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Strategies for Combatting Brucellosis: A Review on Control and Prevention in Bovines

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Abstract

Brucellosis, a widespread zoonotic disease, causes substantial economic losses in the livestock industry. The World Health Organization (WHO) and Office International des Epizooties recommend strategies for control, but only three countries achieved freedom from animal brucellosis after decades-long programs. Notably, the absence of a human vaccine underscores the critical interconnection between human and bovine brucellosis. The WHO recognizes the urgency of developing a human vaccine and implementing robust control programs to address this public health threat. Developing countries face challenges in implementing these strategies due to limited resources, making the control of brucellosis a complex and resource-intensive project. This study reviews brucellosis control or eradication programs worldwide, emphasizing the need for effective strategies in developing countries. Despite the resource-intensive nature of control efforts, well-designed programs have proven economically effective. Delving into the intricate landscape of this disease, the article examines a spectrum of measures including vaccination, testing, surveillance, biosecurity and public awareness campaigns. The analysis covers the importance of the One-Health approach and recognizes the interconnectedness of bovine and human health in the context of this zoonotic disease. The synthesis of current knowledge not only highlights the diverse strategy options available but also emphasizes the ongoing challenges that require continuous adaptation. This review provides a valuable source for researchers, policymakers and practitioners engaged in the global effort to mitigate the impact of brucellosis on both animal welfare and public health.

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Introduction

Brucella is an infectious disease affecting many mammalian species, including humans, cattle, horses, sheep and deer. *Brucella* is a Gram-negative, non-motile coccobacilli belonging to alpha-Proteobacteria and prefers chronic infections [1]. It is mainly transmitted through ingestion of food or water, which are contaminated by uterine discharges and aborted fetal membranes. Infected males can transmit the disease to females through natural mating and artificial insemination [1]. Ice cream and homemade cheese can also spread the disease among humans. *Brucella* was initially considered to be a monospecific genus with six biovars. However, modern molecular tools have shown that several species are related clones of one organism. The discovery of new species has raised questions about their genetic diversity. Currently, at least 12 *Brucella* species are known [2]. Brucellosis, a re-emerging zoonotic disease, is prevalent in the Middle East, China, Africa, and US. It affects humans and livestock with *Brucella melitensis* and *B. abortus* posing significant threats. In humans, symptoms range from mild to severe, with no available vaccine. Animal vaccination and farm sanitation are crucial preventive measures. The One-Health approach addresses disease's complexity, emphasizing medicine, ecology, socioeconomics, policy, science, management and education. Eco health and health promotion principles further enhance prevention strategies [3]. Brucellosis, an ancient disease with historical roots dating back over 2000 years, gained modern recognition when Sir David Bruce isolated *B. melitensis* in 1887. The recent identification of *B. inopinata*, linked to human infection, expands the scope of this genus. The zoonotic potential of marine mammal-associated *Brucella* strains has been evidenced by cases of human infections. *B. abortus*, *B. melitensis*, and *B. suis* are major pathogenic species, causing economic losses through abortions in natural hosts and contributing to human brucellosis [4]. Brucellosis poses health and economic threats as it disrupts daily activities and affects livestock production, ranking high in impact on poor communities. The One-Health approach, integrating human, animal, and environmental health, is crucial for controlling brucellosis. Strengthening collaboration between health-related domains and reducing sectoral overlaps can enhance the efficiency of health policies, contributing to Sustainable Development Goals [5]. In this review, we set a goal to intensively analyze the prevalent measures for combatting brucellosis in bovines, by focusing on control and prevention measures. After synthesizing prevalent literature and examining key findings, our target is to provide awareness about beneficial approaches for mitigating the impact of brucellosis within bovine populations. Additionally, we analyze emerging trends and challenges, offering guidelines for future research directions and practical implementations.

