



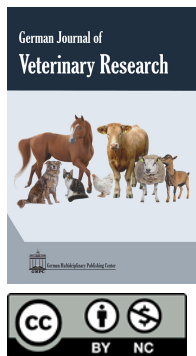
Review

Immune response and recent advances in diagnosis and control of brucellosis

Alyaa Elrashedy, Mohamed Gaafar, Walid Mousa*, Mohamed Nayel, Akram Salama, Ahmed Zaghawa, Ahmed Elsify and Ali S. Dawood

Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Egypt

This article is published in the special issue: Brucellosis from a One-Health Perspective



Article History:

Received: 09-Feb-2022

Accepted: 23-Mar-2022

*Corresponding author:

Walid Mousa

walid.saad@vet.usc.edu.eg

Abstract

Brucellosis is a zoonotic disease that has serious animal welfare and economic consequences worldwide. In mammals, this stealthy intracellular pathogen causes abortion and infertility, and in humans, it produces a terrible febrile illness that can progress into a long-term condition with serious implications. The pathogenicity of brucellae is based on their ability to survive and replicate in host cells, which allows them to escape from the immune system. The gold standard test for diagnosis, which demands competence, is still isolation and identification. Advancements in diagnostic procedures and screening of recently infected animals are required to achieve effective control. Despite their drawbacks, the most widely used vaccine strains to protect against *Brucella* infection and relevant abortions in cattle are *B. abortus* strains S19 and RB51 and in small ruminants is *B. melitensis* Rev1. However, there are no safe vaccine candidates for humans. Therefore, it is critical needs to improve vaccine production using advanced techniques such as subunit vaccines that are both effective and safe. Studying the overview of the *Brucella* immune response mechanism and advances in the diagnosis procedures allow more understanding of effective control strategies. The current review provides an overview on the immune response and updates on the diagnosis and control of brucellosis based on published literature on different search engines.

Keywords: *Brucella*, Diagnosis, Immune response, Immune evasion, Vaccine, Control**Citation:** Elrashedy, A., Gaafer, M., Mousa, W., Nayel, M., Salama, A., Zaghawa, A., Elsify, A. and Dawood, A. S. 2022. Immune response and recent advances in diagnosis and control of brucellosis. Ger. J. Vet. Res. 2 (1): 10-24. <https://doi.org/10.51585/gjvr.2022.1.0033>

Introduction

Brucella spp. is an intracellular zoonotic pathogen that invades and replicates inside the host's cells (Boichenko et al., 2019). Every year, more than 500,000 new human cases are recorded around the world. It causes substantial losses in the livestock industry, particularly among small-scale holders, reducing economic profits and preventing admittance to global markets (Franc et al., 2018). The economic importance of the disease is due to infertility, reduced milk production, high veterinary costs, and high culling rates.

In humans, persistent or intermittent fever, fatigue, excessive sweating, cold, arthritis, aches, weight loss, and possibly fatal problems such as pregnancy failure in women and endocarditis, neurological problems, and testicular or bone abscess formation are the main recorded signs. It also has adverse effects on the liver and spleen, and if not treated, it can persist for a long time. In animals, *Brucella* spreads through the ingestion of infected tissues or discharges from diseased animals. Whereas in humans, it spreads through the consumption of unpasteurized milk and dairy products

and direct contact with infected animals, particularly among occupational personnel such as farmers, laboratory persons, abattoir workers, and veterinarians (Ny-erere et al., 2020).

Brucella virulence is associated with the ability of bacteria to multiply intracellularly inside the phagocytic and non-phagocytic cells. It lacks the classic virulence determinants such as toxins, pili, fimbria, and plasmids (Bennett, 2016). Contrastingly, it has different antigenic components that allow it to establish intracellularly (Głowacka et al., 2018). *Brucella* overcomes the reaction of innate immunity at the early stages of infection through the production of specific Ag structures (Priyanka et al., 2021). It avoids the innate immune response, hindering dendritic cell (DC) maturation, and undergoes macrophage and neutrophil damage. *Brucella* fights and tolerates the bactericidal activity of neutrophils, but does not multiply within these cells, so the anti-infection efficacy of innate cellular immunity is poor (Jiao et al., 2021). Therefore, it is vital to detect diseased animals accurately and quickly to avoid disease extent (Vatankhah et al., 2019).

Bacterial culture techniques are considered the gold standard method despite their need for special laboratories as *Brucella* is a hazardous zoonotic bacteria (Yagupsky et al., 2019). Therefore, serological procedures are employed to diagnose the disease practically. It is also helpful in herds screening during monitoring and eradication programs (Ducrottoy et al., 2018). But, some drawbacks include cross-reactions between *Brucella* antigen and other Gram-negative bacterium species such as *Vibrio cholera*, *Bordetella bronchiseptica*, *Yersinia enterocolitica*, and *Salmonella* spp. have been evident. Molecular approaches, such as polymerase chain reaction (PCR), are fast, specific, sensitive, and safe. The most significant feature of RT-PCR is its quantity tool for more sensitivity, which allows it to detect even the lowest amount in a sample (Ulu Kilic et al., 2013).

The combination of test and slaughter strategy and vaccination of animals is still the practical strategy used to control brucellosis in animals' herds (Vatankhah et al., 2019; El-Diasty et al., 2022). Continuous surveillance is essential to prevent transmission, and detect the new reservoir (Zameer Durrani et al., 2020). Some countries have effectively managed *Brucella* infection to some extent through the application of strong immunization protocols that include the use of appropriate smooth live vaccines, reliable diagnostic technologies, mass vaccination of large populations, and regular culling of *Brucella*-positive animals. However, if the immunization and precise diagnosis are not conducted, the transmission in diverse species may increase especially in the absence of competent immune animals (Moreno, 2014).

This review article discusses brucellosis, with a spotlight on the disease immune response strategy, recent advances in diagnosis, control strategy, and vaccines production.

Antigenic components of *Brucella* as virulence factors

Lipopolysaccharide

Brucella has two colony morphological features: smooth and rough (Liu, 2015). Compared to other Gram-negative lipopolysaccharides (LPS), such as those from the *Enterobacteriaceae* family, *Brucella* LPS is one of its main pathogenic factors (Lapaque et al., 2005). Both *B. abortus* and *B. melitensis* LPS neither stimulate macrophage activity nor enhance the production of pro-inflammatory cytokines. However, *Brucella* spp. LPS seems to de-regulate macrophage and DC antigen-presenting capabilities, inhibiting T lymphocyte activation and proliferation (Barquero-Calvo et al., 2015).

The LPS of *Brucella* spp. comprises three main structures: lipid A, core, and "O" side chain (Smith, 2018). Lipid A is sited into the outer membrane and has a diaminoglucose backbone; the acyl groups are the longest chain, C18–C19 or C29, rather than C12–C14 in typical LPS (Mancilla, 2015). *Brucella* LPS acts as a critical virulence component that hinders identification

by pattern recognition receptors (PRR) (Barquero-Calvo et al., 2015). The smooth LPS-O side chain of *Brucella* has an "O" side chain made up of repeated units of sugars with enough variety to allow for species differentiation (Lapaque et al., 2005).

The absence of the O-chain results in attenuated strains, which enables the infected cell to transport the *Brucella*-containing vesicle to the lysosomal region, where the pathogen is devastated (Smith, 2018). However, the smooth strains can escape from this intracellular destruction pathway, implying that the LPS "O" side chain is involved in transferring *Brucella*-containing vesicles to non-lysosomal compartments or avoiding transport to such lysosomal compartments (Jiao et al., 2021). It also prevents the activation of the complement, avoids opsonization by subcomponent C3b, and reduces recognition by phagocytic cells. Furthermore, it avoids the production of anaphylatoxins C3a and C5a, inhibiting the consequent pro-inflammatory reaction (Stranahan and Arenas-Gamboa, 2021).

Cyclic β -1, 2-Glucan

Cyclic glucans are Gram-negative bacteria's intrinsic portions with substantial structural diversity and are responsible for controlling osmolarity homeostasis (Martirosyan et al., 2012). It has a spine of sugar molecules bound by β -1, 2 linkages with a polymerization degree of 17–25 monomers, and is established in the periplasm (Roset et al., 2014). Mutants of the genes included in the creation and transport of glucan to the periplasm exhibit a decrease in bacterial persistence and multiplying in the spleen of BALB/c mice. A mutant strain of the cyclic glucan synthase (*cgs*) gene responsible for glucan production, creates a Th1 lymphocyte-only response (Briones et al., 2001).

