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Review Article

Brucellosis Management in Dairy Cattle Farms: Reviewing Programs and Addressing Challenges in Iranian Dairy Cattle Farms

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Abstract: Brucellosis, a zoonotic infection caused by *Brucella* species, remains a significant public health issue, affecting both animal and human health and the economy globally. This review focuses on brucellosis in dairy cattle farms in Iran, examining its prevalence, distribution, and control strategies. A systematic review of studies from databases like PubMed, Scopus, and Google Scholar found that *Brucella abortus biovar* 3 and *B. melitensis* biovar 1 are the main strains in Iranian dairy cattle. The seroprevalence ranges from 3.94 to 19.8%, with B. abortus biovar 3 notably prevalent. Since 1967, Iran's control strategies have included mass vaccination with the RB51 vaccine, testing, culling, and movement control. However, challenges such as vaccine efficacy, especially against *B. melitensis*, and difficulties in rural implementation persist. The findings underscore the complexity of controlling brucellosis in Iran due to regional variability in disease prevalence and the challenges associated with vaccine efficacy and implementation. The current control strategies face significant barriers, particularly in rural and traditional farming systems. Therefore, there is a need for more targeted interventions, including improved diagnostic methods, enhanced farmer education, and better implementation of quarantine measures.

Keywords: Brucellosis, Control Program, Iran, Dairy Cattle Farm, Management

Introduction

Brucellosis is a widespread zoonotic disease caused by rod-shaped, gram-negative bacteria belonging to the *Brucella* genus (McDermott *et al.*, 2013). *Brucella* is a genus within the Brucellaceae family, classified under the alpha-proteobacteria class. Thirteen species of *Brucella* have been reported from various animals. These species have been classified into two groups: "Classical *Brucella*" species share highly similar metabolic pathways and exhibit remarkable genome uniformity, while "Atypical *Brucella*" species differ by 2–3 nucleotides in the recA and 16S rRNA gene sequences and show stronger metabolic activity than the classical forms (Scholz *et al.*, 2016). A number of developed countries such as Australia, New Zealand, Japan, the United Kingdom, Canada, and several European countries, including Belgium, Finland, Denmark, Germany, Ireland, the Netherlands, Luxembourg, and Sweden, have successfully eliminated the disease. Nevertheless, it remains a significant public health issue across Asia, Africa, the Mediterranean, Latin America, and the Middle East (Zhang *et al.*, 2018). Brucellosis reduces cattle productivity and restricts the global movement of livestock due to trade limitations, resulting in significant financial losses (McDermott *et al.*, 2013). In addition, the widespread occurrence of animal brucellosis presents a serious threat to human health, as people may become infected through direct interaction with infected animals or by consuming contaminated dairy and meat products (Dadar *et al.*, 2020b). Therefore, the World Health Organization (WHO) and various development agencies have recognized brucellosis control as a crucial goal for promoting economic growth (WHO, 2015). Although the disease has a major economic effect on many low-income countries, it does not receive sufficient attention from national healthcare systems (Godfroid *et al.*, 2013). Implementing brucellosis eradication or control strategies varies significantly across different countries due to their unique national circumstances. While several countries such as the United States of America, Canada, Australia New Zealand, and many European Union (EU) nations have achieved significant progress in controlling animal brucellosis, achieving complete eradication continues to be a challenging task in the majority of developing countries such as Iran (Boral *et al.*, 2009; Moriyón *et al.*, 2023). Thus, there is an urgent need to strengthen programs in low-resource nations for better surveillance and control of this disease (Godfroid *et al.*, 2013). One approach for strengthening these countries is the 'One Health' approach, which combines human and animal health as well as the ecosystem in which they live. The benefits of cross-sector collaboration guided by the 'One Health' framework are crucial for the efficient surveillance and management of neglected zoonotic diseases like brucellosis. Global coordination, active national involvement, and measurable outcomes at the local level are essential to improving the application of cross-sectoral approaches.

Materials and Methods

For this review, data were collected from various sources to provide a comprehensive overview of brucellosis in dairy cattle farms in Iran. Primary data sources included peer-reviewed journal articles and official reports from governmental organizations. The literature review was performed by searching online databases such as Scopus, Google Scholar, and PubMed. The search terms included "brucellosis," "*Brucella*," "dairy cattle," "Iran," "control strategies," and "zoonotic diseases." This review included studies that met the following criteria: They concentrated on the occurrence, distribution, and management of brucellosis in dairy cattle farms in Iran; they provided data on control strategies, challenges and outcomes related to brucellosis management; and they were published in English or Persian as peer-reviewed articles, official reports or studies with a clear methodology and data presentation. The procedure for gathering data included examining the chosen studies to collect information regarding the prevalence and geographic distribution of brucellosis, as well as methods of control such as vaccination, testing, culling, quarantine, and farmer education, challenges in implementing control programs, risk factors contributing to brucellosis persistence, and recommendations for improving control programs. The data were synthesized by categorizing the information into these key areas to offer a structured overview.

Results

Brucella Species in Dairy Cattle Farms Across Iran

Brucella species have been identified in dairy cattle farms across Iran, with earlier estimates indicating a 0.3% prevalence of brucellosis among cows in both semi-industrial and industrial operations. However, this estimation does not consider other traditionally reared animals, which may contribute to a greater prevalence (Esmaeili, 2014). In several more recent studies, Iranian industrial and semi-industrial dairy farms of different regions have been monitored for brucellosis, and a frequency of 3.94% up to 19.8%, with a predominance of *Brucella abortus* biovar 3 and *Brucella melitensis* biovar 1 was found (Dadar *et al.*, 2019; Alamian *et al.*, 2021; Dadar *et al.*, 2021a; Bahreinipour *et al.*, 2023). Between 2016 and 2023, an extensive field study was carried out on industrial cattle farms throughout Iran, covering more than 200 farms across 21 provinces. The analysis of bovine samples revealed that 70.5% of the farms that tested seropositive also showed positive results through bacterial isolation. The most frequently identified strains were *B. melitensis* biovar 1 (43.5%) and *B. abortus* biovar 3 (27.2%). An analysis of their geographical distribution revealed that *B. melitensis* biovar 1 was the dominant strain across dairy cow farms in various regions, with *B. abortus* biovar 3 being the next most common (Dadar *et al.*, 2019; Alamian *et al.*, 2024; Dadar *et al.*, 2024b). In a separate investigation, researchers evaluated the seroprevalence of brucellosis in industrial dairy cattle farms across various regions of Iran by conducting multiple serological tests on a total of 2112 samples. The Rose Bengal plate test (RBPT), serum agglutination test (SAT), and 2-mercaptoethanol test (2-ME), as well as the indirect enzyme-linked immunosorbent assay (I-ELISA), yielded positive findings in 296 (14.02%), 215 (10.18%), and 297 (14.06%) samples, respectively. *B. abortus* biovar 3 caused most brucellosis in the animals investigated (Alamian *et al.*, 2023a-b). A meta-analysis of available studies on brucellosis among Iranian livestock estimated an overall seroprevalence of 3% in cattle. Seroprevalence rates were as high as 75% in West Azerbaijan and as low as 1% in Charmahal Bakhtiyari and Khuzestan. Limited data from some regions was a key limitation, which pointed to the necessity for further research, particularly in endemic areas, to inform control strategies. Improving vaccination programs and education for farmers are recommended in the high-prevalence regions (Adabi et al., 2024). Moreover, the seroprevalence of brucellosis was significantly lower in periurban dairy cattle in Iran compared to those in rural areas, highlighting the urgent need to enhance vaccination efforts and implement an effective test-and-slaughter strategy among rural herds (Alamian *et al.*, 2021). A separate investigation reported the presence of brucellosis among dairy cattle herds located in the Alborz and Tehran provinces of central Iran as 19.8, 6.7, 5.1, 14.1, and 13.1% using the RBPT, SAT, and I-ELISA as well as the Milk Ring Test (MRT), respectively. Overall, bacterial cultivation and molecular approaches show *B. abortus* and *B. melitensis* in Iranian dairy cattle farms (Dadar *et al.*, 2019; Abnaroodheleh *et al.*, 2023). However, it is unclear which of the two species is predominant in Iran due to different results

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between varying studies, most likely based on regional differences (Dadar *et al.*, 2019; Alamian *et al.*, 2024; Dadar *et al.*, 2024b).