Epidemiology of Brucellosis

The global epidemiology of Brucellosis reveals its widespread presence, except in the countries where bovine brucellosis has been eradicated, including Australia, Canada, and some parts of Europe. The prevalence of the disease is particularly high in the Mediterranean, Northern and Eastern Africa, the Near East, India, Central Asia, and some parts of the Americas. Despite successful eradication efforts in many regions, Brucellosis remains a persistent challenge, notably with the highest reported infection rates in Egypt and Palestine. The prevalence of bovine brucellosis differs globally, with higher prevalence in many regions due to multiple factors such as poor management practices, vaccination coverage and wildlife reservoirs. Incidence rates may be affected by the effectiveness of preventive measures [6]. This disease exhibits varying prevalence in different animal species, with significant impacts on economic losses. Human brucellosis is a major concern, affecting over 500,000 individuals annually, mainly in Mediterranean countries, Central Asia, India, the Arabian Peninsula, and Latin America. Incidence rates in specific countries, such as Iran and Saudi Arabia, express the severity of the issue. Factors involved in human infections include consumption of fresh cheese, work at animal husbandry, diagnostic laboratory, and veterinary professionals. Despite control efforts, human brucellosis is still endemic in Iran, emphasizing the complex relationship between human and animal infections. It also highlights the need for continued surveillance, control programs, and public health interventions to combat this persistent zoonotic threat [7]. Bovine brucellosis is endemic in certain regions including parts of Africa, Asia, the Middle East, and Central and South America. Many African countries experience a burden of bovine brucellosis. Factors such as extensive livestock farming, interactions with wildlife and less access to veterinary services contribute to the spread of disease in this region. Bovine brucellosis is widespread in various countries in Asia and the Middle East. In developed regions like Europe and

North America, control programs and strict biosecurity measures have led to a reduction in the prevalence of disease. It is generally well-controlled in Australia and New Zealand, with successful programs in place. Global distribution of disease has trade implications, as infected animals and animal products can pose a risk of transmission. International organizations such as the World Organization for Animal Health (OIE) developed guidelines for safe trade while managing the problem [8]. Brucellosis is a global concern. Its impact is increased in developing countries due to inadequate public health measures, limited animal health programs and insufficient diagnostic facilities. The disease is often misdiagnosed and underreported in many nations, exacerbating its severity. Brucellosis is re-emerging in countries like Saudi Arabia, Brazil, Israel, Kuwait and Colombia, with increased incidences in cattle. In Ethiopia, the disease is endemic, especially affecting cattle, camels, and small ruminants in pastoral areas. Research has mainly focused on intensive dairy cattle herds in urban areas, revealing varying prevalence rates. Recent studies in the Jimma Zone, Southern Sidama Zone, North West and Tigray regions have recorded varying prevalence rates in extensive cattle-rearing systems, emphasizing the need for comprehensive monitoring and control efforts [9].

Diagnostic strategies

Isolation of bacteria

Isolation and culturing of *Brucella* species are considered as gold standard diagnostic tests, despite various other available methods. Due to the slow growth of all *Brucella* strains and the potential chance of specimen contamination, a selective medium like Farrell's medium is recommended for isolation purposes. Incubation takes almost about 72 hours, but a negative diagnosis is confirmed only after a week. Specimens for isolation include various body tissues and body fluids, with preferred choices being supra mammary and retropharyngeal lymph nodes. *Brucella* colonies display distinctive characteristics, appearing elevated, transparent, and convex, with a significant honey color. Culture conditions involve a temperature range of 20°C-40°C, pH between 6.6-7.4, and also some species require CO_2 for growth. A culture is considered negative if no colonies emerge after 2-3 weeks of incubation period [10].

Acid-fast staining

It is used for the confirmation of infection in all species involved, demonstrating the presence of bacterial growth through smears. These smears are obtained from various sources like placenta, colostrum, vaginal discharges, fetal stomach fluid, or aborting cow's lochia and are subjected to modified Ziehl-Nelsen stain. Impression smears can be obtained from freshly cut and blotted tissue surfaces, such as placenta cotyledons, by pressing the slide on the tissue, air drying and then, heat fixing. In MZN-stained smears, Bacteria appear as red intracellular coccobacilli, providing a distinctive color contrast from other bacteria, which stain blue [11].