The cyclic glucan of *Brucella* spp. act on the cell membrane at the host's lipid rafts, followed by the development of the vacuoles and avoiding lysosome fusion and enabling it to access the reproduction site (Jiao et al., 2021). Due to the activation of monocyte, DC, and neutrophil by interleukin (IL)-12 and tumor necrosis factor (TNF- α) production, *Brucella*'s cyclic-1, 2-glucan causes splenomegaly in mice (Roset et al., 2014). According to *in-vitro* studies, *Brucella* spp. triggers DCs via toll-like receptors 4 (TLR4), myeloid differentiation factor 88 (MyD88), and TIR domain-containing adaptor inducing interferon (TRIF), but not CD14, boosting the antigen-specific reaction of CD8⁺ T cells (Barquero-Calvo et al., 2015).

Type IV Secretion System and BvrR/BvrS

The BvrR/BvrS is a two-component system found in *Brucella* spp. This system regulates the expression of operon *virB*, which codes for the type IV secretion system (T4SS) through direct and indirect systems (Ke et al., 2015). It is composed of a cytoplasmic regulator (BvrR) and a histidine-kinase sensor (BvrS) in the cytoplasmic membrane. In several studies, the mutation in the BvrR/BvrS system in mice reduces the ability to penetrate macrophages, and HeLa cells

(Ke et al., 2015). Moreover, the *Brucella* BvrR/BvrS two-component regulatory system is required to detect the changes in the phagosomal environment. It also enables the bacteria to shift from an extracellular to an intracellular lifestyle. Furthermore, the BvrR/BvrS system controls the LPS structure's conformation as well as outer membrane proteins (Omps) expression (Lamontagne et al., 2007). BvrR has been recognized as a likely choice for a DNA vaccine in the murine brucellosis model, but more research is needed (Chen et al., 2019).

Enzyme Superoxide Dismutase

The superoxide dismutases (SODs) are metalloenzymes that catalyze superoxide ions' dismutation, which is the main antioxidant defense tool (Broxton and Culotta, 2016). It is patenting from the cell wall's periplasmic region (Pratt et al., 2015). The comparison between the bacterial counts of *B. abortus* Cu/Zn SOD mutants recovered from BALB/c mice is much lower than the amount recovered from wild *B. abortus* (Tatum et al., 1992). This finding highlighted the value of Cu/Zn SOD in the bacterium's pathogenicity.

Urease

The hydrolysis of urea in carbon and ammonia is performed by urease (Bandara et al., 2007). Several pathogens have been displayed to utilize the urease as a virulence factor, causing direct toxicity in renal tissue and the formation of kidney stones. It also helps bacteria to survive in an acidic environment, which makes them more susceptible to infection (Paixão et al., 2009).

The outer membrane proteins (Omps)

The Omps of *Brucella* spp. have been documented as the main virulence factors that encourage infection and pathogenicity and the co-infection with other pathogenic organisms. In previous studies, Omp25 had been established to be important in reducing tumor necrosis factor- α (TNF- α) and interleukin (IL)-12 expression produced by various microorganisms to stimulate intracellular survival in porcine and murine macrophages (Li et al., 2021).

Proline Racemase Protein A (*prpA*)

The *prpA* is one of the various virulence factors required for *Brucella* to induce chronic infection. It interfaces with macrophages to enhance B-cell proliferation. Also, it regulates IFN- γ , IL-10, TNF- α , and transforming growth factor β TGF- β 1 in the early stage of infection. The findings showed that immunomodulators *prpA*, *Brucella* TIR domain-containing protein (Btp1/TcpB), and LPS could block IFN-secretion while promoting IL-10 production, influencing the Th1 immune response. As well as B Lymphocyte mitogen is a *B. abortus* virulence factor required for persistent infection (Spera et al., 2014).

***Brucella* TIR-containing effector protein-1 (*Btp1*)**
Brucella effectors considerably impact T lymphocyte stimulation and antigen presentation via controlling the TLR signaling pathway associated with the DC development. The Btp1 sequence of *Brucella* is comparable to the Toll/IL-1 domain family (Salcedo et al., 2008). The Btp1 hinders the synthesis of cytokines and the development of DC, stopping TLR2 and TLR4 signals from being stimulated. Although the exact mechanism of this protein is still unknown, research indicates that when it links to the Toll/IL-1 receptor domain-containing adaptor protein (TIRAP/Mal), it fights with the myeloid differentiation factor 88 (MyD88), which not only increases TIRAP/Mal production but also suppresses the TLR4 and TLR2 signal transmission (Snyder et al., 2014).

Different routes of entry of *Brucella*

Oral cavity as the first contact site for *Brucella* spp.

Initially, the oral cavity is the first site of contact of *Brucella* spp., which contains barriers such as saliva, which comprises components that remove or control microbial progress, such as lysozyme, lactoferrin, nystatin, peroxidases, and immunoglobulins, mainly IgA (Meyle et al., 2017). Therefore, *Brucella* should be recognized firstly by the immune response at this site (López-Santiago et al., 2019). In addition, the gingival crevicular fluid that shelters the space among the teeth and the gingiva, known as the gingival sulcus contains complement, antibodies, neutrophils, and plasma cells constituents. This combination of saliva and gingival crevicular fluid is a strong primary hindrance against *Brucella*. In the mucosal tissues, the phagocytic cells and macrophages, antigen-presenting cells (APCs), can capture antigens and migrate to the regional cervical lymph node followed by a proper activation signal (Meyle et al., 2017). The suppressor of tumorigenicity 2 (ST2) receptor is playing a crucial role in the regulation of *B. abortus* oral infection via penetration into the mucosa (Santos et al., 2020).

The interplay of *Brucella* spp. through the gastrointestinal tract

The lamina propria is located where the APCs such as CD68⁺ macrophages and DC are present and exhibit high phagocytic activity, which is the main effector reaction of the intestinal immune response (Avila-Calderón et al., 2020). Polymorphonuclear (PMN) cells (neutrophils, eosinophils mastocytes) are identified in the lamina propria, especially when an inflammatory response is existent (de Figueiredo et al., 2015). It is also inhabited by T and B lymphocytes which migrate from their activation site in the lymphoid tissue to the intestine. The Peyer's patches, isolated lymphoid follicles, and mesenteric lymph node (MLN) make up the gut-associated lymphoid tissue (GALT); these are the primary sites for triggering the adaptive immune response since they are also where the antigen is presented to T cells and B lymphocytes are activated, resulting in germinal centers (López-Santiago et al., 2019).

Table 1: The antigenic structures of *Brucella* and the mode of action.

Virulence factor	Mode of action	Reference
Lipopolysaccharides (LPS)	<ul style="list-style-type: none"> The endotoxic reaction is reduced. Phagocytosis and bactericidal activity are inhibited. Secures from complement attacks. Major histocompatibility complex II (MHC2) antigen presentation is diminished. DC maturation is reduced by interaction with TLR4/MD-2 recognition. 	López-Santiago et al. (2019)
Cyclic β -1, 2-Glucan	<ul style="list-style-type: none"> BCV biogenesis. Regulator fusion between phagosome and lysosome. 	Jiao et al. (2021) López-Santiago et al. (2019) Roset et al. (2014)
BvrR/BvrS regulatory system	<ul style="list-style-type: none"> Activate the genes required for invasion and intracellular survival by turning down extracellular survival genes. Controls the LPS structure's conformation as well as Omp expression. 	Głowacka et al. (2018) Ke et al. (2015)
Type IV Secretion System	<ul style="list-style-type: none"> Intracellular replication. 	López-Santiago et al. (2019) Głowacka et al. (2018) Ke et al. (2015)
Enzyme Superoxide Dismutase	<ul style="list-style-type: none"> Vital mechanism of antioxidant defense. 	López-Santiago et al. (2019) Pratt et al. (2015)
Urease	<ul style="list-style-type: none"> Encourages the survival of bacteria in the surrounding acid environment and thus enhances infection. 	López-Santiago et al. (2019) Paixão et al. (2009)
Omp25	<ul style="list-style-type: none"> Inhibits the generation of TNF-α. IL-12 production is negatively regulated at both the transcriptional and post-transcriptional stages. 	(Li et al., 2021) López-Santiago et al. (2019) Luo et al. (2017)
Proline racemase <i>prpA</i>	<ul style="list-style-type: none"> IL-10 secretion is stimulated by T-independent B cell mitogen. 	López-Santiago et al. (2019) Spera et al. (2014)
Btp1/TcpB	<ul style="list-style-type: none"> TLR2 and TLR4 signaling are disrupted. NFκB activation is induced by blocking MyD88. 	López-Santiago et al. (2019) Salcedo et al. (2008)

Abbreviation: TLR: Toll-like receptors; TNF- α : Interferon alpha; NF- κ B: Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; BCV: *Brucella* containing vacuole; Omp: Outer membrane protein; DC: Dendritic cells

Brucella is migrated via M cells, which are enterocytes specialized in capturing lumen antigens and then transferring the antigen to adjacent DCs ([Paixão et al., 2009](#)). M cells are located in the follicle-associated epithelial region of Peyer's patches ([Nakamura et al., 2018](#)). The cellular prion protein (PrPC) is abundantly expressed at the apical end of M cells, was investigated to achieve a good understanding of the role of M cells. The heat-shock protein Hsp60, released by *B. abortus* through its type-IV secretion system, is one of its known targets. This shows that PrPC and Hsp60 in-

teract to enhance the macrophage infection, indicating that PrPC is a *Brucella* receptor. Thus, PrPC is a crucial module in the *Brucella* presentation ([Watarai et al., 2003](#)).