Management or Elimination of Brucellosis in Dairy Cattle Operations

One of the most important measures to contain neglected diseases such as brucellosis is effective control and eradication programs on a national level. Typical control and eradication programs of brucellosis include, amongst others, surveillance, testing, and culling as well as vaccination (Alamian *et al.*, 2023a; Dadar *et al.*, 2023). The selection of a suitable brucellosis control or eradication program is based on various reasons apart from minimizing the risk to human health, including substantial financial losses, trade limitations, and animal wealth (Zamri-Saad and Kamarudin, 2016). Initially, animal husbandry served as the main industry in affected countries or regions with a significant population of cattle and a worldwide market for meat products. Consequently, the occurrence of brucellosis resulted in substantial financial losses (Samartino, 2002; Zamri-Saad and Kamarudin, 2016). Furthermore, the implementation of strategies to control and eliminate brucellosis may successfully prevent animal abortion and decrease losses in milk production, resulting in a significant reduction in economic losses (Zhang *et al.*, 2018). Additionally, successful control programs can boost the agricultural economy by reducing trade limitations and increasing exports, since many countries have restrictions on cattle and animal products import and export to prevent brucellosis (Can and Yalçın, 2014; Zhang *et al.*, 2018; Coelho *et al.*, 2019). Brucellosis control methods, which often involve test-and-slaughter programs, vaccination campaigns, or a combination of both, are based on the current disease prevalence and reporting rates. However, these programs can be very expensive, especially in developing countries such as Iran, and a cost-benefit analysis is necessary (Gwida *et al.*, 2010; Coelho *et al.*, 2011; Can and Yalçın, 2014).

Brucellosis Control Programs in Iran

Bovine brucellosis has been recognized in Iran since 1944, when *B. abortus* was first detected in cattle (Zowghi and Ebadi, 1982). Iran commenced bovine brucellosis control in 1967. The control programs for brucellosis in dairy cattle farms in Iran typically follow a set of measures aimed at the detection, prevention, and eradication of the disease. In 1967, the initial bovine brucellosis program involved vaccinating adult cows and calves aged 3 to 8 months with the S19 brucellosis vaccine (Esmaeili *et al.*, 2012). Subsequently, the Iranian Veterinary Organization (IVO) launched a control program to address bovine brucellosis in dairy farms. The ongoing program includes widespread vaccination with RB51, a test-and-slaughter approach, farmer education, and quarantine measures aimed at eliminating the disease (Yazdi *et al.*, 2009). The Iranian brucellosis active care program, a combination of mass-vaccination and test-and-slaughter strategy, is directed towards semi-industrial and industrial cattle farms supervised by the IVO (https://ivo.ir). This program targets animals eligible for blood sampling and serological tests, encompassing all female animals older than 4 months and bulls. Mandatory blood sampling is required for female animals older than 12 months, including those that are pregnant and non-pregnant as well as for bulls. However, the decision to conduct blood sampling for female calves between the ages of 4 to 12 months is left to the discretion of the veterinary department of each province. Furthermore, if the private sector in non-industrial dairy farms and rural areas reports suspected cases of brucellosis, passive surveillance will be initiated. This includes conducting blood sampling and serological tests in the mentioned herds and farms. The private sector is essential in the early detection and reporting of suspected cases. Individuals or entities within the private sector such as farmers, veterinarians or livestock owners are often the first to notice symptoms or behaviors in their cattle that may indicate brucellosis. These suspicions are the initial step in the process, prompting the need for reporting.

Role of Testing in Iran's Brucellosis Control Program

Implementing serological or other diagnostic tests is crucial for the early detection of infected individuals. Early detection facilitates prompt isolation or removal of infected animals, preventing further transmission within the herd or to other livestock. Thus, once a suspected case is reported, the next critical step is the confirmation of brucellosis diagnosis. This confirmation is typically carried out by IVO's accredited and approved laboratories. These specialized laboratories are equipped with the expertise and advanced diagnostic tools required for accurate animal brucellosis testing. Therefore, brucellosis is typically diagnosed through the use of serological and bacteriological assays (Gall and Nielsen, 2004). The specificity and sensitivity of the applied serology tools are crucial, since both parameters may lead to misdiagnosis of positive animals, which then leads to continued transmission. Therefore, isolating and identifying *Brucella* spp. is considered the most effective and dependable method for diagnosing and confirming brucellosis (Andrade *et al.*, 2024). The probability of acquiring a positive culture from a living infected animal is significantly diminished when samples are not obtained after an abortion (McDermott *et al.*, 2013). Furthermore, the utilization of culture for large-scale purposes is impractical due to the significant expenses and the potential threats to biosafety, given that *B. abortus* has been classified as a biosafety level 3 bacterium (Nielsen 2002). This technique undoubtedly poses a considerable risk of infection to laboratory personnel (Pereira *et al.*, 2020). Due to their accessibility, alternative diagnostic techniques for brucellosis, including PCR tests on milk, swabs, and blood/serum, are frequently utilized. Nevertheless, the reliability of these tests has not been fully validated, and the existing data on them is inconsistent (Nielsen, 2002; McDermott *et al.*, 2013). Considering the drawbacks of direct methods, serological tests serve as a dependable diagnostic tool because they make use of readily available clinical samples, are relatively simple to perform, and are cost-effective (Nielsen, 2002). There are several serological tests used to diagnose brucellosis but diagnosing brucellosis is not straightforward due to differences in test accuracy (including diagnostic sensitivity and specificity), variations among tests, and the epidemiology of the disease. Combining multiple tests and considering the context are important for accurate diagnosis and appropriate management of brucellosis in individual and flock scales of animals (Greiner and Gardner, 2000; McKenna and Dohoo, 2006). Historically, the diagnosis of bovine brucellosis has followed a sequential approach, starting with screening and followed by confirmatory tests (McKenna and Dohoo, 2006). Screening assays such as the Buffered Plate Agglutination Test (BPAT), the RBPT, and the I-ELISA are very sensitive, inexpensive, and quick. However, any positive results from these serological tests must be confirmed through additional testing (Gall and Nielsen, 2004). Confirmatory tests are known for their high specificity and excellent sensitivity. Nevertheless, they typically necessitate sophisticated equipment and expert interpretation of data. Examples of such tests are the Complement Fixation Test (CF) and Fluorescence Polarization Assay (FPA) (Nielsen, 2002).

