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Mini-Review

Brucellosis in Iranian buffalo: prevalence and diagnostic methods

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Abstract

The water buffalo (*Bubalus bubalis*) in Iran represent an important source of meat and milk products with high biological value. Given the importance of water buffalo in Iran and the prevalence of brucellosis as one of the most important zoonotic diseases in this ruminant species, this study summarized available data on history, epidemiology, diagnosis, and control of brucellosis in water buffalo from previous studies that have been carried out in Iran. According to the documented data, there are three main groups of Iranian buffalo, including the Khuzestan ecotype (Khuzestan province); the Azary ecotype (Western/ Eastern Azarbaijan and Ardabil provinces); and the North ecotype (Gylan and Mazandaran provinces). Preliminary studies conducted on Iranian buffaloes either by serological or molecular tools reported that buffaloes' infection occurred due to natural exposure to a wild strain of *Brucella abortus* and *Brucella melitensis*. Previous studies dealing with brucellosis in Iranian buffalo are next to none. This review notifies the importance of reliable and detailed epidemiological investigations of Iranian buffaloes through continuous monitoring systems of the health status of buffalo populations. Continuous test and slaughter strategy, vaccination, and re-planning of veterinary activities are required to mitigate buffalo's role in disseminating and maintaining brucellosis in Iran.

Keywords: Water buffaloes, *Bubalus bubalis*, Brucellosis, *Brucella abortus*, *Brucella melitensis*, Iran

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Introduction

Brucellosis is a highly contagious zoonotic infection caused by facultative intracellular bacteria of the genus *Brucella* (*B.*). The pathogen affects a wide variety of livestock and wildlife species and humans. The incidence of brucellosis is fluctuating, and new hot spots continue to appear, especially in the Middle East and African countries (Wang and Jiang, 2020). Brucellosis is a true one-health disease in the Middle East region, prevalent in humans, animals and reported in several studies on milk and milk products (Abedi et al., 2020; Bagheri Nejad et al., 2020). It has significant impacts on developing countries with high economic losses in animal production due to sterility, abortions, decreased milk production, costs of replacement animals, and veterinary fees (McDermott et al., 2013; Dadar and Alamian, 2020).

The main aetiological agents in large and small ruminants are *B. abortus* and *B. melitensis*, respectively. Brucellosis in livestock animals in Iran has been reported in different parts of the country since the early 1930s (Esmaeili, 2014). Iran represents an endemic region for animal and human brucellosis in the Middle East (Dadar et al., 2019a, 2021), and bovine brucellosis in cattle population has been extended across Iran. *B. abortus* was isolated for the first time from an aborted fetus in 1944 (Esmaeili, 2014). Previous investigations identified various biovars of *B. abortus* (predominantly biovar 3) from cattle and sheep. Besides, *B. melitensis* biovars 1, 2, and 3 (predominantly bv1) were isolated from sheep, goats, camels, cattle, dogs, and humans (Zowghi et al., 1990; Dadar et al., 2019a). However, other *Brucella* species, including *B. ovis*, *B. suis*, *B. neotomae*, and *B. canis* were not documented.

The water buffalo (*Bubalus bubalis*) is an economically important livestock species in different parts of the world, including Iran. Until now, only four studies in Iran investigated the presence of *B. abortus* and *B. melitensis* in aborted fetuses, blood, and semen samples of buffaloes by Real-time Polymerase Chain Reaction (RT-PCR) and serological methods (Nowroozi-Asl et al., 2007; Dehkordi et al., 2012, 2014; Alizadehmofrad and Parvini, 2017). The present review highlights the importance of epidemiological analysis and investigations of brucellosis in Iranian buffalo, considering the importance of buffalo in Iran.

Brucellosis in Iranian livestock

Over the last decades, Iran faces serious challenges in controlling animal and human brucellosis, which induce significant economic losses and public health is-

sues (Dastjerdi et al., 2012; Esmaeili, 2014; Dadar and Alamian, 2020). In Iranian livestock, there are several reports of *Brucella* spp. infections that have destructive effects on fetal development and reproductive systems led to eradicating failure, infertility, and abortions (Dadar et al., 2019b; Alamian and Dadar, 2019; Dadar et al., 2020). Although long and sustainable efforts in several developed countries led to eradicating brucellosis from livestock (Godfroid, 2017), the infection remains a major issue in endemic countries such as Iran. Moreover, the eradication or control of livestock brucellosis needs sustainable budgets for long-term and systematic surveillance strategies (Zhang et al., 2018). *B. abortus* biovar 3 and *B. melitensis* biovar 1 are the most prevalent isolates in different Iran regions (Zowghi et al., 2008; Dadar et al., 2019a; Alamian et al., 2020; Dadar and Alamian, 2020).