The mucosa of the respiratory tract

Following inhalation of *Brucella*, it comes in contact with epithelial and phagocytic cells. The interaction between the bacteria and epithelial cells initiates acquired temporary immunity ([Virji, 2005](#)). *Brucella* also attacks lung epithelial cells and alveolar

macrophages and this may encourage the extension and dissemination of the pathogen outside pulmonary tissue (Hanot Mambres et al., 2016). Although the IL-1, IL-6, and TNF are necessary regulators for topical and systemic inflammatory processes (Skendros and Boura, 2013), it has been observed that the absence of both IL-1 and IL-6 does not affect the course of *Brucella* infection in the lungs, liver, or spleen. On the other hand, deficits in T Cell Receptor (TCR), IL-17 receptor A, Transporter associated with antigen-processing-1 (TAP1), and IFN- γ R have an impact on *Brucella* regulation in the lungs (Hanot Mambres et al., 2016). Recently, it was found that the inflammatory components the IL-1 receptor (IL-1R), Nucleotide-binding oligomerization domain-3 (NLRP3), and absent in melanoma 2 (AIM2) protect against respiratory *Brucella* infection, most likely through IL-1 activity (Hielpos et al., 2018).

Immune evasion

Brucella interferes with innate immune recognition and response of host

The innate immune response is primarily composed of macrophages, DCs, granulocytes (neutrophils, basophils, and eosinophils), and natural killer cells, in addition to physical barriers and soluble substances such as complement proteins (Oliveira et al., 2008). *Brucella* can activate PMN neutrophils, which improve their microbicidal action and allow leukocytes to survive for long periods. So, it is considered a stealthy pathogen that evades innate immunity, stimulates PMNs, and resists the mechanisms of phagocytes (Moreno and Barquero-Calvo, 2020). The innate immunity identifies bacteria by their distinctive chemicals, such as Gram-negative LPS Lipid A, via TLRs, which trigger pro-inflammatory compounds and small GTPases of the p47 group involved in the intracellular pathogen. The interplay of PRR and pathogen-associated molecular patterns (PAMPs) permits host cells to recognize hazardous chemicals, prompting the body to elucidate the immune response to destroy pathogens (Li et al., 2014). However, *Brucella* takes an additional character to avoid recognition by innate immunity, changing PAMPs into critical components (Kumar et al., 2011).

The TLR4 is activated by the LPS of *Brucella*, but only at very high concentrations (Ke et al., 2015). As well as *Brucella* flagellin lacks the unique aspect recognized by TLR5 and is critical in immune evasion (Andersen-Nissen et al., 2005). Because LPS affects the complement system, the O-chain missing a free hydroxyl is beneficial for binding with C3 which inhibits the synthesis of C3a and C5a by associating with the specific O-chain *Brucella* LPS, allowing it to evade host immunity (Kim et al., 2004). The main mechanisms of immune evasion were discussed by Skendros and Boura (2013) and summarized in Figure 1.

The adaptive immune response

The immune response against brucellosis is illustrated in Figure 2. The humoral immune response and the cellular immune response are the two main mechanisms

of the organism's adaptive immune response (Cannella et al., 2012). Adaptive immune response mechanisms in brucellosis are divided into three stages. In the first, it inhibits *Brucella*'s intracellular survival, IFN- γ produced by CD4⁺, CD8⁺, and T cells initiate bactericidal function in macrophages. However, in the second, infected macrophages are destroyed, causing CD8⁺ and T lymphocytes to cause cytotoxicity. Finally, IgG2a and IgG3 opsonize *Brucella* in the endocytic compartments, which improves phagocytosis (Martirosyan et al., 2011). In addition, IL-12, IFN- γ , and TNF- α initiate and regulate innate and adaptive immunological responses in brucellosis (Durward et al., 2012).

Brucella stimulates antigen-presenting cells to produce IL-2 and activates natural killer cells (NK) (Gao et al., 2011). TNF- α , IFN- γ , GM-CSF, and other cytokines are secreted by NK cells and play a major role in Th1 and type 1 CD8⁺ T cells (Tc1) responses (Li et al., 2014). In macrophages, *Brucella* can decrease IFN- γ mediated phagocytosis and TNF expression (Barrionuevo et al., 2008). TNF- α can increase macrophage bactericidal ability, while IL-12 can trigger a Th1 immune response and create IFN- γ (Kianmehr et al., 2015). *Brucella* modulates MHC-I and MHC-II expression through regulation of the IFN- γ secretion. So, removal of *Brucella* needs an IFN- γ , which mediates the Th1 immune response (Mora-Cartín et al., 2019). Moreover, the reduction in CD8⁺ T lymphocyte activation, IL-12, and TNF- α , results in immunosuppression, which stimulates *Brucella* replication and persistent infection (Guimarães et al., 2020).

Diagnosis of brucellosis

Isolation and identification

Bacterial isolation is the gold standard for diagnosis (Ledwaba et al., 2020). Culture procedures require ideal biosafety procedures, which can be difficult to achieve anywhere (Stear, 2005). Tryptose soy agar (TSA) is the widely used dehydrated basal medium for *Brucella*. In some strains like *B. abortus* biovar2, bovine, or horse serum (2–5%) must be supplied (Sylla et al., 2014). Other suitable media are serum-dextrose agar (SDA) and glycerol dextrose agar, which aid in examining morphological characteristics. When *Brucella* is isolated from blood, other body fluids, or milk, Castaneda's medium (nonselective biphasic media) are preferred because they provides enrichment and eliminates confusion in biotyping (Mantur et al., 2008).

For some *B. abortus* biovars, serum and carbon dioxide are necessary for growth, but *B. melitensis* does not. Selective media, such as Farrell's selective media, are needed to prevent contaminants and are used to isolate bacteria from milk samples (Ledwaba et al., 2020). *Brucella* spp. can be identified and confirmed using colony morphology, Gram staining, H₂S production, the requirement of CO₂, lysis of phages, and biochemical tests such as catalase, oxidase, urease,

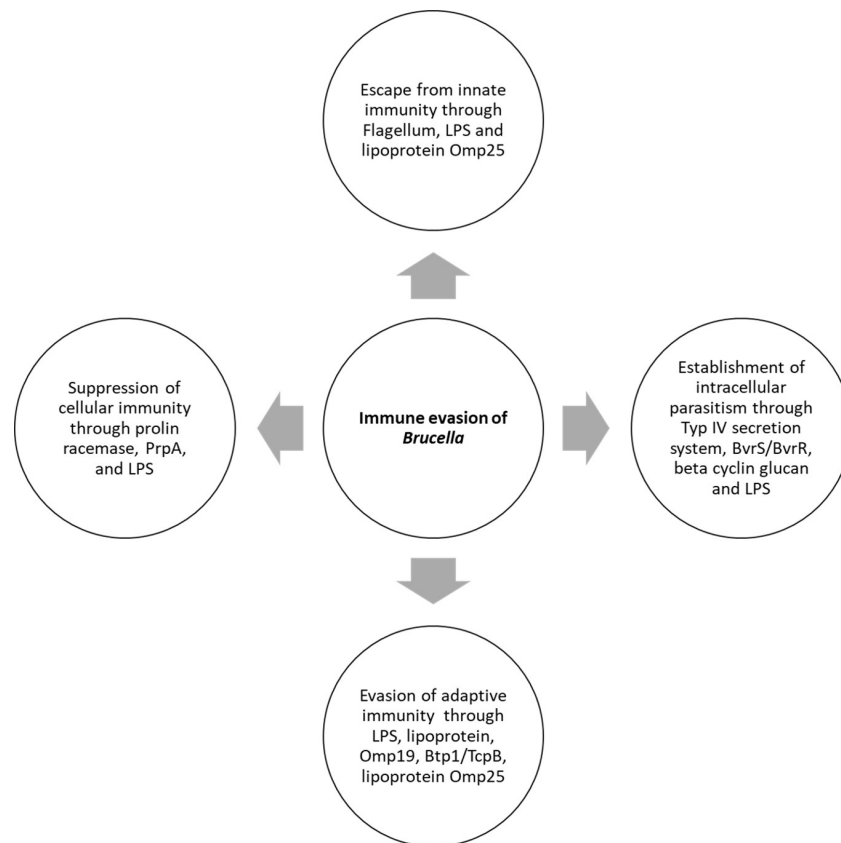


Figure 1: Immune evasion of *Brucella*. The escape from innate immunity, suppression of cellular immunity, evasion of adaptive immunity, and establishment of intracellular parasitism are the main mechanisms.