Serological testing

Serological tests play a vital role in diagnosing bovine brucellosis, forming the necessary cornerstone of control and eradication programs for bovine brucellosis (Table 1). These tests are classified into screening and confirmatory methods. Some common serological tests include the serum agglutination test (SAT), Rose Bengal plate test (RBPT) and indirect enzyme-linked immunosorbent assay (ELISA). RBPT is mostly used because of its simple procedure, but its interpretation can be subjective. The Milk Ring Test (MRT) is effective for screening of disease in dairy cattle, but it has some limitations, especially in large animal herds and in male animals [12]. The complement fixation test serves as a confirmatory diagnostic test, recommended by the Organization for Animal Health (OIE), but it faces some challenges

Table 1 Adaptation of testing strategy against brucellosis [17].

Country	Duration	Milk test	Screen test	Confirmation test	Slaughter	Time of Launch
Fiji	2009-2013	MRT	RBT	ELISA/CFT	Yes	2009
Ireland	1998-2005	MRT, ELISA	MSAT	CFT	Yes	1998
Korea	2004-2011	MRT	RBT	ELISA/CFT/Tube agglutination test	Yes	1996
Malta	1987-1996	MRT	RBT, SAT	CFT	Within 14 days	-
Malaysia	1979-2016	-	RBPT	CFT	Yes	2016
Macedonia	2004-2006	-	RBT	CFT/ELISA	Within 1 month	2008

CFT = complement fixation test; ELISA = enzyme- linked immunosorbent assay; MRT = milk ring test; RBT/RBPT = Rose Bengal test; RIVT= Rivanol test; SAT = serum agglutination test; MSAT = macroscopic slide agglutination test.

like subjectivity in interpretation and potential interference from vaccination. ELISA has gained popularity due to its vast ability to screen large populations and to differentiate between acute and chronic phases of infection. However, its complexity limits the applicability of ELISA, especially in vaccinated areas. SAT is a standardized and easy-to-perform test to measure agglutinating antibodies, but it has a few drawbacks and potential cross-reactions. Modifications, like the addition of ethylenediaminetetraacetic acid (EDTA) or antihuman globulin, are employed to cover some of these limitations [13].

Molecular testing

Polymerase chain reaction (PCR) is the most widely used *in vitro* technique for amplification of nucleic acid. It plays an important role in diagnosing infectious diseases including human brucellosis. PCR along with its variants, focuses on specific genomic sequences of *Brucella* species, and it is considered an extensively used molecular technique for brucellosis diagnosis. Real-time PCR is known for its sensitivity and rapidity as compared to conventional PCR. It eliminates the need for post-amplification handling and reduces the risk of contamination and the chance of false positives. Recent advancements now include the development of real-time PCR assays which are specially designed for testing *Brucella* cells [13].

Control and eradication of Brucellosis

Efforts to control bovine brucellosis have faced many challenges. Factors involved in this difficulty include prolonged latent period of disease, limited sensitivity of diagnostic tests and environmental resistance of some of the species including *B. abortus* and *B. melitensis*. Despite these challenges, three effective strategies, strict biosecurity, test and slaughter programs and immunization have proven much more effective when employed together [14]. Optimal results are received from the combined application of at least two of these control measures, with the most relevant strategy depending on epidemiological context and available resources. Additionally, some complementary tools including animal identification, movement control and economic compensations play a significantly vital role in the success of control program. This portion comprehensively reviews the current tools for control and eradication of bovine brucellosis in livestock, emphasizing diagnostic tools and immunization strategies (Table 3). New approaches arising from evolving knowledge of the disease are now explored [15]. The OIE outlines requirements for a country or zone to be declared free from bovine brucellosis, including low prevalence rates, no recent need for vaccination, periodic testing, culling of reactors and introduction of animals from official brucellosis-free herds. Control measures usually include vaccination, test and slaughter programs and complementary prevention measures. Wildlife reservoirs can need relevant focus in specific scenarios. Two vaccines, including S19 and RB51, target *B. abortus* in cattle but there are many concerns regarding diagnostic interference and efficacy. Control of *B. melitensis* in cattle hinges the disease management in small ruminants. Some societal factors also influence control strategies, with vaccination as a primary tool despite drawbacks.