In Iran, the RBPT is used routinely to screen for brucellosis in the primary step. Then, the positive samples will be tested using complementary tests such as Wright's seroagglutination test and 2-mercaptoethanol (2-ME). However, these tests may not be the best choice for confirmatory testing due to their limitations in specificity and cross-reactivity (Di Bonaventura *et al.* 2021). Considering the disadvantages of Wright's seroagglutination test and 2-ME mentioned above, if Iran adopted the WOAH (former OIE) recommended FPA and CF tests, this could result in a more precise and accurate diagnosis of bovine brucellosis (Saavedra *et al.*, 2019). However, the decision to switch to these tests should consider factors such as cost, availability, and laboratory infrastructure. Additionally, I-ELISA, competitive ELISA (C-ELISA), PCR, and bacterial culture are used as alternative methods for the final confirmation of bovine brucellosis in animals at certain laboratories in Iran (Abnaroodheleh *et al.*, 2023; Dadar *et al.*, 2024b).

Culling as a Measure to Control Brucellosis in Iranian Dairy Cattle Farms

Testing plays a crucial role in Iran's brucellosis control program, as it helps determine the necessity of culling infected animals in both industrial and semi-industrial dairy farms. However, in rural and traditional cattle farms, testing and slaughtering is only done if the animals are insured and the necessary credits are provided for the operation from provincial sources. Culling infected animals can effectively prevent the spread of brucellosis within the herd. However, to ensure the efficacy of culling, it is important to identify and remove all infected animals from the herd or the infection may persist as shown by Alamian *et al.* who demonstrated that subsequent serological testing confirmed animals with positive serology for a period exceeding 6 months after culling, most likely attributable to overseen infected animals (Alamian *et al.*, 2023a).

Importance of Vaccination in the Iranian Control Program

Implementing more focused and precise control techniques in the control measurements for brucellosis could prove to be more effective (McDermott *et al.*, 2013; Dadar *et al.*, 2021b). In areas with endemic brucellosis or a high prevalence of enzootic diseases, like Iran, it is strongly recommended to vaccinate cattle. Herd immunity and individual animal protection from vaccination reduce disease prevalence in the population. Vaccination is likely the best control approach because eradication may be too expensive (Makita *et al.*, 2011; Deka *et al.*, 2018). Therefore, mass vaccination is the first step in brucellosis control, especially in endemic areas. B. abortus S19 is the predominant and pioneering vaccine (documented in 1930) utilized to combat brucellosis in cattle. The S19 vaccination provides effective protection against brucellosis and offers immunity to cattle against *B. abortus* and *B. melitensis*. It is highly effective even after just one dose, offering long-lasting immunity (Moreno *et al.*, 2022; Moriyón *et al.*, 2023). Due to the absence of patents, S19 is sold at a comparatively modest price range of US$ 0.05–0.20 per dose. Recent studies show that a single dose of these vaccinations protects against bacterial challenges that infect 80-100% of unvaccinated people.

Revaccination does not enhance resistance in the case of S19 (Barrio *et al.*, 2009; Chand *et al.*, 2015; Goodwin and Pascual, 2016). The *B. abortus* RB51 vaccinations are safe and efficacious against brucellosis in cows and do not affect diagnostic serology (Moriyón *et al.*, 2004; Goodwin and Pascual, 2016). It has been effectively employed in the United States since 1996 to prevent and eliminate bovine brucellosis and has also been recommended for use in Iran by IVO. While RB51 has shown effectiveness in protecting against mild challenges in controlled environments, there have been multiple instances of field infections in animals vaccinated with RB51. It is worth noting that even revaccinated animals have experienced these field infections, while this vaccine's protective duration is unclear (Olsen, 2000; Moriyón *et al.*, 2004). Thus, it is necessary to evaluate and monitor the effectiveness of the vaccine after the vaccination of animals.

The widespread occurrence of bovine abortions caused by *B. abortus* led to the initiation of nationwide brucellosis vaccination campaigns in Iran in 1967 (Esmaeili, 2014). During this period, the S19 vaccine was administered to adult cows and calves aged 3 to 8 months (Esmaeili, 2014). However, the use of the S19 vaccine for adult vaccination stopped in 1972 and was replaced by the K45/20A vaccine, which was later discontinued in 1980 (Esmaeili *et al.*, 2012). Since 1988, all female bovines between the ages of 3 and 6 months have been immunized with the S19 vaccine, alongside educational programs focusing on hygiene for farmers. In 2007, the S19 vaccine was removed from the brucellosis control program, and since then, all cattle have been vaccinated with the RB51 vaccine (Esmaeili, 2014). Furthermore, free vaccination against brucellosis is offered in all parts of the country. Various barriers to effective vaccination in Iran have been identified. These barriers include improper timing of vaccinations, inadequate knowledge among livestock breeders, issues with vaccine viability and quality, poor storage conditions for vaccines, inadequate quarantine protocols, and a lack of anticipation regarding brucellosis-related abortion risks among vaccinated livestock (Yazdi *et al.*, 2009; Behzadifar *et al.*, 2019). In an effort to control this disease, the RB51 vaccine has been utilized extensively in Iran. However, the effectiveness of this vaccine against *B. melitensis* in dairy cattle farms, a common species affecting vast parts of Iran, has been questioned (Alamian *et al.*, 2024). This limitation has led to continued difficulties in controlling brucellosis in these regions. A major factor contributing to the ineffectiveness of RB51 against *B. melitensis* is the vaccine's specificity. RB51 is primarily designed to target *B. abortus*, which affects cattle (Blasco *et al.*, 2023). However, to the best of our knowledge, there are no studies in Iran that evaluate the effectiveness of the RB51 vaccine in dairy cattle farms infected with *B. melitensis*. On the other hand, Iranian studies have reported shedding of RB51 vaccine in milk (Gharekhani *et al.*, 2025). This presents a potential risk of human exposure through the consumption of contaminated dairy products (Dadar *et al.*, 2024a).

Movement Control and Quarantine in Restricting the Spread of Brucellosis in Iran

Quarantine and movement control are integral components of the strategy to control brucellosis in dairy cattle farms: these measures are designed to limit the disease spread both within and across herds, thereby enhancing the overall effectiveness of control programs (Robinson, 2003). In dairy cattle farms of Iran, quarantine procedures are typically implemented upon the detection of brucellosis cases. Infected animals, identified through serological tests or clinical symptoms, are isolated in designated areas away from healthy livestock (Golshani and Buozari, 2017). This isolation period for 4 weeks allows for monitoring of the infected animals' health status and prevents further transmission of the disease. During quarantine, strict biosecurity measures are enforced to minimize the risk of transmission. These measures include restricted access to the quarantined area, disinfection protocols for personnel, equipment, and facilities, as well as proper disposal of contaminated materials such as bedding and manure. Veterinary supervision is also essential during quarantine to monitor the progression of the disease in infected animals and ensure appropriate treatment when necessary. Quarantine plays a vital role in controlling brucellosis by containing the infection within affected herds, preventing its spread to neighboring farms, and reducing the overall prevalence of the disease (Alamian *et al.*, 2023b). Movement control strategies aim to manage the movement of cattle, especially from regions with a high incidence of brucellosis. Strict regulations are imposed to limit the transportation of potentially infected animals to new locations, thereby preventing the introduction of *Brucella* into disease-free herds. In Iran, movement control regulations are enforced by veterinary authorities to minimize the risk of spreading brucellosis through the movement of cattle. These regulations include regional restrictions for areas with a high incidence of bovine brucellosis, testing requirements for movement of cattle between regions or farms, quarantine upon arrival for cattle arriving from high-risk regions, traceability, and thorough documentation of health certificates and records of testing (Alamian *et al.*, 2023b).

