Abstract

Brucellosis is one of the five most common bacterial zoonotic diseases worldwide, including in Iran. Although *Brucella canis* is the known cause of brucellosis in dogs, infections with *Brucella abortus*, *Brucella melitensis*, and *Brucella suis* have also been reported. The presence of non-specific *Brucella* species in dogs may make them a potential reservoir for the main hosts of these species. This study investigated *Brucella's* presence in stray and herding dogs and compared their contamination. Blood samples were collected from 156 dogs, including 60 stray dogs from Tehran province and 96 herding dogs from some villages in West Azarbaijan Azerbaijan province, Iran, in contact with cattle and sheep herds. Then the level of contamination was evaluated using the Rose Bengal Plate Test (RBPT), Wright, and Polymerase Chain Reaction (PCR) tests and compared. *Brucella* was found in 66 (42.3%) samples through Rose Bengal, 16 samples through Wright (10.2%), and 1 (0.006%) sample through PCR methods. The herding dogs were more infected than the stray dogs. The higher contamination levels in the herding dogs compared to the stray dogs may be due to their close contact with farm animals, which are natural reservoirs of bacteria. This study confirmed the possibility of *Brucella* transmission from cattle and sheep to dogs and possibly to humans, as well as confirming the role of dogs in the dissemination of disease to cattle and sheep.

Introduction

Brucellosis is an infectious disease that is commonly transmitted between humans and animals and a serious health problem that causes heavy economic losses (1-3). *Brucella* consists of 12 species that cause diseases in different animals (4). *B. abortus* and *B. melitensis* are the most important species concerning zoonosis, economic losses, and pathogenicity (5, 6). Meanwhile, cattle and small ruminants are specific hosts of *B. abortus* and *B. melitensis,*

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respectively; however, humans can also be secondary hosts for these two species (4). The presence of this bacterium in non-specific hosts has been identified by serological and molecular tests (7). Dogs can be infected with other *Brucella* species such as *B. abortus*, *B. melitensis*, and *B. suis*; however, the main cause of brucellosis in dogs is *B. canis* (8). On the other hand, cross-contamination between animal species can occur due to animal husbandry practices, in which dogs are often in close contact with reservoir animals, such as cattle and sheep, and may be infected with *B. abortus* and *B. melitensis*. Dogs can also consume entrails, placentas, aborted fetuses, or raw dairy products, such as raw milk from cows and sheep, and therefore may become infected (2, 9). In Iran, dogs, especially those kept as guards or herding dogs in rural areas, are in close contact with host animals (10). Serologic studies have reported seroprevalence rates, ranging from 15.8% to 3.5% in different provinces (11). Brucellosis is recognized as endemic in Iran, with significant incidence rates reported in both humans and animals, including dogs (12). The interconnectedness between canine and livestock populations complicates control measures, as dogs can serve as reservoirs for the disease (13). A systematic review underscored the need for comprehensive epidemiological data on *Brucella* infections across various animal species, including dogs, to develop effective public health strategies (14).

In Brazil, a notable prevalence of *B. canis* was recorded in commercial breeding kennels, indicating the economic ramifications and public health concerns associated with canine brucellosis (15). Furthermore, a serological study in Nigeria revealed low but significant seroprevalence rates of both *B. abortus* and *B. canis* in household dogs, stressing the need for further investigation into the factors contributing to brucellosis transmission (16).

This study aimed to investigate the presence of *Brucella* in stray dogs in Tehran province and herding dogs in West Azerbaijan province through serological and molecular tests to understand the epidemiology of this disease. Moreover, a comparison was drawn between the herding dogs that were in close contact with specific hosts of *B. abortus* and *B. melitensis*, such as cattle and sheep, and stray dogs that were not in direct contact with these animals in terms of *Brucella* infection.

Materials and Methods

A total of 156 blood samples, including 60 samples from stray dogs in Tehran province and 96 samples from herding dogs in West Azerbaijan province in close contact with sheep in rural areas, were collected between May and August 2022. Blood samples were transported on ice packs to the Research Laboratory of the Brucellosis Department of Razi Vaccine and Serum Research Institute (Karaj, Iran). All applicable international and national guidelines for the care and use of animals were followed.