A preliminary epidemiological study of animal brucellosis in Iran reported the high prevalence of the disease in livestock population, reaching 17.6% in cattle and 14.7% in sheep and goats in different areas (Sabbaghian and Nadim, 1974; Zowghi et al., 2008). Different livestock species, including cattle, buffaloes, sheep, goats, and camels, were infected (Akbarmehr and Ghiyamirad, 2011; Alizadehmofrad and Parvini, 2017; Alamian and Dadar, 2019; Dadar et al., 2019a; Dadar and Alamian, 2020; Dadar et al., 2020). Although important epidemiological studies have been performed at regional levels on different livestock in various Iranian districts, some areas remain uncovered with a real lack of prevalence data regarding animal brucellosis. Furthermore, the prevalence value of animal brucellosis in Iran could not be reported easily because of a lack of population statistics.

Water buffalo population of Iran

Water buffalo production was settled and domesticated in Iran since 2500 B.C. and then migrated to southern Europe through this region (Naserian and Saremi, 2007). The ancestry of Iranian buffaloes has phenotypic similarity to Indian buffaloes such as Murrah, although their origin is not clearly identified. Iranian buffaloes can be classified according to climate conditions in three main groups: the Khuzestan ecotype in Khuzestan province, the Azary ecotype in Western and Eastern Azarbaijan and Ardabil and the North ecotype in Gylan and Mazandaran provinces. According to the Ministry of Agriculture statistics, the water buffalo population in Iran is about 200,000 animals in 2021. The total production of buffalo milk and meat in Iran is 293K tons and 24.7K tons, respectively (Madad et al., 2013).

Most of this population is scattered in the provinces of Khuzestan (28%), West Azerbaijan (26%), East Azerbaijan (20%), Ardabil (16%), the provinces of Gilan, Mazandaran and Golestan (about 8%), as well as in other provinces of Iran. More than 28% of Iran's water buffalo population is reported in Khuzestan, with a herd size of 5 to 300 animals and an average annual population growth of 2.6% (Naderfard and Qanemy, 1997; Ahmadzadeh et al., 2019). Iranian water buffalo is one of the most important livestock with a significant contribution to meat and milk production. These animals have a critical role in Iran's rural family economy because of their unique properties, such as resistance to parasites and diseases, showing a higher capability of consuming low-quality forage and having a long productive lifespan (Safari et al., 2018). The commercial value of Iranian water buffaloes is approximately equal to a pure Holstein dairy cow. More than 75% of water buffaloes in Iran are dairy breeds. It has been reported that 16% of the Iranian buffaloes are slaughtered for meat production, with a total meat yield of 12,960 tons per year and an average carcass weight of 162 kg per buffalo (Naserian and Saremi, 2007).

Brucellosis in water buffaloes of Iran

There are few data regarding the prevalence of brucellosis in Iranian water buffaloes. There are no reports for specific clinical signs of brucellosis in Iranian buffaloes. The clinical signs are similar to those seen in cattle or goats and sheep and are most often associated with reproductive failure, causing abortion in the last trimester of gestation accompanied by grayish or white mucoid discharges from the vagina, decreased fertility in females and males, and weak calves (Ayala et al., 2019). Brucellosis also can reduce milk production in buffaloes (Sousa et al., 2017). A serological investigation to evaluate the prevalence of brucellosis in 400 blood samples collected from water buffalo in Khuzestan province revealed a prevalence of 20.5%, 19.5% and 11% by Rose Bengal Test (RBT), tube agglutination test and 2-mercaptoethanol test, respectively. The study also proposed that the water buffaloes in Khuzestan province are a reservoir for *Brucella* spp. and significantly impact the epidemiologic pattern of livestock and human brucellosis in Southwestern Iran (Nowroozi-Asl et al., 2007).