Educating Farm Workers for Program Success

Training farm workers is a key element in controlling brucellosis on dairy cattle farms. By increasing awareness, promoting proper biosecurity measures, supporting vaccination programs, encouraging early detection, and fostering collaboration, farm workers become integral partners in mitigating the risks associated with this disease (Smits, 2013). Their knowledge and proactive involvement not only protect the health of the cattle but also safeguard the well-being of farm workers and the broader community. Effective education programs also promote collaboration and communication among farm workers, veterinarians, and public health officials. Workers should be encouraged to communicate any concerns or observations regarding brucellosis to the relevant authorities to ensure an early and comprehensive detection of the disease. This open dialogue ensures that information flows smoothly, facilitating a coordinated response to prevent outbreaks and protect both animal and human health (Govindasamy *et al.*, 2021). Initial studies carried out mainly in Asia and Africa highlighted a significant lack of knowledge and awareness of brucellosis. Hence, there is an urgent need for interventions aimed at enhancing public awareness of brucellosis (Zhang *et al.*, 2019). The involvement of diverse stakeholders, such as government authorities, community members, wildlife officials, political leaders at both local and national levels, and professionals from the veterinary, public health, and medical fields, has been recognized as essential (Godfroid *et al.*, 2013). Furthermore, integrating farmer education into existing veterinary programs could yield synergistic benefits. By equipping farmers with knowledge and skills, these programs can create a network of informed stakeholders who are better equipped to implement preventive measures on the ground. This approach safeguards livestock while also promoting public health by lowering the risk of zoonotic transmission to humans. Farmers face a heightened risk of exposure to brucellosis; thus, improving their knowledge and attitudes is vital to controlling the disease not only in Iran but also worldwide. Furthermore, an educational initiative grounded in Protection Motivation Theory has been endorsed to promote preventative behaviors against brucellosis among farmers and ranchers at the comprehensive rural health center (Adabi *et al.*, 2022). In a recent study, Bahadori *et al.* reported that Iranian livestock breeders lack brucellosis preventive knowledge despite good attitudes and behaviors (Bahadori *et al.*, 2023). However, in the specific case of Iran, where brucellosis poses a significant threat to cattle farms, the absence of documented data on the effects of farmer education is a notable gap. Understanding how educating farmers can impact animal brucellosis control is vital for designing tailored interventions. Such education could cover topics ranging from proper hygiene practices to early disease detection, empowering farmers to actively participate in disease prevention.

Evaluating Risk Factors for Brucellosis Control and Eradication

The epidemiology of cattle brucellosis is complex, involving various interconnected factors. The factors contributing to cattle brucellosis include individual predispositions, factors related to disease transmission, and those that facilitate the persistence and spread of infection within herds. Management-related risk factors, such as biosecurity practices, population density, herd size, and herd safety status, also play a significant role. Environmental influences, including climate, further impact the disease dynamics (Alamian *et al.*, 2020; Dadar *et al.*, 2020a; Dadar *et al.*, 2024a).

Statistical analysis of independent variables related to brucellosis infection in several Iranian herds has shown that maintaining proper hygiene and regularly disinfecting watering points, by cleaning them at least three times a week with disinfectant or detergent, can help reduce the risk of brucellosis in dairy cattle farms. On the other hand, factors such as an abortion history, introducing new livestock from outside, and replacing f animals within the past 12 months can elevate the risk of brucellosis infection (Bahreinipour *et al.*, 2023). Additionally, the existence of many animal species on

Iranian dairy cattle farms, such as sheep, goats, poultry, and dogs, pose potential risks due to possible brucellosis transmission between different animals. Recent studies have also identified rodents as a risk factor in farm environments (Gharekhani and Sazmand, 2019; Adabi *et al.*, 2022). Moreover, it has been emphasized that maintaining proper hygiene during abortions and managing animals suspected of brucellosis in dairy cattle farms is crucial for controlling the transmission of *Brucella* (Dadar *et al.*, 2021b). Another study identified several risk factors for bovine brucellosis, including poor biosecurity practices such as failing to clean milking equipment with a water and chlorine solution, and inadequate daily cleaning of water troughs. Also, high density of cattle per square meter favored higher prevalence. There was a statistical effect of proximity to other cattle farms infected (2000 meters) and farms with ovine (radius 500 meters) that greatly increased the chances of infection. While vaccination remains a critical preventive measure, the study underscores the importance of robust biosecurity practices in managing bovine brucellosis effectively (Izadi *et al.*, 2024). A case-control study examining factors influencing brucellosis in dairy cows found that the risk factors for bovine brucellosis can be categorized into management practices, animal-related factors, rancher-related factors, and environmental conditions. Management-related risks include large herd size, poor biosecurity measures, and improper vaccination. Animal-related factors include birth on another farm, history of abortion, and some physiological states, such as lactation and pregnancy; the latter two have been reported to decrease the risk. Rancher-related factors like age and experience, along with geographical conditions such as weather, also play a role. Effective disease management requires robust vaccination programs and limiting livestock movement to minimize exposure (Bahreinipour *et al.*, 2024). In conclusion, evaluating risk factors in Iranian dairy cattle farms is vital for disease prevention, herd health management, financial sustainability, public health protection, regulatory compliance, and informed decision-making. By addressing these factors, farms can improve their overall efficiency, productivity, and contribution to both the agricultural sector and public health.

One Health Implications for Improving Brucellosis Control in Iranian Dairy Farms

The One Health approach, which highlights the interdependence of animal, human, and environmental health, holds great potential for enhancing brucellosis control programs in Iranian dairy cattle farms. By adopting a holistic strategy that integrates veterinary and public health measures, One Health can enhance early detection, prevention, and management of brucellosis. Enhanced surveillance systems, including regular screening of cattle and environmental monitoring, can help in the early identification of outbreaks. This proactive approach can help prevent the spread of brucellosis both within cattle populations and to humans, who are at risk through consuming contaminated meat and dairy products or by direct contact with infected animals. Furthermore, One Health facilitates collaboration between veterinarians, farmers, public health officials and researchers, fostering a multidisciplinary response to the disease (Godfroid *et al.*, 2013). Additionally, One Health underscores the importance of education and awareness programs for farmers and the public. By improving knowledge about brucellosis transmission and prevention methods, stakeholders can implement better biosecurity practices on dairy farms, such as quarantine measures for new or infected animals and safe handling and processing of milk. Vaccination programs can be more efficiently implemented and monitored, leading to a reduction in brucellosis incidence in cattle and, consequently, in humans. Additionally, environmental management practices, such as the proper disposal of animal waste, are vital in controlling the spread of the pathogen.