Successive steps are involved in the transition from mass vaccination to restricted vaccination and eventually, proposing an eradication and control program based on testing and culling. Decisions should include vaccination coverage, complementary measures and resource availability [3]. Policymaking in control of bovine brucellosis involves important decisions such as the selection of diagnostic tests, determination of specific cut-off levels and formulation of vaccination policies. These decisions have a huge impact on disease prevalence assessment, herd destruction criteria and compensation for affected animal owners. Animal vaccination, an important key policy decision, requires very careful consideration of epidemiological factors, selection of appropriate vaccines and vaccination frequency. Preparedness is an essential tool, that involves national priorities aligned with legal and financial

Table 2 Adaptation of test and slaughter strategy against brucellosis [19].

Country	Duration	Test and slaughter		Vaccination	
		Tests	Slaughter	Vaccine	Time of Launch
Australia	1970-1989	MRT, RBT, CFT, ELISA	Yes	S19	1970-1985
Egypt	1981-1997	SAT, RBPT, ELISA, CFT	Yes	S19	2002
Iran	1983-1996	RBPT, SAT	Yes	Rev.1	1983
America	1934-2001	SAT, CFT, RIVT	Yes	RB51	1997
New Zealand	1966-1989	CFT, Card test	Yes	S19	1969-1975

CFT = complement fixation test; ELISA = enzyme- linked immunosorbent assay; MRT = milk ring test; RBT/RBPT = Rose Bengal test; RIVT = Rivanol test; SAT = serum agglutination test structures. In brucellosis-free countries, monitoring animal movement, herd registration and implementation of a real-time outbreak identification system should be priorities. In endemic countries, addressing abortion waves requires surveys of populations, proper diagnosis of *Brucella* cases in bovines and enforcement of quarantine and test-and-cull policies. Herd destruction is advised for herds with high infection rates, while educational campaigns involving society leaders and children play a vital role in the success of the program [16].

Brucellosis, classified by the World Health Organization as a neglected zoonotic disease, significantly contributes to poverty in many developing countries. Control programs include sanitation, vaccination, testing and removal of animals are vital for protecting dairy herds. Vaccination, particularly in cattle aged 4-12 months and those over 12 months is an economically effective measure. RB51 and S19 are live vaccine strains, which are widely used for the control of brucellosis in cattle, while the *B. melitensis* REV-1 vaccine is considered as most efficient for small ruminants in high-prevalence areas. The success of control programs relies on factors like regional epidemiological patterns, infrastructure support, cross-sectoral coordination, surveillance, husbandry practices, community awareness and social customs. Strategies in areas of low prevalence involve test-and-slaughter techniques or preventive measures like certification of brucellosis-free herds and vaccination of female bovines. Strict surveillance at the national level is crucial to identify and address infected herds. Effective control necessitates various approaches including serological tests for animal surveillance, prevention of transmission and the elimination of carriers like dogs, cats and mice. Farmer cooperation, awareness and access to resources are important for long-term eradication and control programs, which emphasize the need for continuous education and training by veterinary organizations [17].