Dehkordi et al. (2012) have also evaluated the presence of *B. abortus* and *B. melitensis* in water buffalo fetuses by conventional and RT- PCR. Statistical analysis showing remarkable differences between the prevalence of *B. abortus* and *B. melitensis* in abomasal contents of aborted buffaloes. In total, 32.94% of samples showed positive results for *Brucella* spp. by molecular methods. Among buffalo semen samples collected from four major provinces of Iran (Khuzestan, Sistan Va Balochestan, Boshehr and Hormozgan) in various seasons, 14.28% were positive for *B. abortus* and *B. melitensis* by molecular methods. Sistan Va Balochestan province had the lowest prevalence, while Khuzestan had the highest prevalence of *Brucella* spp. This study also showed that 1.09% and 15.38% of buffalo semen samples were positive for *B. melitensis* and *B. abortus*, respectively (Dehkordi et al., 2014).

In another study, 40 raw buffalo milk samples from dairy farms in Tabriz were randomly selected in the winter of 2017 and used for *Brucella* investigations by Milk ring test and ELISA. The analyzed samples showed that *B. abortus* was found in 14.11% of raw milk samples. This finding indicated that buffaloes' raw milk could be a significant source of infection with

B. abortus, and it is necessary to prevent the distribution of contaminated milk with careful and appropriate handling (Mohsen Azizi Allah, 2018). *Brucella* infections were also confirmed in female buffaloes slaughtered in Khuzestan province by serological and bacteriological examination. The contamination rate was reported as 17% by RBT and 14% by Wright and 2-mercaptoethanol test (Azarkamand et al., 2017). However, *B. abortus* has been reported as the predominant agent causing brucellosis in water buffaloes of Iran (Dehkordi et al., 2012). Still, a comprehensive epidemiological investigation of *Brucella* infection has not been performed in Iran.

Diagnostic, control, and prevention of brucellosis in buffalo

Diagnosis of brucellosis in buffaloes can be performed by direct and indirect approaches. Indirect methods such as serological assays can detect anti-*Brucella* antibodies in the serum and are an excellent tool due to the convenience, speed, and low cost. The methods recommended by OIE for brucellosis diagnosis in buffaloes are the same recommended for cattle, including the milk ring test (MRT) and the Rose Bengal test (RBT), which are used as screening tests, and serum agglutination test (SAT), 2-Mercaptoethanol (2-ME), and the complement fixation tests (CFT), which are used as confirmatory tests. It is worth mentioning that Iranian literature cites several serological tests such as RBT, i-ELISA, SAT and 2-ME to investigate the presence of antibodies against *Brucella* on the serum samples of water buffaloes (Nowroozi-Asl et al., 2007; Azarkamand et al., 2017).

Among these methods, the most accurate approach is the i-ELISA (Azarkamand et al., 2017). However, other serological tests are also used for detecting *Brucella* antibodies in buffaloes as alternative methods in serological surveillance for primary detection of infected buffaloes (Nowroozi-Asl et al., 2007; Ramnanan et al., 2012). The direct diagnostic methods for brucellosis can be performed by bacterial culture and nucleic acid detection. RT-PCR has been validated for direct diagnosis of *Brucella* spp. with high levels of specificity and sensitivity in Iranian buffalo (Dehkordi et al., 2012, 2014).

Perspective and future recommendations

Brucellosis control is a critical program to preserve the genetic diversity of water buffaloes in Iran. Domestic water buffalo in Iran are raised in small herds. However, there are a few large herds of dairy and beef buffaloes that are intensively managed. It has been revealed that the RB51 vaccine does not adequately protect against *Brucella* infection in water buffaloes (Fosgate et al., 2011; Sousa et al., 2017). However, the development of effective vaccination protocols is an essential requirement for brucellosis control in buffaloes. Control of brucellosis in buffaloes in several countries has been performed according to the vaccination of three- to eight-month-old females with the *B. abortus* vaccine strain B19 (similar to cattle) with successful results. The vaccine strain B19 has been successfully applied in buffaloes and cattle since 1930, with adequate results, as indicated by inducing adequate immunization to prevent brucellosis (Crasta et al., 2008). However, there are no active control programs of brucellosis in buffaloes in Iran.

Control of brucellosis in buffaloes is challenging in Iran due to the lack of specific health control programs for buffaloes and the difference in these animals' ecotype populations. The best way for brucellosis control in Iranian buffaloes is the simultaneous approaches of test-and-slaughter program, elimination of positive animals, and vaccination of three- to eight-month-old females. Finally, it is important to evaluate all differences in the brucellosis epidemiology between cattle and buffaloes and within the buffalo ecotypes population. Furthermore, isolation, identification, and molecular characterization of *Brucella* spp. in water buffaloes are highly recommended to identify the source of infection and define an accurate conceptual framework for appropriate control programs.

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