Serological tests

Serological diagnosis are essential for global screening, surveillance, control, and eradication programs. It includes serum agglutination test (SAT), rose Bengal test (RBT), complement fixation test (CFT), milk ring test (MRT), Coombs test, lateral flow assay (LFA), and ELISA tests (Chisi et al., 2017). Serological tests are easy, cheap, and fast. However, the false-positive results consider a diagnostic problem because of the similarity of the LPS-O chain of *Brucella* with other Gram-negative bacteria such as *Escherichia coli* O157, *Vibrio cholera*, *Francisella tularensis*, and *Yersinia enterocolitica* (Ali Hussei et al., 2019). In comparison to RBT and indirect ELISA, the in-house fluorescence polarization assay (FPA) and competitive ELISA (cELISA) can be applied to detect *B. abortus* S19 post-vaccinal antibodies (Kallehamurthy et al., 2020). As a result, the detection of *Brucella* antibodies in milk can be done by using MRT and immunological methods like the i-ELISA, being the most common and widely used tests for indirectly confirming *Brucella* spp. in milk (Alamian and Dadar, 2019).

Polymerase chain reaction (PCR) assay

Real-time PCR, PCR restriction fragment length polymorphism (RFLP), and *B. abortus*, *B. melitensis*, *B. ovis*, and *B. suis* PCR (AMOS-PCR) are PCR-based molecular approaches that have provided significant sensitivity in identifying *Brucella* DNA (Kolo et al., 2019). Several specific genes have been recognized

as candidates for genus-specific PCR-based methods for *Brucella* species, including Omp2 and *bcs*31, 16S rRNA, *recA* gene, the 16S-23S region, and RNA Polymerase Beta Subunit (*rpoB*) (Bazgari et al., 2020). The polymorphism deriving from the species-specific location of the insertion sequence IS711 in the *Brucella* chromosome was applied to construct the first species-specific multiplex PCR assay, known as AMOS-PCR, to differentiate *Brucella* species (Bricker and Halling, 1994). Several *Brucella* species and the vaccine strains e.g. *B. melitensis* Rev.1, *B. abortus* strain 19 (S19), and *B. abortus* RB51 have been well-known and differentiated in one step by using a multiplex PCR method named the Bruce ladder PCR (López-Goñi et al., 2011).

Furthermore, real-time quantitative PCR (qPCR) appeared to be quite valuable in determining the presence of *Brucella* infection in milk, providing the differentiation of vaccine strains from virulent strains (Kaynak-Onurdag et al., 2016). In addition, it has been supposed that the newly discovered loop-mediated isothermal amplification (LAMP) is a fast, direct, and accurate diagnostic technique for detecting *Brucella* from clinical samples (Moeini-Zanjani et al., 2020). In comparing the PCR to the culture approach, the sensitivity and specificity of the PCR can detect minute amounts of *Brucella* in milk (Dadar et al., 2019). Moreover, PCR-based methods save roughly three days in *Brucella* detection (Yagupsky et al., 2019).

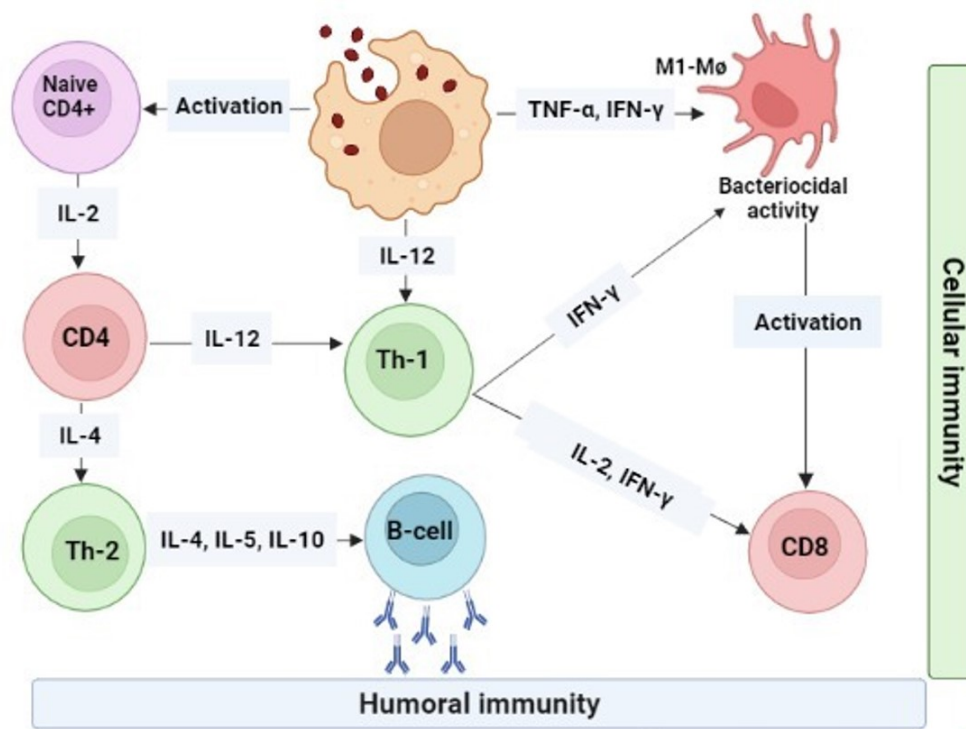


Figure 2: Immune response against *Brucella*, modified after Skendros and Boura (2013). The APCs exhibit several functions against *Brucella* such as i) Xenophagy through degradation by hydrolytic enzymes of phagolysosomes, ii) antibacterial peptides defensins, iii) secretion of $\text{TNF-}\alpha$ that enhances phagocytosis, iv) secretion of IL-2 that activates Th1.

Immunodominant proteins of *Brucella* and their role in the immunogenicity and antigenicity of a novel *Brucella* multi-epitope

Various *Brucella* spp. proteins have been used as diagnostic antigens, with pure natural protein(s) or generated recombinant protein(s) substituting S-LPS (s). This includes *Brucella* protein26 (BP26), Cu-Zn superoxide dismutase (SodC), VirB12, Serine protease, BAB1-1885, twin-arginine translocation pathway signal sequence domain-containing protein (Twin arginine), solute-binding family 5 protein, Leu/Ile/Val-binding family protein, branched-chain amino-acid ABC transporter substrate-binding protein, bacterioferritin (Bfr), thiamine transporter binding protein, Invasion protein B (InvB), *Brucella* lumazine synthase (BLS), malate dehydrogenase (Mdh), and Aldehyde dehydrogenase (Nagalingam et al., 2021). Except for BLS, Bfr, Mdh, VirB12, and Aldehyde dehydrogenase, cytoplasmic proteins. All designated proteins are outer membrane or periplasmic.

All these proteins were tested for amino acid sequence similarity using BLASTP and showed a 60% match with other cross-reacting pathogens were further expressed. The type IV secretion system (T4SS) protein VirB12 has been detected for possible diagnostic for diagnosis of brucellosis (Chen et al., 2019). Furthermore, Nagalingam et al. (2021) reported that BP26 is the optimal recombinant protein for the diagnosis, as BP26 based ELISA can be used as a screening test to identify infected herds to avoid cross-reactivity. In a previous study, both BP26 and BLS were the best im-

munogenic proteins (Tian et al., 2020). *Brucella* Omps have been proven to be highly immunogenic, and they could be used as novel subunit vaccines and diagnostic antigens (Yin et al., 2021). The recombinant protein was utilized as an antigen to evaluate the immunological response of immunized mice, and goat serum was used to confirm the diagnostic performance of the recombinant protein (Yin et al., 2021).

Meanwhile, in the vaccinated mice, antibody production was successfully induced, and the T cell subsets improved the percentage of CD4, CD8, and CD4/CD8 ratios in mouse splenocytes and could be used as a target antigen for the development of brucellosis vaccines and serological diagnostics. A comparative study (Yang et al., 2020) described that Omp25-specific mAbs are valuable tools for detecting brucellae in clinical samples. In practical practice, FCM based on mAb 6C12 could be utilized to monitor the therapy efficacy for brucellosis. Bulashev et al. (2021) revealed that the three kinds of *Brucella* chimeric proteins (rOmp19+25, rOmp19+31, and rOmp25+31) elicit a strong immune response as compared to the single Omps taken separately and that they'd be utilized to detect infected animals in an unvaccinated herd by improving an ELISA test for precise diagnosis of brucellosis.

