Brucella Infection in Horses From Hamedan Province, Iran

Jamal Gharekhani*, Ehsan Barati, Ehsan Abbasi Doulatshahi, Mahdi Talaei, Mahmoud Reza Rasouli

Iranian Veterinary Organization, Hamedan, Iran

Abstract
Background: Domestic animals play a significant role in the distribution of Brucella infection to humans. Particularly, horses have a high impact on the transmission of zoonoses due to long life and multi-function. This project was conducted in Hamedan Veterinary Office for the primary assessment of Brucella infection in horses in Hamedan province using the serology technique.

Methods: Between April 2019 and January 2019, a total of 495 horse blood samples was collected from different equestrian clubs of Hamedan province. The animals were of different breeds without the clinical signs of disease. The samples were evaluated for the presence of anti-Brucella antibodies using the Rose-Bengal plate test (RBPT).

Results: All animals were negative for Brucella antibodies. To the best of our knowledge, this is the first evaluation of Brucella infection in horses from an intensive system in Iran. The prevalence of brucellosis in horses was low and it had no significant impact on the transmission of infection to humans.

Conclusions: Regular screening programs, along with the quarantine and elimination of animal transfer are essential for controlling the disease.

Keywords: Brucella, Brucellosis, Rose Bengal, Horse, Quarantine, West of Iran

Background
Brucellosis, as a common zoonotic disease from Iran, is a particularly essential animal disease that is occasionally observed in horses (1,2). Livestock plays an important role in supplying milk, meat, and wool in Iran. Additionally, horses are used as farmworkers, and for transport, equitation, and sports in rural and suburb areas of urban regions. Therefore, they play a significant role in the distribution of infection in animal to animal and/or animal to human models (3). Three species of Brucella (i.e., B. abortus, B. suis, and B. canis) are the causes of brucellosis in the horse but B. abortus is the dominant species (4).

Genitally tract disorders and reproductive failures such as abortion and stillbirth are the routine findings of infections in animals. Clinical symptoms in horses are termed as "poll-evil" or "fistulous withers", leading to abscess and fistulation in the affected location (5). The isolation of bacteria is a gold standard for diagnosis although techniques based on immuno-serology such as Rose-Bengal plate test (RBPT) and standard tube agglutination test (STAT) are user friendly because of rapid function and low cost (2,5).

There are 1750 horse populations in the equestrian clubs, breeding farms, and rural regions of Hamedan province. According to the Iranian Veterinary Organization (IVO) approach, there is no vaccination program against brucellosis in horses (1). Brucellosis in horses from Iran and other countries (Figure 1) was reported in limit scales (3,6-11). To compensate for this fracture, the current project was designed to screen Brucella infection in the horses of the region based on serology.

Methods
In a cross-sectional study conducted during April 2019-January 2019, 495 blood samples (5 mL from the jugular vein) of the horses were collected from different equestrian clubs (n=24) in Bahar, Razan, Asadabad, and Hamedan in Hamedan province (Figure 1). The animals were of different breeds without the clinical signs of disease. The samples were taken from the animals after obtaining official permissions and under the supervision of the Institutional Animal Ethics and Research Committee of IVO (Certificate No. 98/3127/27838).

After the preparation of sera samples (centrifuge at 1200 xg for 12 minutes), they were screened for the presence of anti-Brucella antibodies using the RBPT. Briefly, equal volumes (30 μL) of RBPT antigens (Razi Vaccine and Serum Institute, Iran) and serum samples were placed on a white ceramic tile and then mixed and rocked gently for 4 minutes. The reaction was recorded positive when the agglutination was observed and the antigens could detect B. abortus, B. melitensis, and B. suis (12).
Results and Discussion