Serum samples

After the blood samples were transported, sera were separated by centrifugation at 1000 rpm for 10 minutes. The sera were deactivated for 30 minutes at 56°C and kept at -20°C until use.

Rose Bengal Plate Test

First, the serum and antigen samples were placed at room temperature (22 ± 4°C). Then, 30 mL of each serum sample was placed on the slide followed by gentle shaking, and an equal volume of Rose Bengal Antigen (Razi Vaccine and Serum Research Institute, Karaj, Iran) was placed near each spot of the serum and mixed. The mixture was then gently stirred on a rocking shaker for 4 minutes at room temperature, and agglutination was read immediately after this period. The formation of distinct pink granules (agglutination) was recorded as a positive result. A modified RBPT was applied; however, the amount of serum in this test was 60 λ, and 30 λ of antigen was used (17).

Tube agglutination test (Wright)

First, the tubes were arranged in a tube holder. Then, 800 µL (800 λ) of physiological serum and 200 µL of serum were added to the first tube of each row, and 500 µL of physiological serum was added to the remaining four tubes of each row. The liquid contents of the tubes were serially diluted (500 µL from each tube into the next tube up to the fifth tube). Then, 500 µL of diluted Wright antigen (Razi Vaccine and Serum Research Institute, Karaj, Iran) was added to all 5 tubes of each row. The tubes were placed in an incubator at 37°C and read after 24 hours. The tubes were evaluated for the presence of agglutination; the last dilution in which agglutination was observed was considered the titer based on Wright's test (18).

DNA extraction and PCR

DNA was extracted from the samples proven positive by screening tests, using a kit (Dyna Bio, Iran) according to the manufacturer's instructions. A NanoDrop spectrophotometer was used to control the quality of extracted DNAs. Then, the isolates were stored at -20°C until PCR. The following primers (SinaClon, Tehran, Iran) (shown in Table 1) were used for the PCR test. This primer confirms the presence of *Brucella* at the genus level. The following schedule was used to assess the presence of the *BCSP31* sequence: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 65°C for 1 minute, and elongation at 72°C for 1 minute. The final elongation step was performed at 37°C for 5 minutes. The final product was electrophoresed through 1% agarose gel and the final image of the gel was recorded. For negative controls, template DNA was replaced with sterile water. The *B. abortus* ATCC23457 was used as a positive control.

Table 1. Primer Sequences of *BCSP31* Gene

Target Gene	Primer Name	Sequence	Reference
BCSP31	B4 (S)	5′-TGG CTC GGT TGC CAA TAT CAA-3′	(14)
	B5 (AS)	5′-CGC GCT TGC CTT TCA GGT CTG-3′	

Statistical analysis

Test results were performed by SPSS for Windows using a *t-test*. Differences were considered significant at $p \leq 0.05$. Additionally, Kappa analysis was performed to assess the agreement between the brucellosis-positive rates in stray dogs and herding dogs. The results of this analysis indicated a significant difference in positivity rates based on the Wright test ($p<0.05$).

Results

The normal and modified RBPT were performed on blood samples obtained from 96 herding dogs and 60 stray dogs. Out of these samples, 20 (12.8%) tested positive for *Brucella*, including 4 samples from stray dogs and 16 samples from herding dogs. Additionally, 66 samples (42.3%) were also positive, comprising 28 samples from stray dogs and 38 samples from herding dogs. A T-test showed a significant difference between the stray and herding dogs in terms of the brucellosis-positive rate based on the normal and modified Rose Bengal test ($p<0.05$). Moreover, 16 samples (10.2%), including 5 stray-dog samples and 11 herding-dog samples were positive for *Brucella* as evidenced by the Wright test. Kappa analysis showed a significant difference between the stray and herding dogs in the brucellosis-positive rate based on the Wright test ($p<0.05$). Furthermore, one herding dog (0.006%) was found positive for *Brucella* as confirmed by PCR test (Figure 1).