Proteomics of brucellosis

Nowadays, proteomic technologies are important post-genomic techniques for determining the effects of regulatory systems on microbial protein composition (Schmidt and Völker, 2011). It is a useful tool for

studying microbial physiology, gene expression, and bacteria-host cell interactions. LC-MS-based proteomics technology has been developed recently and is now frequently used in *Brucella* diagnosis. It can identify a larger number of proteins. Comparative and comprehensive proteomics evaluation of many strains of brucellae from various host populations may aid in detecting critical virulence proteins. Also, it can improve the progress in diagnostics and safer vaccines to control brucellosis in animals and humans effectively (Wareth et al., 2020).

Nanotechnology methods used for diagnosis of brucellosis

Flower-like gold nanoparticles labeled and silver deposition rapid vertical flow technology for highly sensitive detection of *Brucella* antibodies

The rapid vertical flow technique (RVFT) was discovered to identify brucellosis antibodies. It can successfully avoid the false negative problem in lateral flow assay. It is easy to use, with a quick time of 2–3 minutes visible to the naked eye and no special equipment. LPS were utilized to detect brucellosis antibodies to improve the procedure's sensitivity. The advantages of the lateral flow immunoassay were kept while a single multipurpose buffer was developed in whole blood and other biological samples to enhance the sensitivity of serum antibody detection (Rey et al., 2017).

The RVFT sensitivity can be increased in two ways: by utilizing flower-like gold nanoparticles to fix to more labeled proteins and by silver deposition to develop the experimental results (Fang et al., 2021). It was observed that increasing the surface area of gold nanoparticles caused more proteins/antigens to be bound to the surface. Nonspherical particles have a higher surface area than spherical particles since they are smaller. The reaction concept is that the microporous membrane serves as a carrier for the appropriate antibody or antigen. Following, the antigen or antibody in the sample will infiltrate through the microporous membrane and bind with the antibody or antigen in the solid carrier, and the gold nanoparticles marker probe will mix with the present antigen-antibody complex. Therefore, these will be colored dots in a positive reaction due to the gold nanoparticles.

Quantum dot-based immunochromatographic test strip

Developing new diagnostic methods for brucellosis studies is a major issue, particularly in developing countries. A Quantum dot (QD) immunochromatographic system is developed for the quick detection of brucellosis antibodies in the field. To investigate the sensitivity, specificity, and stability of brucellosis serum, QD test strips were used (Qiu et al., 2014).

The rose bengal test, standard agglutination test, and colloidal gold immunochromatographic assay were all compared to the test strips. *Brucella*'s total protein that has been labeled showed high specificity and no

cross-reactivity. The labeled bacterial protein concentration was 3.9 mg/ml, the coating concentration was 2.0 mg/ml, and the lowest detection sensitivity serum titer was 1:25. The ideal reaction temperature for the test strip was between 25 and 30°C. The test strip was stable after being stored at room temperature, and it had a good degree of repeatability, with a coefficient of variation of 4.0 percent. The sensitivity of the QD test strip was 98.53 percent, the specificity was 93.57 percent, and the correlation rate with the standard agglutination test was 96.98 percent after analyzing 199 serum samples (Li et al., 2020).

Latex agglutination: A Rapid, specific immunoassay for diagnosis of ruminant brucellosis

Advances in nanotechnology have boosted the use of latex agglutination immunoassays in diagnosis. Because of its specificity, simplicity, speed, easy to interpret technique, and cheapness, the latex agglutination test coated with SBPs 50 was found to be an effective tool for diagnosing bovine brucellosis (M. Roushdy et al., 2021). In this study, by differential precipitation with 50 percent ammonium sulfate, soluble *Brucella* proteins (SBPs 50) fractions were obtained from a reference strain of *B. abortus* (strain 99). Compared to low-molecular-mass markers, the obtained proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and revealed a dominating 37.6 kDa protein, a medium-sized 30.6 kDa protein, and a low molecular weight protein of 17.2 kDa (14 kDa to 92 kDa).

The covalent coupling of obtained SBPs 50 to carboxylated polystyrene microsphere latex beads ($0.81 \pm 0.15 \mu\text{m}$ diameter) was assessed. The efficacy of latex beads coated with the obtained SBPs had high diagnostic specificity (DSp) in all tested species; cattle, buffaloes, sheep, and goats (92.3%, 99.0%, 94.1%, and 95.9%), respectively. The diagnostic sensitivity (DSe) of LAT was recorded as 97.2%, 95.0%, 91.7%, and 85.6% for cattle, buffaloes, sheep, and goats, respectively, which were comparatively lower than other conventional screening immunoassays such as buffered antigens (BAPA and RBT). Kappa agreement values (κ) between the latex agglutination test (LAT) and complement fixation test (CFT) were (0.90, 0.95, 0.83, and 0.76) for cattle, buffaloes, sheep, and goats, respectively representing an almost perfect correlation in tested species except in goats which displayed substantial correlation.

Control of brucellosis

Strategies of control

The planned control program for brucellosis must consider many aspects, including knowledge of regional and local variations in epidemiological patterns, cross-sectoral brucellosis epidemiological coordination and surveillance, farming methods, supporting infrastructure, awareness programs, and social customs (Seimenis et al., 2019). For an ideal control strategy, combining vaccination, test and slaughter, sanitary conditions, and personal protection in humans is the best control system, as it has a significant effect at a lower cost.

Annual reports about the numbers of positive cases in the United States have dropped significantly to about 100 cases per year due to aggressive animal vaccination programs and milk pasteurization. Most United States cases are now due to the consumption of illegally imported unpasteurized dairy products from Mexico, and approximately 60% of human brucellosis cases occur in California and Texas (Nyerere et al., 2020).

Vaccination

The most cost-effective and crucial method for controlling and eradicating brucellosis is animal vaccination, particularly in endemic areas; this would also reduce the risk of human infections (Zamri-Saad and Kamarudin, 2016). Vaccination of calves aged 4 to 12 months is the most cost-effective method of brucellosis control (Al Hamada et al., 2021). The live vaccine strains of *B. abortus* RB51 and S19 are the most commonly used to control brucellosis in cattle (Hou et al., 2019). Furthermore, the *B. melitensis* Rev-1 strain is a widely used vaccine candidate to prevent brucellosis in young and adult small ruminants (Khurana et al., 2021).

The test and slaughter policy is used to control diseases in dairy cows in countries with low *Brucella* infections (Tesfaye et al., 2011). Other preventive measures for brucellosis control include importation from brucellosis-free herds, and immunization of females may be an efficient tool (Avila-Granados et al., 2019). In addition, veterinary authorities should continuously provide education and training programs to achieve farmer awareness of prevention and transmission routes and to help eradicate the disease in other animals like dogs, cats, and mice to eliminate infection reservoirs (Kiros et al., 2016).

Different strategies for developing safe and effective vaccines

Due to the side effects and insufficient protection of the available live attenuated vaccines (RB51, S19, 45/20, and SR82) (Goodwin and Pascual, 2016), a greater focus on molecular pathobiology immunological features of *B. abortus* is required to generate better and safer vaccines. The pathophysiology, immunologic mechanisms, gene interaction networks, and protective indicators of *Brucella* are required to obtain safer and more efficient vaccines. New potential vaccines, such as recombinant *B. abortus* subunits, DNA, and a live vector, have recently been used in a mouse model (Gheibi et al., 2018). A variety of techniques have been used to develop safe and effective vaccines, including the invention of subunit vaccines (Bobbala and Hook, 2016), bacterial vectors (Baloglu et al., 2005), and the over-expression of homologous protective antigens. In addition, immunization with DNA vaccines produces a protecting antigen (Wang and Wu, 2014).

DNA vaccines

DNA vaccines have been considered potentially useful vaccines because of their high cell-mediated immune response, which plays a significant role in protecting against intracellular organisms (Chen et al.,

2021). Several previous studies have shown that particular DNA vaccines, e.g., SEN1002 and SEN1395 genes, can prevent brucellosis (Bello et al., 2016), lumazine synthase (Velikovskiy et al., 2002), as well as Cu Zn superoxide dismutase (Retamal-Díaz et al., 2014), were capable of making a significant level of protection in mice. In China, the *B. abortus* 2308 strain A19 vaccine is frequently used and provides excellent protection for cattle. The antibody response produced by the A19 vaccine's O-side chain interacts with serological diagnosis, which can't differentiate between vaccinated and infected animals (Perkins et al., 2010). Likely, gene-deleted vaccines are being developed and used to overcome this problem (Lin et al., 2020). In mice and cattle, the safety and protective effectiveness of the A19D virB12 vaccination have been reported (Yang et al., 2021).