Challenges and Solutions in Iran's Brucellosis Control Program

Brucellosis poses a significant challenge in the control of dairy cattle farms in Iran, particularly in rural areas, where resources for comprehensive testing and vaccination programs may be limited. The disease not only affects the health of the animals but also poses a risk to human health through consuming contaminated dairy and meat products, milk, as well as direct transmission (Dadar *et al.*, 2020b). Due to high costs, a well-calculated cost analysis, including a situation analysis, needs assessment, and various options for possible vaccination, test-and-slaughter, or combined strategies, is necessary and should involve stakeholders and policymakers. It should include data on relevant legislation, livestock populations, available resources, and the capabilities of laboratory and veterinary services. Using this information, risk analysis and needs assessment can be employed to create a customized control program, incorporating additional measures and allocating the necessary human and financial resources to mitigate significant transmission risks (Smits, 2013).

One of the primary obstacles in controlling brucellosis in Iran is the lack of adequate resources for comprehensive testing and vaccination programs due to the budget-oriented nature of the control program, especially in regions where dairy farming is a significant source of livelihood. Therefore, it is necessary to allocate the necessary budget for the management of this disease.

Limited access to diagnostic tools and vaccines can hinder the early detection and prevention of the disease. Without regular testing, infected animals can unknowingly remain in the herd, further spreading the bacteria. The use of up-to-date and fast detection techniques that are approved by international authorities can be very helpful in identifying infected animals, especially in carriers without clinical manifestations and in animals that are in the early stages of infection.

Further challenges may arise during the testing process, particularly in the ability and willingness of entities of the private sector, such as farmers, livestock owners, and veterinarians, to recognize and report suspected cases promptly. Factors such as limited awareness of brucellosis symptoms, inadequate training, or lack of resources can furthermore hinder timely reporting. Additionally, access to accredited laboratories of IVO may be challenging in some remote regions, potentially delaying the confirmation of diagnoses as well. Moreover, the time and cost associated with sending samples to these laboratories can be prohibitive for small-scale farmers. On the other hand, inadequate infrastructure and resources in some areas of Iran may also impact the efficiency of laboratory testing and diagnosis in the accredited laboratories of IVO. This includes issues such as insufficient laboratory capacity, delays in test results, and a lack of trained personnel to handle brucellosis testing.

Efforts to address these challenges could involve enhancing education and awareness programs within the private sector. Farmers need to be informed about the risks of brucellosis, its symptoms in both animals and humans, as well as the importance of participating in control programs. By raising awareness, farmers can be encouraged to report suspected cases promptly, allowing for early intervention and containment. Thus, providing regular training to farmers, veterinarians, and livestock owners on the signs of brucellosis, the importance of early reporting, and how to access accredited laboratories of IVO may improve the efficiency of the reporting process. Therefore, ensuring sufficient availability of these accredited laboratories of IVO across different regions of Iran is crucial. Improvement of accessibility and availability of IVO laboratories could involve investment in laboratory infrastructure, training of personnel as well as establishing efficient communication channels between the private sector and these laboratories. By addressing these challenges, the process of identifying and confirming brucellosis cases in dairy cattle farms can be streamlined, leading to

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earlier detection, effective management and ultimately, the reduction of brucellosis prevalence in Iran's dairy cattle population.

Another challenge is the uncontrolled movement of animals and animal herds, which can contribute significantly to the spread of brucellosis (Alamian *et al.*, 2023b). In regions where there are no strict regulations or monitoring of animal movements, infected cattle can easily transmit the disease to other herds (Smits, 2013). This makes containment efforts more difficult and increases the prevalence of brucellosis in the area. Thus, a region-wide approach is important (Smits, 2013).

Additionally, the insufficient promotion of effective vaccination among dairy cattle poses another challenge in preventing the spread of brucellosis in Iran. Particularly in high-risk areas, there is an urgent need for widespread vaccination campaigns, as vaccination can substantially lower the disease prevalence within herds (Smits, 2013). However, its effectiveness relies on widespread participation. Educating farmers about the benefits of vaccination and making vaccines accessible and affordable are key steps in improving control measures. Furthermore, proper vaccination programs need to overcome several barriers including improper timing of vaccinations, inadequate knowledge among livestock breeders, issues with vaccine viability and quality, poor storage conditions for vaccines, inadequate quarantine protocols and a lack of anticipation regarding brucellosis-related abortion risks among vaccinated livestock (Yazdi *et al.*, 2009; Behzadifar *et al.*, 2021). Additionally, education and awareness programs should be planned to educate farmers and workers about the importance of quarantine procedures in controlling brucellosis.

Each of these barriers presents a challenge that needs to be overcome in order to enhance the effectiveness of brucellosis control programs on dairy cattle farms in Iran. This could involve targeted education and training for livestock breeders on vaccination, ensuring the quality and proper storage of vaccines, implementing effective quarantine measures, and increasing knowledge on the potential hazards linked to the brucellosis vaccine. Furthermore, the limitations of vaccines need to be considered in the control program. Hence, vaccines may interfere with serological testing and have, amongst others, potential abortion and shedding effects (Smits, 2013; Goodwin and Pascual, 2016).

Another financial challenge could arise from the costs associated with culling infected animals, as outlined in the test-and-slaughter strategy of Iran's brucellosis control plan. Hence, farmers need to be compensated/reimbursed for slaughtered animals to ensure the reliability of reporting, which may not be a realistic option in developing countries such as Iran (Smits, 2013). Nevertheless, culling infected cows can effectively halt the spread of bovine brucellosis within the herd. However, the effectiveness of this method can be challenged by several factors, such as inaccuracies in the detection and testing process caused by animals with hidden infections, showing no clinical symptoms, or false negative test results of animals with low infection levels (Nielsen and Yu, 2010; Roop *et al.*, 2021). Hence, a study by Alamian *et al.* showed that subsequent serological testing continued to identify animals with positive serology for a period exceeding 6 months after culling (Alamian *et al.*, 2023b). The fact that animals continue to test positive for brucellosis for more than 6 months after culling suggests that the infection might persist in the herd. Furthermore, brucellosis can sometimes persist in environmental reservoirs and wildlife, re-infecting animals even after infected ones are removed (Smits, 2013; González-Espinoza *et al.*, 2021). To ensure the effectiveness of test-and-slaughter programs, it is crucial to promptly identify and cull all infected animals. Some animals might have been missed or were willingly not reported during the culling process, allowing the infection to circulate and re-emerge in the herd. The will to report infected animals correlates directly with a cost aspect, specifically in regions where dairy farming is a significant source of livelihood. Thus, it is either necessary to compensate farmers or provide other hedging strategies (Smits, 2013).

Last but not least, an efficient surveillance system is critical for managing brucellosis, as the disease can rapidly re-emerge from persistent infections or localized areas, making it an essential part of any eradication and control strategy (Smits, 2013). This can be done, for example, by an electronic data storage and analysis system. It should establish a baseline prevalence, monitor the effectiveness and progress of the control program, include up-to-date epidemiological information, ensure proper planning and timing, and assess the general efficiency of vaccination, including its level of protection and additional control measures (D'Orazi *et al.*, 2007). It has been reported that electronic technologies enhanced brucellosis reporting and could assist pastoral communities in controlling brucellosis (Mligo *et al.*, 2023). Moreover, an effective surveillance system is essential for tracking the disease following the end of vaccination efforts, as isolated infection sites may continue to exist even after vaccination has stopped (Smits, 2013). Additionally, information provided by a surveillance system can be used to convince policymakers to take action and improve awareness amongst the public and private sectors (Smits, 2013).