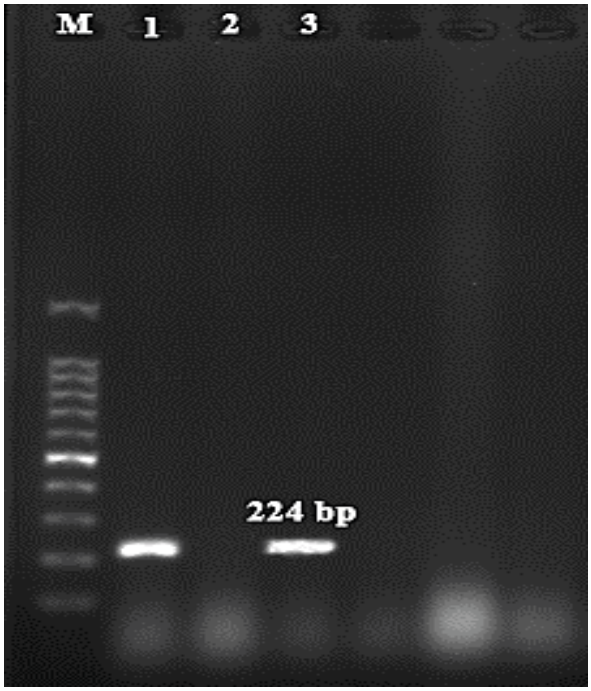

Fig. 1. Agarose gel electrophoresis of PCR product: Lane M: Gene Ruler TM 100 bp plus DNA ladder, Lane 1: Positive control; Lane 2: Negative control; Lane 3: Positive sample

Overall, the prevalence of *Brucella* infection in the samples examined by serological and molecular tests was significantly different as these tests showed a low prevalence of brucellosis in stray dogs compared to that in the herding dogs (Table 2).

Discussion

Brucellosis is sometimes neglected due to its difficult and challenging definitive diagnosis in dogs (9). This disease poses a significant threat to public health, particularly given the absence of an effective vaccine, the high incidence of relapses, and the limited treatment options available (19). Although the known cause of brucellosis in dogs is primarily *B. canis*, dogs are also susceptible to brucellosis by *B. abortus* and *B. melitensis*, and a higher prevalence has been reported in dogs in close contact with specific hosts (20). Stray dogs are more prone to be in contact with *Brucella*-infected environments and are usually infected through the ingestion of *Brucella*-infected tissues (21).

Table 2. Results of serological and molecular tests performed on stray and herding dogs

Dog	Positivity (%) (serological test)			Positivity (%) (molecular test)
	RBPT	RBPT(modified)	Wright	PCR
Stray Dog	4(6.66%)	28(46.66%)	5(8.33%)	0
Herding Dog	16(16.66%)	38(39.58%)	11(11.45%)	1