To improve the accuracy of the VirB12-based method's differential diagnosis, it was tested for extra antigens using a combination of proteomics and protein chip technologies. Five of the 26 proteins discovered in S2308 induced a greater IgG response in challenged sera than in immune serum. They also looked at virulence-related proteins and pathways in comparison to S2308 and A19DvirB12. Luo et al. (2006) found that a divalent DNA vaccination expressing both the *B. abortus* L7/L12 and Omp16 genes boosted cellular and humoral immunity in mice by increasing IFN- γ and IgG2a production. In addition, as compared to univalent Omp16 or L7/L12 DNA vaccines, this divalent DNA vaccine induced higher levels of protection, even though the protective efficacy of the divalent Omp16 and L7/L12 was lower than that of the conventional *B. abortus* RB51 vaccine.

Subunit vaccines

Several *Brucella* fragments, such as recombinant peptides, proteins, DNA, LPS, and outer membrane vesicles (OMVs) used as *B. abortus* subunit vaccines (Lalsiamthara et al., 2018). Recombinant subunit vaccinations have induced high amounts of antibodies and eliminated the safety concerns associated with live vaccines (Zai et al., 2021). The subunit vaccines are more homogeneous and can be better regulated, and the raised antibodies are easy to differentiate from other antigens, thus facilitating the differentiation between wild and vaccine strains. Although subunit immunizations often need an adjuvant and are less protective than standard immunizations due to their limited antigenic epitopes, they are thought to be safer vaccines. In the study of (Zhu et al., 2020), the immunological effect of Taishan Pinus massoniana pollen polysaccharides (TPPPS), a proven natural adjuvant, was tested on the recombinant proteins. These findings suggest that a brucellosis subunit vaccine combining recombinant Omp10-Omp28-L7/L12 antigen with TPPPS adjuvant has potential and that *Pichia pastoris* is the best expression system for producing this recombinant subunit antigen.

Nanotechnology vaccines

Recombinant vaccines, in comparison to entire bacterial vaccines, are less immunogenic. They require immune-stimulating or adjuvant-stimulating molecules that can particularly increase immune responses to these compromised antigens to improve the efficacy (Mohan et al., 2013). Furthermore, the importance of the vaccine delivery method and administration route of vaccination is considered (Baumann, 2008). Increased antigen absorption by APCs is achieved by incorporating antigens into nanoparticles as an adjuvant and effective delivery method. Nano-adjuvants can also shield these antigens against harmful conditions, such as low pH and enzyme activity. For all these reasons, they can be highly advantageous for vaccines that enter the body through the mucosal surfaces, such as oral and nasal vaccines.

The spray freeze dryer (SFD) is a drying method that uses low-liquid nitrogen temperatures to create ultra-fast freezing rates. This can result in homogeneous antigen embedding and minimize the likelihood of phase separation between protein and the phase of excipients around (Karevan et al., 2021). Furthermore, the use of SFD can increase the stability, dissolving rates, and aerosol function of dry powder inhalers (Eggerstedt et al., 2012). Adding other amino acids, such as glycine, to the spray drying formula prevents powder clumping and significantly improves spray characteristics. Therefore, the chimeric antigen-loaded glycine nanoparticles can be used as a vaccine candidate to induce a cellular and humoral immune response to brucellosis (Karevan et al., 2021).

Use of Postbiotics for immunomodulation of the immune response against brucellosis

Postbiotics, functional fermentation compounds, have beneficial actions such as gut microbiota change, competitive adhesion to mucosa and epithelium, improved epithelial barrier performance, and immune system ability (Bermudez-Brito et al., 2012). Postbiotics have numerous benefits over probiotics regarding safety, such as the reduced risk of microbial translocation, infection, or increased inflammatory responses, safe dosing settings, and longer shelf life (Tomar et al., 2015). In a study by Santos et al. (2021) the postbiotic supplements was suggested as a safe, healthy, and cost-effective method to increase animal productivity. Cattle can also contract better with different pathogenic pathogens, such as *Brucella* spp. if their immune systems are strengthened. Because reducing the prevalence of brucellosis has significant socioeconomic consequences, the use of postbiotics could become a crucial tool with a favorable cost-benefit ratio in control of brucellosis (Santos et al., 2021).

Summary and conclusion

Brucellosis is considered one of the most serious zoonotic diseases worldwide, particularly in the Middle East and the Mediterranean basin. brucellae affect a wide range of domesticated and wildlife species, as

well as humans. It is also able to stay in the environment and foodstuff for a long time. Therefore, collaboration between human, veterinary, food, and environmental sectors in fighting against brucellosis is urgently needed to implement a One-Health strategy. Additionally, several aspects of its biology are not well covered and still, many gaps are existing in brucellosis such as host-pathogen interaction, pathogenicity, and virulence determinants, as well as resistance development.

The specific different strategies of *Brucella* to evade the immune system are regulated by its special antigenic components including LPS, T4SS, BvrR/BvrS, Omp, *prpA*, and Btp1. These mechanisms include intracellular survival, retarded phagocytosis, bactericidal activity, and the generation of TNF- α . Definitive diagnosis is an obvious inquiry in the control of the disease, and serology is the most popular tool because it is easy, cheap, and rapid. However, one test is not enough to cull seropositive animals from the herd, and cross-reaction with other bacteria is still the main drawback. Therefore, the implementation of at least two screening tests and one confirmatory test is necessary for the accurate diagnosis of brucellosis. Although culturing is the gold standard diagnostic technique, it needs professional skills and special laboratory facilities due to the hazards. Moreover, successful of culturing is very low due to the fastidious nature of brucellae. Molecular methods have a major role in diagnosis but can't be applied practically at the field level. So, the need to develop in-site diagnostic approaches with high sensitivity and specificity is urgent to get the proper diagnosis. A combination of serology and molecular diagnosis is recommended to detect the true seropositive animals, particularly the seronegative/culture-positive cases.

Looking for a species-specific protein antigen used in the diagnosis of brucellosis is a matter of discussion and is required to avoid the cross-reaction with other Gram-negative bacteria that reduce the sensitivity and specificity of available serological tools. Current research focuses mainly on the development of suitable immunodominant proteins to serve as promising diagnostic tools. The use of BP26 protein-based ELISA and VirB12, as a specific screening test for detecting the infection in livestock, was evaluated. Furthermore, the proteomic tools are helpful to understand the consequences of regulatory pathways on microbial protein composition and help in the diagnosis and safe vaccine production. In addition, a few recent studies referred to using postbiotics as an essential tool with a favorable cost-benefit ratio in controlling the infection.

Although the S19, RB51, Rev1 vaccines are the most common vaccines applied under field conditions, some drawbacks were reported such as serological interference, abortion in pregnant animals, and the risk of human infection. The control strategies through mass vaccination and test and slaughter are the main applied protocols in almost countries. Therefore, the development of effective vaccine candidates that is able to protect from different *Brucella* species, have

a high immune response, and are non-pathogenic to humans is required. Increasing the awareness and implementation of high biosafety and biosecurity measures are supreme in the control of brucellosis.

Article Information

Acknowledgments. The authors would like to thank the staff members of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, as well as the Academy of Scientific Research Technology.

Funding. This research received no specific grant or funding agency.

Conflict of Interest. The authors have no conflict of interest to declare.