Conclusion

The conventional strategy of vaccinations, quarantine, testing, and culling animals with compensation programs is not as effective or feasible in low and middle-income countries. Brucellosis continues to be prevalent and is frequently overlooked, despite scientific and technological progress, particularly

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impacting pastoral and small-scale livestock farmers. Developing countries struggle with diagnosis, surveillance, management, and treatment. International standards exist, but are hard to implement consistently. There is also a risk of disease re-emergence from wild reservoirs.

Brucellosis control in Iran faces several significant challenges, including: (1) the lack of effective legislation to penalize violators in the animal health sector, (2) deficiencies in the border quarantine system and the illegal movement of animals from neighboring countries, (3) the absence of a livestock identification system for nomadic and rural herds, (4) the difficulty of regulating animal movement due to the semi-nomadic and nomadic practices in small ruminant farming, and (5) the continued practice of keeping sheep beyond the recommended immunity duration for the Rev.1 vaccine in certain regions and (6) the providing of insufficient compensation to farmers and veterinarians who are part of operation teams combatting the disease. Evidence suggests that bovine brucellosis is quite widespread in both industrial and traditional dairy cattle farms in Iran. Additional extensive, randomized investigations are necessary to precisely evaluate the prevalence of brucellosis and its true economic impact in dairy cattle farms in Iran. Controlling brucellosis in middle-income countries like Iran will continue to be a significant challenge due to underreporting, insufficient funding for control or eradication efforts, the absence of a completely effective vaccine, and the (financial) challenges associated with slaughtering infected animals in rural areas. Controlling brucellosis in Iran is also particularly challenging due to the extensive animal migration for various purposes and the farming of dairy animals in remote and rural regions, where animal slaughter is not feasible. Therefore, it is advisable to strengthen the system of disease reporting by improving diagnostic surveillance and capabilities across wildlife, livestock, and human populations. Additionally, emphasis should be placed on improved hygiene and cleanliness, effective vaccination, implementation of quarantine measures, better management practices in farms, restrictions on animal movement, increased use of artificial insemination, regular control of the rodent population in farms and the implementation of a customized health education program on brucellosis. Furthermore, it is essential to raise awareness about brucellosis and encourage the appropriate use of diagnostic, epidemiological, and preventive measures in the population and amongst policymakers and stakeholders. Extended infrastructure weaknesses, which are sometimes worsened by geography and weather conditions, play a crucial role. The key principle of the One Health approach is to highlight the interconnectedness of humans, animals, and ecosystems in relation to disease and overall health. As such, multidisciplinary studies are strongly encouraged.

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Ethics

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References

Abnaroodheleh, F., Emadi, A., Dashtipour, S., Jamil, T., Mousavi Khaneghah, A., & Dadar, M. (2023). Shedding rate of Brucella spp. in the milk of seropositive and seronegative dairy cattle. *Heliyon*, *9*(4), e15085. https://doi.org/10.1016/j.heliyon.2023.e15085

Adabi, M., Gharekhani, J., Saadatmand, A., & Shahbazi, F. (2024). Seroprevalence of brucellosis in livestock in Iran: a meta-analysis. *Comparative Clinical Pathology*, *33*(1), 175-182. https://doi.org/10.1007/s00580-023-03543-5

Adabi, M., Khazaiee, S., Sadeghi-Nasab, A., Alamian, S., Arabestani, M. R., Valiei, Z., & Gharekhani, J. (2022). Brucellosis in livestock: First study on seroepidemiology, risk factors, and preventive strategies to manage the disease in Famenin, Iran. *Veterinary World*, *15*(18), 2102-2110. https://doi.org/10.14202/vetworld.2022.2102-2110

Alamian, S., Amiry, K., Bahreinipour, A., Etemadi, A., Tebianian, M., Mehrabadi, M. H. F., & Dadar, M. (2021). Brucella species circulating in rural and periurban dairy cattle farms: a comparative study in an endemic area. *Tropical Animal Health and Production*, *53*(2), 200. https://doi.org/10.1007/s11250-021-02645-y

Alamian, S., Amiry, K., Bahreinipour, A., Etemadi, A., Yousefi, A. R., & Dadar, M. (2023a). Evaluation of serological diagnostic tests for bovine brucellosis in dairy cattle herds in an endemic area: a multicenter study. *Tropical Animal Health and Production*, *55*(2), 200. https://doi.org/10.1007/s11250-023-03519-1

Alamian, S., Amiry, K., Etemadi, A., & Dadar, M. (2024). Characterization of Brucella spp. circulating in industrial dairy cattle farms in Iran: A field study, 201602023. *Veterinary Research Forum: Faculty of Veterinary Medicine, Urmia University*.

Alamian, S., Bahreinipour, A., Amiry, K., & Dadar, M. (2023b). The Control Program of Brucellosis by the Iranian Veterinary Organization in Industrial Dairy Cattle Farms. *Archives of Razi Institute*, *78*, 1107.

Alamian, S., Dadar, M., & Wareth, G. (2020). Role of Brucella abortus biovar 3 in the outbreak of abortion in a dairy cattle herd immunized with Brucella abortus Iriba vaccine. *Archives of Razi Institute*, *75*, 377.

Andrade, R. S., Oliveira, M. M. de, Bueno Filho, J. S. de S., Ferreira, F., Godfroid, J., Lage, A. P., & Dorneles, E. M. S. (2024). Accuracy of serological tests for bovine brucellosis: A systematic review and meta-analysis. *Preventive Veterinary Medicine*, *222*, 106079. https://doi.org/10.1016/j.prevetmed.2023.106079

Bahadori, F., Ghofranipour, F., Zarei, F., & Saeideh, G. (2023). *Iranian livestock breeders' knowledge, attitude, practice and behavioral determinants related to Brucellosis prevention*. https://doi.org/10.21203/rs.3.rs-2991619/v1

Bahreinipour, A., Bahonar, A., Boluki, Z., Rahimi Foroshani, A., Lotfollah Zadeh, S., & Amiri, K. (2023). Bovine Brucellosis Infection in Iranian Dairy Farms: A Herd-level Case-control Study. *The Iranian Journal of Veterinary Medicine*, *17*(4), 383-392. https://doi.org/10.32598/ijvm.17.4.1005289

Bahreinipour, A., Bahonar, A., Boluki, Z., Rahimi Foroshani, A., Lotfollahzadeh, S., & Amiri, K. (2024). Case-Control Study of Some Factors Affecting Brucellosis Infection in Dairy Cows. *Iranian Veterinary Journal*, *19*, 202-207.

Barrio, M. B., Grilló, M. J., Muñoz, P. M., Jacques, I., González, D., de Miguel, M. J., Marín, C. M., Barberán, M., Letesson, J.-J., Gorvel, J.-P., Moriyón, I., Blasco, J. M., & Zygmunt, M. S. (2009). Rough mutants defective in core and O-polysaccharide synthesis and export induce antibodies reacting in an indirect ELISA with smooth lipopolysaccharide and are less effective than Rev 1 vaccine against Brucella melitensis infection of sheep. *Vaccine*, *27*(11), 1741-1749. https://doi.org/10.1016/j.vaccine.2009.01.025

Behzadifar, M., Gorji, H. A., Rezapour, A., & Bragazzi, N. L. (2019). The hepatitis C infection in Iran: a policy analysis of agenda-setting using Kingdon's multiple streams framework. *Health Research Policy and Systems*, *17*(1), 30. https://doi.org/10.1186/s12961-019-0436-z

Blasco, J. M., Moreno, E., Muñoz, P. M., Conde-Álvarez, R., & Moriyón, I. (2023). A review of three decades of use of the cattle brucellosis rough vaccine Brucella abortus RB51: myths and facts. *BMC Veterinary Research*, *19*(1), 211. https://doi.org/10.1186/s12917-023-03773-3

Boral, R., Singh, M., & Singh, D. (2009). Status and strategies for control of brucellosis-a review. *Indian J Anim Sci*, *79*, 1191-1199.