Biosecurity and management

Effective management strategies and hygiene measures are crucially important in reducing the risk of *Brucella* infection in bovines. Prevention strategies should focus on minimizing contact with viable bacteria, whether from infected animals, humans or contaminated environments. Common routes of bacterial entry in herds or animal populations include the purchase of infected animals and contact with contaminated pastures or materials, given *Brucella*'s environmental resilience. Biosecurity measures have a pivotal role in encompassing quarantine protocols, animal movement control and strict sanitation practices [18]. For artificial insemination, it is important to minimize the contact between livestock and wildlife. In infected areas, some additional hygienic measures are required such as removal of abortive materials through cleaning and disinfection. Traditional farming practices including nomadism and shared pastures can complicate the control efforts and emphasize the need for tailored strategies [15].

Culling

The primary objective of early case detection and removal of potential sources of infection is to prevent the circulation of brucellosis in the bovine and human populations. Despite its effective diagnostic strategies, the risk of the presence of silent carriers always remains. This approach proves most valuable and reliable in low-prevalence settings with sufficient resources. Test and slaughter strategies are also applicable during disease outbreaks, especially when stamping-out measures are impractical. Tests used for disease detection are usually categorized based on their ability to identify the pathogen or the host's immune response. In certain conditions, stamping out, followed by thorough cleaning practices, routine disinfection and replacement with *Brucella*-free animals are the only methods to ensure the complete elimination of bacteria from the flock or animal population [19].

Vaccination

Immunization against bovine brucellosis has some unique challenges. It usually targets less susceptible populations due to its negative effects on pregnant animals. The "ideal vaccine" should provide solid and long-lasting immunity without inducing abortion, should be safe for humans and must be cost-effective. Current vaccines like Rev.1 and S19/RB51 have some limitations; Rev. 1 induces abortions and S19/RB51 has some efficacy concerns. Live vaccines usually aim for a balance between protection and virulence, while killed or subunit vaccines face fewer efficacy perceptions (Table 3). Residual virulence is always considered as a concern, which leads to complications like abortions. Strategies include restricted vaccination, reduced doses and modified administration routes, each has varying success and challenges. Addressing these issues is important for enhancing immunization efficacy in controlling bovine brucellosis [20].

Table 3 Adaptation of vaccination strategies against brucellosis [21].

Country	Duration	Vaccine	Dose	Time of Launch
Kuwait	1985-1988	Rev.1	10^9 CFU	1987
Greece	1975-2004	Rev.1	1×10^9 CFU	1998
Tajikistan	2004-2009	Rev.1	-	2004

CFU = colony-forming unit

International cooperation

International cooperation is indispensable in tackling the challenges posed by bovine brucellosis on a global scale. Brucellosis knows no borders. Sharing information, coordinating surveillance efforts and harmonizing control strategies across countries help to prevent the spread of disease globally. Collaboration between countries and international organizations involves the exchange of information, capacity building, cross-border surveillance, standardization of control measures, research collaboration, emergency response coordination and policy harmonization. By sharing relevant knowledge, aligning strategies and coordinating efforts, nations can successfully address the complex epidemiology of bovine brucellosis, enhance control measures and mitigate the impact of the disease on animal and public health. This collaborative approach is a key component in fostering an effective response to brucellosis, ensuring a sustained and coordinated effort toward its control and eradication [17].

Other complementary measures

Complementary measures for the prevention of bovine brucellosis focus on a range of strategies beyond vaccination and test-and-slaughter programs. These include controlling animal movements, ensuring a robust laboratory infrastructure, implementing surveillance systems and establishing effective animal identification systems. Other critical points involve economic compensation for farmers, strict controls on animal movement and comprehensive monitoring strategies. Success in the prevention of disease relies on a multifaceted approach that focuses on various aspects of disease control and considers local epidemiological factors, along with available resources [15]. Addressing the need for resources is of huge importance in the effective control of disease. Successful strategies need sufficient economic support for vaccination programs, comprehensive surveillance and robust laboratory facilities. Adequate funding facilitates the implementation of control measures such as animal movement control and economic compensation for farmers. The availability of resources is an important factor that influences the overall success of disease control efforts, emphasizing the importance of enough financial and technical support [7].