References

- Al Hamada, A., Bruce, M., Barnes, A., Habib, I., Robertson, I.D., 2021. Cost-benefit analysis of a mass vaccination strategy to control brucellosis in sheep and goats in Northern Iraq. *Vaccines* 9. [10.3390/vaccines9080878](https://doi.org/10.3390/vaccines9080878).
- Alamian, S., Dadar, M., 2019. *Brucella abortus* contamination of camel milk in two Iranian regions. *Preventive Veterinary Medicine* 169, 104708. [10.1016/j.prevetmed.2019.104708](https://doi.org/10.1016/j.prevetmed.2019.104708).
- Ali Hussei, H., Hassan Moh, R., Mohammed A, A., Mahmoud Ab, E., Salah Moha, R., Abd El-Nas, A., Wehrend, A., 2019. Diagnosis of brucellosis in recently aborted ewes using serological tests and polymerase chain reaction. *Journal of Applied Sciences* 19, 77–81. [10.3923/jas.2019.77.81](https://doi.org/10.3923/jas.2019.77.81).
- Andersen-Nissen, E., Smith, K.D., Strobe, K.L., Barrett, S.L.R., Cookson, B.T., Logan, S.M., Aderem, A., 2005. Evasion of toll-like receptor 5 by flagellated bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 102, 9247–9252. [10.1073/pnas.0502040102](https://doi.org/10.1073/pnas.0502040102).
- Avila-Calderón, E.D., Flores-Romo, L., Sharon, W., Donis-Maturano, L., Becerril-García, M.A., Arreola, M.G.A., Reynoso, B.A., Güemes, F.S., Contreras-Rodríguez, A., 2020. Dendritic cells and *Brucella* spp. interaction: the sentinel host and the stealthy pathogen. *Folia Microbiologica* 65, 1–16. [10.1007/s12223-019-00691-6](https://doi.org/10.1007/s12223-019-00691-6).
- Avila-Granados, L.M., Garcia-Gonzalez, D.G., Zambrano-Varon, J.L., Arenas-Gamboa, A.M., 2019. Brucellosis in Colombia: current status and challenges in the control of an endemic disease. *Frontiers in Veterinary Science* 6, 321. [10.3389/fvets.2019.00321](https://doi.org/10.3389/fvets.2019.00321).
- Baloglu, S., Boyle, S.M., Vemulapalli, R., Sriranganathan, N., Schurig, G.G., Toth, T.E., 2005. Immune responses of mice to vaccinia virus recombinants expressing either *Listeria monocytogenes* partial listeriolysin or *Brucella abortus* ribosomal L7/L12 protein. *Veterinary Microbiology* 109, 11–17. [10.1016/j.vetmic.2005.04.011](https://doi.org/10.1016/j.vetmic.2005.04.011).
- Bandara, A.B., Contreras, A., Contreras-Rodríguez, A., Martins, A.M., Dobrean, V., Poff-Reichow, S., Rajasekaran, P., Sriranganathan, N., Schurig, G.G., Boyle, S.M., 2007. *Brucella suis* urease encoded by ure1 but not ure2 is necessary for intestinal infection of BALB/c mice. *BMC Microbiology* 7, 57. [10.1186/1471-2180-7-57](https://doi.org/10.1186/1471-2180-7-57).
- Barquero-Calvo, E., Mora-Cartín, R., Arce-Gorvel, V., de Diego, J.L., Chacón-Díaz, C., Chaves-Olarte, E., Guzmán-Verri, C., Buret, A.G., Gorvel, J.P., Moreno, E., 2015. *Brucella abortus* induces the premature death of human neutrophils through the action of its lipopolysaccharide. *PLoS Pathogens* 11, e1004853. [10.1371/journal.ppat.1004853](https://doi.org/10.1371/journal.ppat.1004853).
- Barrionuevo, P., Cassataro, J., Delpino, M.V., Zwerdling, A., Pasquevich, K.A., García Samartino, C., Wallach, J.C., Fosati, C.A., Giambartolomei, G.H., 2008. *Brucella abortus* inhibits major histocompatibility complex class II expression and antigen processing through interleukin-6 secretion via toll-like receptor 2. *Infection and Immunity* 76, 250–262. [10.1128/IAI.00949-07](https://doi.org/10.1128/IAI.00949-07).
- Baumann, U., 2008. Mucosal vaccination against bacterial respiratory infections. *Expert Review of Vaccines* 7, 1257–1276. [10.1586/14760584.7.8.1257](https://doi.org/10.1586/14760584.7.8.1257).
- Bazrgari, N., Imam, K., Garosi, G.A., Imam, K., Dadar, M., 2020. Genetic diversity and phylogenetic relationship of clinical isolates of *Brucella melitensis* based on gene polymorphism of β subunit of RNA polymerase (*rpoB*) gene in Iran. *Iranian Journal of Medical Microbiology* 14, 425–440. [10.30699/ijmm.14.5.425](https://doi.org/10.30699/ijmm.14.5.425).
- Bello, J., Sáez, D., Escalona, E., Velozo, P., Santiviago, C.A., Contreras, I., Onate, A., 2016. Mucosal immunization of BALB/c mice with DNA vaccines encoding the SEN1002 and SEN1395 open reading frames of *Salmonella enterica* serovar enteritidis induces protective immunity. *Epidemiology and Infection* 144, 247–256. [10.1017/S095026881500120X](https://doi.org/10.1017/S095026881500120X).
- Bennett, N.J., 2016. Brucellosis: Background, pathophysiology, etiology. URL: <https://emedicine.medscape.com/article/213430-overview>.
- Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., Gil, A., 2012. Probiotic mechanisms of action. *Annals of Nutrition & Metabolism* 61, 160–174. [10.1159/000342079](https://doi.org/10.1159/000342079).
- Bobbala, S., Hook, S., 2016. Is there an optimal formulation and delivery strategy for subunit vaccines? *Pharmaceutical Research* 33, 2078–2097. [10.1007/s11095-016-1979-0](https://doi.org/10.1007/s11095-016-1979-0).
- Boichenko, M.N., Kravtsova, E.O., Zverev, V.V., 2019. Mechanism of intracellular bacterial parasitism. *Journal of Microbiology, Epidemiology and Immunobiology*, 61–72. [10.36233/0372-9311-2019-5-61-72](https://doi.org/10.36233/0372-9311-2019-5-61-72).
- Bricker, B.J., Halling, S.M., 1994. Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv. 1 by PCR. *Journal of Clinical Microbiology* 32, 2660–2666. [10.1128/jcm.32.11.2660-2666.1994](https://doi.org/10.1128/jcm.32.11.2660-2666.1994).
- Briones, G., Iñón de Iannino, N., Roset, M., Vigliocco, A., Paulo, P.S., Ugalde, R.A., 2001. *Brucella abortus* cyclic beta-1,2-glucan mutants have reduced virulence in mice and are defective in intracellular replication in HeLa cells. *Infection and Immunity* 69, 4528–4535. [10.1128/IAI.69.7.4528-4535.2001](https://doi.org/10.1128/IAI.69.7.4528-4535.2001).
- Broxton, C.N., Culotta, V.C., 2016. SOD enzymes and microbial pathogens: surviving the oxidative storm of infection. *PLoS Pathogens* 12, e1005295. [10.1371/journal.ppat.1005295](https://doi.org/10.1371/journal.ppat.1005295).
- Bulashev, A.K., Ingirbay, B.K., Mukantayev, K.N., Syzdykova, A.S., 2021. Evaluation of chimeric proteins for serological diagnosis of brucellosis in cattle. *Veterinary World* 14, 2187–2196. [10.14202/vetworld.2021.2187-2196](https://doi.org/10.14202/vetworld.2021.2187-2196).
- Cannella, A.P., Tsohis, R.M., Liang, L., Felgner, P.L., Saito, M., Sette, A., Gotuzzo, E., Vinetz, J.M., 2012. Antigen-specific