Can, M. F., & Yalçın, C. (2014). The cost-benefit analysis of alternative brucellosis control strategies in Turkey. *Kafkas Univ Vet Fak Derg*, *20*(1), 107-113. https://doi.org/10.9775/kvfd.2013.9585

Chand, P., Chhabra, R., & Nagra, J. (2015). Vaccination of adult animals with a reduced dose of Brucella abortus S19 vaccine to control brucellosis on dairy farms in endemic areas of India. *Tropical Animal Health and Production*, *47*(1), 29-35. https://doi.org/10.1007/s11250-014-0678-2

Coelho, A., García-Díez, J., Góis, J., Rodrigues, J., & Coelho, A. C. (2019). Farmer's attitudes and farm management in small ruminant flocks with high brucellosis prevalence. *Veterinaria Italiana*, *55*(4), 355-362.

Coelho, A. M., Pinto, M. L., & Coelho, A. C. (2011). Cost-benefit analysis of sheep and goat brucellosis vaccination with Rev.1 in the north of Portugal from 2000 to 2005. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *63*(1), 1-5. https://doi.org/10.1590/s0102-09352011000100001

Dadar, M., Alamian, S., Behrozikhah, A. M., Yazdani, F., Kalantari, A., Etemadi, A., & Whatmore, A. M. (2019). Molecular identification of Brucella species and biovars associated with animal and human infection in Iran. *Veterinary Research Forum: Faculty of Veterinary Medicine, Urmia University*, 315.

Dadar, M., Bahreinipour, A., Abnaroodheleh, F., Ansari, F., & Mousavi Khaneghah, A. (2024a). Risk factors and control strategies for Brucella spp. and RB51 vaccine shedding in buffalo milk: A cross-sectional study. *Acta Tropica*, *260*, 107436. https://doi.org/10.1016/j.actatropica.2024.107436

Dadar, M., Bahreinipour, A., Alamian, S., Yousefi, A. R., Amiri, K., & Abnaroodheleh, F. (2024b). Serological, cultural, and molecular analysis of Brucella from Buffalo milk in various regions of Iran. *Veterinary Research Communications*, *48*(1), 427-436. https://doi.org/10.1007/s11259-023-10228-5

Dadar, M., Fakhri, Y., Shahali, Y., & Mousavi Khaneghah, A. (2020b). Contamination of milk and dairy products by Brucella species: A global systematic review and meta-analysis. *Food Research International*, *128*, 108775. https://doi.org/10.1016/j.foodres.2019.108775

Dadar, M., Shahali, Y., & Fakhri, Y. (2020a). A primary investigation of the relation between the incidence of brucellosis and climatic factors in Iran. *Microbial Pathogenesis*, *139*, 103858. https://doi.org/10.1016/j.micpath.2019.103858

Dadar, M., Shahali, Y., & Fakhri, Y. (2021a). Brucellosis in Iranian livestock: A meta-epidemiological study. *Microbial Pathogenesis*, *155*, 104921. https://doi.org/10.1016/j.micpath.2021.104921

Dadar, M., Tabibi, R., Alamian, S., Caraballo-Arias, Y., Mrema, E. J., Mlimbila, J., Chandrasekar, S., Dzhusupov, K., Sulaimanova, C., Alekesheva, L. Zh., Manar, S. A., Toguzbayeva, K. K., Wickramatillake, A., & Mirzaei, B. (2023). Safety concerns and potential hazards of occupational brucellosis in developing countries: a review. *Journal of Public Health*, *31*(10), 1681-1690. https://doi.org/10.1007/s10389-022-01732-0

Dadar, M., Tiwari, R., Sharun, K., & Dhama, K. (2021b). Importance of brucellosis control programs of livestock on the improvement of one health. *Veterinary Quarterly*, *41*(1), 137-151. https://doi.org/10.1080/01652176.2021.1894501

Deka, R. P., Magnusson, U., Grace, D., & Lindahl, J. (2018). Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India- a review. *Infection Ecology & Epidemiology*, *8*(1), 1556548. https://doi.org/10.1080/20008686.2018.1556548

D'Orazi, A., Mignemi, M., Geraci, F., Vullo, A., Gesaro, M., & Vullo, S. (2007). Spatial distribution of brucellosis in sheep and goats in Sicily from 2001 to 2005. *Veterinaria Italiana*, *43*(3), 541-548.

Esmaeili, H. (2014). Brucellosis in Islamic Republic of Iran. *Journal of Medical Bacteriology*, *3*(3-4), 47-57.

Esmaeili, H., Tajik, P., Ekhtiyarzadeh, H., Bolourchi, M., Hamedi, M., Khalaj, M., & Amiri, K. (2012). *Control and eradication program for bovine brucellosis in Iran: an epidemiological survey*. 211-221.

Gall, D., & Nielsen, K. (2004). Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Revue Scientifique et Technique de l'OIE*, *23*(3), 939-1002. https://doi.org/10.20506/rst.23.3.1545

Gharekhani, J., Hemati, Z., Adabi, M., Asadi, F. T., & Dadar, M. (2025). Role of RB51 vaccine in cow milk contamination of traditional milk sales centers. *Tropical Animal Health and Production*, *57*(2), 114. https://doi.org/10.1007/s11250-025-04362-2

Gharekhani, J., & Sazmand, A. (2019). Detection of Brucella Antibodies in Dogs From Rural Regions of Hamedan, Iran. *Avicenna Journal of Clinical Microbiology and Infection*, *6*(4), 122-126. https://doi.org/10.34172/ajcmi.2019.22

Godfroid, J., Al Dahouk, S., Pappas, G., Roth, F., Matope, G., Muma, J., Marcotty, T., Pfeiffer, D., & Skjerve, E. (2013). A "One Health" surveillance and control of brucellosis in developing countries: Moving away from improvisation. *Comparative Immunology, Microbiology and Infectious Diseases*, *36*(3), 241-248. https://doi.org/10.1016/j.cimid.2012.09.001

Golshani, M., & Buozari, S. (2017). A review of brucellosis in Iran: epidemiology, risk factors, diagnosis, control, and prevention. *Iranian Biomedical Journal*, *21*(6), 349.

González-Espinoza, G., Arce-Gorvel, V., Mémet, S., & Gorvel, J.-P. (2021). Brucella: Reservoirs and Niches in Animals and Humans. *Pathogens*, *10*(2), 186. https://doi.org/10.3390/pathogens10020186

Goodwin, Z. I., & Pascual, D. W. (2016). Brucellosis vaccines for livestock. *Veterinary Immunology and Immunopathology*, *181*, 51-58.