Background on the prevalence and risk factors associated with brucellosis in animals is extremely important because of the zoonotic impact (1). In our serology work, all animals were negative for Brucella antibodies using the RBPT parallel to other studies conducted in the central regions (Yazd and Kerman) and the north (Torkaman-Sahra) of Iran (13-15). In prior serology projects implemented in Iran, the seroprevalence of Brucella infection in horses was recognized 0.5% in the rural regions of Hamedan (16) and zero to 11.1% in other districts (15,17). This rate was detected zero in Arab (14) and Turkaman (13) breeds and 4.9% in native breed horses (18). Seronegative latent infections can occur in animals without antibodies (10,19). In the study by Badiei et al (4), the seroprevalence of brucellosis was found to be 9.9, 8, and 7% by RBPT, STAT, and 2-mercaptoethanol techniques, respectively. RBPT has a high sensitivity of about 99% (4). In addition, the sensitivity and specificity of the STAT are 95.6 and 100%, respectively (20). In a study using molecular tools, one of the three seropositive horses with the fistula sign was positive with the field strain of Brucella spp. (4). According to Keid et al (21), a negative blood culture or polymerase chain reaction cannot always be relied upon for diagnosis of brucellosis, especially in chronically infected cases since the stage of Brucella infection may influence the number and location of bacteria (22).

Hamedan province with the incidence of 31-41 per 100,000 in human brucellosis is classified as the “very high” category in Iran (2). This rate was reported as 3%, 4.6%, and 3.3% in the sheep, goats, and dogs in the region, respectively (12,23). The detection of the infection source and related risk options is very helpful for reducing the infection rate. Therefore, the direct contact of different types of animals together and with humans is a predominant risk factor for the transmission of the disease to livestock and humans (9). For limiting the infection in humans, the disease must be controlled in animals. Brucellosis is one of the important obstacles to animal commerce in national and/or international levels. To achieve this goal, the close cooperation of medical and veterinary officers is essential using a ‘One Health’ approach (24). Animals transfer in border regions is fused in the culture and economics of people. Consequently, the regional decision approach is a practical method for controlling the disease. Further, the high infection in border regions is a risk factor, especially in Iran. Thus, the observance of hygienic standards, the guidelines of quarantine and animal spirit is highly recommended (24). In conclusion, to the best of our knowledge, this is the first evaluation of Brucella infection in horses from an

Figure 1. Sampling Sites in Hamedan Province and the Seroprevalence Rate of Brucella Infection in Horses From Different Regions of Iran and Neighboring Countries.
intensive system in Iran, which was applied for designing and developing future investigations. According to our outcomes and those of other researchers in Iran, the prevalence of brucellosis in horses was low and its impact on the transmission of the infection to humans was not significant. Complex risk factors such as the climate and close contact of different types of animals influence the prevalence and distribution of infections. In the exact control and eradication process, a combination of different measures including regular screening programs, test and slaughter, as well as the vaccination and quarantine of suspected animals is necessary.

Conflict of Interest
None.

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Ethical Approval
The samples were taken from the animals with official permission and under the supervision of the Institutional Animal Ethics and Research Committee of Iranian Veterinary Organization, Hamedan office, Iran (Certificate No. 98/3127/27938).

Author’s contributions
JG designed the study and writing the paper. EAD and EB helped for laboratory techniques. MT helped for sampling and animals’ data, and MRR helped for designing the study and revised the final version of manuscript. All authors reviewed the manuscript.

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References
14. Rafeie-Isharebaki M. Seroprevalence of Brucella antibodies in Arab horses from Yazd province [dissertation]. Kerman: Faculty of Veterinary Medicine, Shahid-Bahonar University; 2018. [Persian].
15. Taheri O. Seroprevalence of Brucella infection in horses from Kerman province [dissertation]. Kerman: Faculty of Veterinary Medicine, Shahid-Bahonar University; 2018. [Persian].
17. Lotfi Z. Seroprevalence of Brucella infection in horses from Khouzestan province [dissertation]. Kerman: Faculty of Veterinary Medicine, Shahid-Chamran University; 2017. [Persian].
18. Nemati Y. Seroprevalence of Brucella abortus in native horses from Tabriz suburb [dissertation]. Kerman: Faculty of Veterinary Medicine, Tabriz University; 2017. [Persian].