Note: RBPT: Rose Bengal Plate Test

Conversely, herding dogs are more likely to be in contact with domestic animals as the main hosts for this bacterium, and the contaminated environment. Therefore, the seroprevalence of brucellosis in herding dogs is high, which shows the possibility of the horizontal transmission of brucellosis from cattle and sheep to dogs and possibly from dogs to other dogs and humans (11, 22). It is not clear whether dogs play a role in the dissemination of disease to the sheep, but it should not be ignored, because the transmission of *Brucella* species through even non-specific hosts is possible, and cross-contamination can occur between the farm animals due to their constant contact with each other (23, 24). Infected dogs can transmit *B. abortus* and *B. melitensis* to specific hosts, humans, and other animal species, and cause adverse outcomes, such as abortion and stillbirth (11, 25). Furthermore, infected dogs can play a vital role in the persistence of *Brucella* infection in ruminants (26). On the other hand, stray dogs were less infected. In recent years, there has been an increase in the number of adopted stray dogs, which pose a health threat to their owners in case of being infected with brucellosis (27). Considering that *B. abortus* and *B. melitensis* are more pathogenic to humans, those in contact with infected dogs should maintain high-levels of standards for personal hygiene when touching their urine, feces, reproductive tissues, or aborted fetuses (28), and patients exposed to infected dogs should be tested for infection and monitored for clinical signs (25). In Iran, sheep, goats, and cattle are among the main farm animals, and *B. melitensis* and *B. abortus* have been reported in many regions (10). Therefore, the infection of dogs with *B. abortus* and *B. melitensis* is not far from expected, especially in enzootic environments where domestic animals share the same habitat with other animals, especially dogs (29). Moreover, another reason for the high prevalence of *Brucella* infection in the herding dogs can be the area (West Azerbaijan), where the dogs are kept with a very high prevalence of brucellosis (30). The high prevalence of *Brucella* infection in herding dogs in West Azerbaijan can be attributed to several factors. This region has a significant population of livestock, such as cattle and sheep, which are known reservoirs for *Brucella* species, leading to increased exposure for herding dogs. Additionally, environmental conditions, including contaminated pastures and limited veterinary care, further exacerbate the risk of infection. Consequently, herding dogs in this area not only face a higher likelihood of contracting *Brucella* but also pose a potential risk for transmission to other animals and humans (30). In general, the north and northwest of Iran are the most affected areas (14).

In this study, although the presence of bacteria was confirmed using the serological test, the molecular test, except for one case, did not show the presence of bacteria in blood samples, which may be due to the presence of bacteria in specific anatomical sites, such as reticuloendothelial organs. According to the initial invasion, the bacteria can transit into the lymph nodes and spread through the lymph and blood to other organs, including the liver, spleen, bone marrow, and other parts of the reticuloendothelial system. Temporary bacteremia also causes the spread and localization of bacteria in the genital organs and glands of adult animals (31). *Brucella* survives for a long time in the reticuloendothelial system (e.g., liver, spleen, lung, and lymph nodes) (32), and dogs also harbor the organisms for a long time in their lymph nodes, stomach, and intestines (4). *Brucella* has also been isolated from the lung, liver, spleen, and kidney of aborted sheep fetuses (33). The presence of *Brucella* in the reticuloendothelial organs of ruminants was also confirmed in Iran through the PCR test (2); however, it is not clear whether this is true for dogs. We clarify that the serological tests indicated a higher prevalence of Brucella infection, while the PCR method yielded only one positive case. This discrepancy suggests that serological tests may detect past exposure rather than active infection, potentially due to false positives in serology and the limitations of PCR in identifying bacteria residing in specific anatomical sites. Further studies on the reticuloendothelial organs, such as the liver or spleen, may confirm or deny this claim

through a PCR test. We had limitations in examining reticuloendothelial organs.

In this study, the presence of *Brucella* was confirmed in dogs and its higher prevalence was observed in dogs that were in contact with cattle and sheep and their results were compared to the stray dogs without this contact. This study confirmed the possibility of *Brucella* transmission from cattle and sheep to dogs and possibly to humans, as well as confirming the role of dogs in the disease dissemination to cattle and sheep. More studies are needed in this regard.

Conclusion

This study highlights the prevalence of *Brucella* infection among herding and stray dogs, revealing significant differences in positivity rates between the two groups. The normal and modified RBPT identified 12.8% and 42.3% of samples as positive for *Brucella*, respectively, with a notably higher rate in herding dogs. Additionally, the Wright test confirmed *Brucella* positivity in 10.2% of samples, further supporting the findings from the RBPT. Kappa analysis demonstrated a significant difference in brucellosis-positive rates based on the Wright test, underscoring the reliability of these serological methods. The PCR test provided molecular confirmation of *Brucella* in one herding dog, indicating that while the overall prevalence is low in stray dogs, herding dogs are at a higher risk for infection. These findings emphasize the need for continued surveillance and preventive measures in dog populations, particularly among herding dogs, to mitigate the risk of *Brucella* transmission to humans and other animals.

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Conflict of Interest Statement

The authors have not declared any conflicts of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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