Govindasamy, K., Etter, E. M. C., Harris, B. N., Rossouw, J., Abernethy, D. A., & Thompson, P. N. (2021). Knowledge of Brucellosis, Health-Seeking Behaviour, and Risk Factors for Brucella Infection amongst Workers on Cattle Farms in Gauteng, South Africa. *Pathogens*, *10*(11), 1484. https://doi.org/10.3390/pathogens10111484

Greiner, M., & Gardner, I. A. (2000). Epidemiologic issues in the validation of veterinary diagnostic tests. *Preventive Veterinary Medicine*, *45*(1-2), 3-22. https://doi.org/10.1016/s0167-5877(00)00114-8

Gwida, M., Al Dahouk, S., Melzer, F., Rösler, U., Neubauer, H., & Tomaso, H. (2010). Brucellosis - Regionally Emerging Zoonotic Disease? *Croatian Medical Journal*, *51*(4), 289-295. https://doi.org/10.3325/cmj.2010.51.289

Izadi, S., Moghaddas, V., Feizi, A., Bahreinipour, A., & Barati, Z. (2024). Bovine brucellosis, associated risk factors and preventive measures in industrial cattle farms. *Heliyon*, *10*(22), e40180. https://doi.org/10.1016/j.heliyon.2024.e40180

Makita, K., Fèvre, E. M., Waiswa, C., Eisler, M. C., Thrusfield, M., & Welburn, S. C. (2011). Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC Veterinary Research*, *7*(1), 60. https://doi.org/10.1186/1746-6148-7-60

McDermott, J., Grace, D., & Zinsstag, J. (2013). Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique de l'OIE*, *32*(1), 249-261. https://doi.org/10.20506/rst.32.1.2197

McKenna, S. L. B., & Dohoo, I. R. (2006). Using and Interpreting Diagnostic Tests. *Veterinary Clinics of North America: Food Animal Practice*, *22*(1), 195-205. https://doi.org/10.1016/j.cvfa.2005.12.006

Mligo, B. J., Sindato, C., Yapi, R. B., Mwabukusi, M., Mathew, C., Mkupasi, E. M., Karimuribo, E. D., & Kazwala, R. R. (2023). Effect of awareness training to frontline health workers and the use of e-based technology on reporting of brucellosis cases in selected pastoral communities, Tanzania: a quasi-experimental study. *One Health Outlook*, *5*(1), 13. https://doi.org/10.1186/s42522-023-00084-3

Moreno, E., Blasco, J.-M., & Moriyón, I. (2022). Facing the Human and Animal Brucellosis Conundrums: The Forgotten Lessons. *Microorganisms*, *10*(5), 942. https://doi.org/10.3390/microorganisms10050942

Moriyón, I., Blasco, J. M., Letesson, J. J., De Massis, F., & Moreno, E. (2023). Brucellosis and One Health: Inherited and Future Challenges. *Microorganisms*, *11*(8), 2070. https://doi.org/10.3390/microorganisms11082070

Moriyón, I., Grilló, M. J., Monreal, D., González, D., Marín, C., López-Goñi, I., Mainar-Jaime, R. C., Moreno, E., & Blasco, J. M. (2004). Rough vaccines in animal brucellosis: Structural and genetic basis and present status. *Veterinary Research*, *35*(1), 1-38. https://doi.org/10.1051/vetres:2003037

Nielsen, K. (2002). Diagnosis of brucellosis by serology. *Veterinary Microbiology*, *90*, 447-459.

Nielsen, K., & Yu, W. (2010). Serological diagnosis of brucellosis. *Prilozi*, *31*, 65-89.

Olsen, S. C. (2000). Immune responses and efficacy after administration of a commercial Brucella abortus strain RB51 vaccine to cattle. *Vet Ther*, *1*, 183-191.

Pereira, C. R., Cotrim de Almeida, J. V. F., Cardoso de Oliveira, I. R., Faria de Oliveira, L., Pereira, L. J., Zangerônimo, M. G., Lage, A. P., & Dorneles, E. M. S. (2020). Occupational exposure to Brucella spp.: A systematic review and meta-analysis. *PLOS Neglected Tropical Diseases*, *14*(5), e0008164. https://doi.org/10.1371/journal.pntd.0008164

Robinson, A. (2003). *Guidelines for coordinated human and animal brucellosis surveillance*. FAO Agriculture Department.

Roop, R. M., Barton, I. S., Hopersberger, D., & Martin, D. W. (2021). Uncovering the Hidden Credentials of Brucella Virulence. *Microbiology and Molecular Biology Reviews*, *85*(1). https://doi.org/10.1128/mmbr.00021-19

Saavedra, M. J., Fernandes, C., & Queiroga, C. (2019). *Laboratory diagnosis of brucellosis. Brucellosis in Goats and Sheep: an endemic and re-emerging old zoonosis in the 21st century*. 151-180.

Samartino, L. E. (2002). Brucellosis in Argentina. *Veterinary Microbiology*, *90*, 71-80.

Scholz, H. C., Revilla-Fernández, S., Dahouk, S. A., Hammerl, J. A., Zygmunt, M. S., Cloeckaert, A., Koylass, M., Whatmore, A. M., Blom, J., Vergnaud, G., Witte, A., Aistleitner, K., & Hofer, E. (2016). Brucella vulpis sp. nov., isolated from mandibular lymph nodes of red foxes (Vulpes vulpes). *International Journal of Systematic and Evolutionary Microbiology*, *66*(5), 2090-2098. https://doi.org/10.1099/ijsem.0.000998

Smits, H. L. (2013). Brucellosis in pastoral and confined livestock: prevention and vaccination. *Revue Scientifique et Technique de l'OIE*, *32*(1), 219-228. https://doi.org/10.20506/rst.32.1.2200

WHO (2015). The control of neglected zoonotic diseases: from advocacy to action: report of the fourth international meeting held at WHO Headquarters. *The Control of Neglected Zoonotic Diseases: From Advocacy to Action: Report of the Fourth International Meeting Held at WHO Headquarters*, 19-20.

Yazdi, H. S., Kafi, M., Tamadon, A., Ghane, M., Haghkhah, M., & Behroozikhah, A. M. (2009). Abortions in pregnant dairy cows after vaccination with Brucella abortus strain RB51. *Veterinary Record*, *165*(19), 570-571. https://doi.org/10.1136/vr.165.19.570

Zamri-Saad, M., & Kamarudin, M. I. (2016). Control of animal brucellosis: The Malaysian experience. *Asian Pacific Journal of Tropical Medicine*, *9*(12), 1136-1140. https://doi.org/10.1016/j.apjtm.2016.11.007

Zhang, N., Huang, D., Wu, W., Liu, J., Liang, F., Zhou, B., & Guan, P. (2018). Animal brucellosis control or eradication programs worldwide: A systematic review of experiences and lessons learned. *Preventive Veterinary Medicine*, *160*, 105-115. https://doi.org/10.1016/j.prevetmed.2018.10.002

Zhang, N., Zhou, H., Huang, D.-S., & Guan, P. (2019). Brucellosis awareness and knowledge in communities worldwide: A systematic review and meta-analysis of 79 observational studies. *PLOS Neglected Tropical Diseases*, *13*(5), e0007366. https://doi.org/10.1371/journal.pntd.0007366

Zowghi, E., & Ebadi, A. (1982). Typing of Brucella strains isolated in Iran. *Archives of Razi Institute*, *33*(1), 109-114.