Prevention and control of Brucellosis through One-Health approach

The global recognition of the One-Health approach in controlling and preventing brucellosis is increasing day by day. This collaborative strategy involves veterinarians, physicians, environmental experts and allied health professionals working together to identify risk factors and to develop effective control measures against the spread of disease. In developing countries, where people usually live in proximity to animals, multidisciplinary efforts like farmer involvement are crucial. Bovine brucellosis is an important global public health issue, primarily affecting dairy workers, shepherds, butchers, veterinarians, and animal husbandry personnel. It is transmitted directly or indirectly to humans, posing a significant public health problem in developing countries [22]. Prevention methods encompass the importance of health education to mitigate the occupational and food-borne risks associated with this disease, such as pasteurizing dairy products. However, the ultimate goal is to eliminate or control the infection in animals through vaccination, herd immunity and culling of infected animals. Collaboration within society is of vital significance, especially where specific control strategies for bovine brucellosis are lacking. Personal responsibilities like avoiding the use of unpasteurized products and practicing good hygiene play a significant role in reducing the risk of bovine brucellosis [9].

Challenges in controlling animal Brucellosis

Control of animal brucellosis poses a multifaceted challenge due to the complex nature of the disease and its impact on both human and bovine health. One of the primary challenges lies in the confusing epidemiology of brucellosis, which includes various host species and multiple *Brucella* species. This diversity usually complicates surveillance, diagnosis and control efforts of disease. Moreover, the zoonotic potential of disease necessitates a holistic One-Health approach, integrating important veterinary and human health interventions. In a few cases, resistance or reluctance among bovine farmers to adopt recommended control and preventive measures, like vaccination or culling of infected animals, can affect the progress in disease control. Vaccines such as S19 and RB51 are the most widely used, but major concerns about safety and efficacy persist. Some vaccines cannot provide complete protection and the potential for adverse and negative reactions or incomplete immunity can pose challenges [23]. Implementation of effective control measures, such as vaccination campaigns faces hurdles because of logistical constraints, limited resources and compliance issues within livestock farming communities. Limited financial resources, infrastructure and skilled personnel in many regions may affect the implementation of effective control programs in bovine populations. Adequate funding and financial support are necessary for sustained control efforts. The continuous movement of livestock, both within and among regions or countries can contribute to the spread of bovine brucellosis. Implementing effective preventive measures is challenging when there are different levels of biosecurity and surveillance practices across different areas. Furthermore, the asymptomatic nature of disease in many infected animals makes it difficult to identify carriers timely and to prevent the spread of disease. International collaboration and standardization of control strategies become vital to address the global impact of bovine brucellosis and to reduce the associated economic losses and public health risks [24].

Conclusions

This review explores the multifaceted landscape of control strategies for brucellosis within bovine populations. The intricate interplay of factors such as confusing epidemiology, zoonotic potential and economic restrictions underscores the need for a multifaceted approach. The pre-mentioned control measures including vaccination, biosecurity, testing, surveillance, public awareness and the vital role of One-Health perspective collectively contribute to the management of bovine brucellosis. Additionally, international cooperation appears as a cornerstone in the global battle against this challenging disease. As nations are struggling with the diverse facets of bovine brucellosis, this review covers the importance of collaborative efforts including information sharing and harmonized policies to fortify the collective capacity for control and prevention. Moving forward, sustained research, capacity building and cross-border coordination will be pivotal in shaping effective control strategies, thereby reducing the prevalence of disease and safeguarding both human and animal health worldwide. Additionally, the pivotal importance of the One-Health perspective has been underscored, which emphasizes the interconnectedness of bovine and human health. Furthermore, international cooperation has been focused as a linchpin in the global effort to combat this challenging disease. In short, this review not only consolidates current knowledge of bovine brucellosis control but also emphasizes the current ongoing challenges that necessitate continuous improvement and adaptation in our global approach. By devising the collective efforts of nations, researchers and policymakers, we can aspire to a bright future where brucellosis is effectively controlled, reducing its impact on both animal welfare and public health worldwide.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

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