- acquired immunity in human brucellosis: implications for diagnosis, prognosis, and vaccine development. *Frontiers in Cellular and Infection Microbiology* 2, 1. [10.3389/fcimb.2012.00001](https://doi.org/10.3389/fcimb.2012.00001).
- Chen, B., Liu, B., Zhao, Z., Wang, G., 2019. Evaluation of a DNA vaccine encoding *Brucella* BvrR in BALB/c mice. *Molecular Medicine Reports* 19, 1302–1308. [10.3892/mmr.2018.9735](https://doi.org/10.3892/mmr.2018.9735).
- Chen, Z., Zhu, Y., Sha, T., Li, Z., Li, Y., Zhang, F., Ding, J., 2021. Design of a new multi-epitope vaccine against *Brucella* based on T and B cell epitopes using bioinformatics methods. *Epidemiology and Infection* 149, e136. [10.1017/S0950268821001229](https://doi.org/10.1017/S0950268821001229).
- Chisi, S.L., Marageni, Y., Naidoo, P., Zulu, G., Akol, G.W., Van Heerden, H., 2017. An evaluation of serological tests in the diagnosis of bovine brucellosis in naturally infected cattle in KwaZulu-Natal province in South Africa. *Journal of the South African Veterinary Association* 88, e1–e7. [10.4102/jsava.v88i0.1381](https://doi.org/10.4102/jsava.v88i0.1381).
- Dadar, M., Shahali, Y., Whatmore, A.M., 2019. Human brucellosis caused by raw dairy products: A review on the occurrence, major risk factors and prevention. *International Journal of Food Microbiology* 292, 39–47. [10.1016/j.ijfoodmicro.2018.12.009](https://doi.org/10.1016/j.ijfoodmicro.2018.12.009).
- Ducrotoy, M.J., Muñoz, P.M., Conde-Álvarez, R., Blasco, J.M., Moriyón, I., 2018. A systematic review of current immunological tests for the diagnosis of cattle brucellosis. *Preventive Veterinary Medicine* 151, 57–72. [10.1016/j.prevetmed.2018.01.005](https://doi.org/10.1016/j.prevetmed.2018.01.005).
- Durward, M., Radhakrishnan, G., Harms, J., Bareiss, C., Magnani, D., Splitter, G.A., 2012. Active evasion of CTL mediated killing and low quality responding CD8+ T cells contribute to persistence of brucellosis. *Plos One* 7, e34925. [10.1371/journal.pone.0034925](https://doi.org/10.1371/journal.pone.0034925).
- Eggerstedt, S.N., Dietzel, M., Sommerfeld, M., Süverkrüp, R., Lamprecht, A., 2012. Protein spheres prepared by drop jet freeze drying. *International Journal of Pharmaceutics* 438, 160–166. [10.1016/j.ijpharm.2012.08.035](https://doi.org/10.1016/j.ijpharm.2012.08.035).
- El-Diasty, M., Salah, K., El-Hofy, F.I., Abd El Tawab, A.A., Soliman, E.A., 2022. Investigation of an outbreak of brucellosis in a mixed dairy farm and evaluation of a test and slaughter strategy to release the herd out of the quarantine. *German Journal of Veterinary Research* 2, 1–9. [10.51585/gjvr.2022.1.0028](https://doi.org/10.51585/gjvr.2022.1.0028).
- Fang, A., Sun, Y., Feng, D., Ma, M., Xu, Z., Zhang, T., Shi, F., 2021. Flower-like gold nanoparticles labeled and silver deposition rapid vertical flow technology for highly sensitive detection of *Brucella* antibodies. *The Analyst* 146, 5362–5368. [10.1039/d1an01075a](https://doi.org/10.1039/d1an01075a).
- de Figueiredo, P., Ficht, T.A., Rice-Ficht, A., Rossetti, C.A., Adams, L.G., 2015. Pathogenesis and immunobiology of brucellosis: review of *Brucella*-host interactions. *The American Journal of Pathology* 185, 1505–1517. [10.1016/j.ajpath.2015.03.003](https://doi.org/10.1016/j.ajpath.2015.03.003).
- Franc, K.A., Krecek, R.C., Häslér, B.N., Arenas-Gamboa, A.M., 2018. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health* 18, 125. [10.1186/s12889-017-5016-y](https://doi.org/10.1186/s12889-017-5016-y).
- Gao, N., Jennings, P., Guo, Y., Yuan, D., 2011. Regulatory role of natural killer (NK) cells on antibody responses to *Brucella abortus*. *Innate immunity* 17, 152–163. [10.1177/1753425910367526](https://doi.org/10.1177/1753425910367526).
- Gheibi, A., Khanahmad, H., Kashfi, K., Sarmadi, M., Khorramizadeh, M.R., 2018. Development of new generation of vaccines for *Brucella abortus*. *Heliyon* 4, e01079. [10.1016/j.heliyon.2018.e01079](https://doi.org/10.1016/j.heliyon.2018.e01079).
- Goodwin, Z.I., Pascual, D.W., 2016. Brucellosis vaccines for livestock. *Veterinary Immunology and Immunopathology* 181, 51–58. [10.1016/j.vetimm.2016.03.011](https://doi.org/10.1016/j.vetimm.2016.03.011).
- Guimarães, E.S., Martins, J.M., Gomes, M.T.R., Cerqueira, D.M., Oliveira, S.C., 2020. Lack of interleukin-6 affects IFN- γ and TNF- α production and early *in vivo* control of *Brucella abortus* infection. *Pathogens* 9. [10.3390/pathogens9121040](https://doi.org/10.3390/pathogens9121040).
- Głowacka, P., Żakowska, D., Naylor, K., Niemcewicz, M., Bielawska-Drózd, A., 2018. *Brucella* - virulence factors, pathogenesis and treatment. *Polish Journal of Microbiology* 67, 151–161. [10.21307/pjm-2018-029](https://doi.org/10.21307/pjm-2018-029).
- Hanot Mambres, D., Machelart, A., Potemberg, G., De Trez, C., Ryffel, B., Letesson, J.J., Muraille, E., 2016. Identification of immune effectors essential to the control of primary and secondary intranasal infection with *Brucella melitensis* in mice. *Journal of Immunology* 196, 3780–3793. [10.4049/jimmunol.1502265](https://doi.org/10.4049/jimmunol.1502265).
- Hielpos, M.S., Fernández, A.G., Falivene, J., Alonso Paiva, I.M., Muñoz González, F., Ferrero, M.C., Campos, P.C., Vieira, A.T., Oliveira, S.C., Baldi, P.C., 2018. IL-1R and inflammasomes mediate early pulmonary protective mechanisms in respiratory *Brucella abortus* infection. *Frontiers in Cellular and Infection Microbiology* 8, 391. [10.3389/fcimb.2018.00391](https://doi.org/10.3389/fcimb.2018.00391).
- Hou, H., Liu, X., Peng, Q., 2019. The advances in brucellosis vaccines. *Vaccine* 37, 3981–3988. [10.1016/j.vaccine.2019.05.084](https://doi.org/10.1016/j.vaccine.2019.05.084).
- Jiao, H., Zhou, Z., Li, B., Xiao, Y., Li, M., Zeng, H., Guo, X., Gu, G., 2021. The mechanism of facultative intracellular parasitism of *Brucella*. *International Journal of Molecular Sciences* 22. [10.3390/ijms22073673](https://doi.org/10.3390/ijms22073673).
- Kalleshmurthy, T., Skariah, S., Rathore, Y., Ramanjinappa, K.D., Nagaraj, C., Shome, B.R., Rahman, H., Barman, N.N., Shome, R., 2020. Comparative evaluation of fluorescence polarization assay and competitive ELISA for the diagnosis of bovine brucellosis vis-a-vis sero-monitoring. *Journal of Microbiological Methods* 170, 105858. [10.1016/j.mimet.2020.105858](https://doi.org/10.1016/j.mimet.2020.105858).
- Karevan, G., Ahmadi, K., Taheri, R.A., Fasihi-Ramandi, M., 2021. Immunogenicity of glycine nanoparticles containing a chimeric antigen as *Brucella* vaccine candidate. *Clinical and Experimental Vaccine Research* 10, 35–43. [10.7774/cevr.2021.10.1.35](https://doi.org/10.7774/cevr.2021.10.1.35).
- Kaynak-Onurdag, F., Okten, S., Sen, B., 2016. Screening *Brucella* spp. in bovine raw milk by real-time quantitative PCR and conventional methods in a pilot region of vaccination, Edirne, Turkey. *Journal of Dairy Science* 99, 3351–3357. [10.3168/jds.2015-10637](https://doi.org/10.3168/jds.2015-10637).
- Ke, Y., Wang, Y., Li, W., Chen, Z., 2015. Type IV secretion system of *Brucella* spp. and its effectors. *Frontiers in Cellular and Infection Microbiology* 5, 72. [10.3389/fcimb.2015.00072](https://doi.org/10.3389/fcimb.2015.00072).
- Khurana, S.K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M.Z., Chhabra, R., Karthik, K., Patel, S.K., Pathak, M., Iqbal Yatoo, M., Gupta, V.K., Dhama, K., Sah, R., Chaicumpa, W., 2021. Bovine brucellosis - a

- comprehensive review. *The Veterinary Quarterly* 41, 61–88. [10.1080/01652176.2020.1868616](https://doi.org/10.1080/01652176.2020.1868616).
- Kianmehr, Z., Soleimanjahi, H., Ardestani, S.K., Fotouhi, F., Abdoli, A., 2015. Influence of *Brucella abortus* lipopolysaccharide as an adjuvant on the immunogenicity of HPV-16 L1VLP vaccine in mice. *Medical Microbiology and Immunology* 204, 205–213. [10.1007/s00430-014-0356-z](https://doi.org/10.1007/s00430-014-0356-z).
- Kim, S., Watarai, M., Suzuki, H., Makino, S.i., Kodama, T., Shirahata, T., 2004. Lipid raft microdomains mediate class a scavenger receptor-dependent infection of *Brucella abortus*. *Microbial Pathogenesis* 37, 11–19. [10.1016/j.micpath.2004.04.002](https://doi.org/10.1016/j.micpath.2004.04.002).
- Kiros, A., Asgedom, H., Abdi, R.D., 2016. A review on bovine brucellosis: epidemiology, diagnosis and control options. *ARC Journal of Animal and Veterinary Sciences* 2. [10.20431/2455-2518.0203002](https://doi.org/10.20431/2455-2518.0203002).
- Kolo, F.B., Adesiyun, A.A., Fasina, F.O., Katsande, C.T., Dogonyaro, B.B., Potts, A., Matle, I., Gelaw, A.K., van Heerden, H., 2019. Seroprevalence and characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa. *Veterinary Medicine and Science* 5, 545–555. [10.1002/vms3.190](https://doi.org/10.1002/vms3.190).
- Kumar, H., Kawai, T., Akira, S., 2011. Pathogen recognition by the innate immune system. *International Reviews of Immunology* 30, 16–34. [10.3109/08830185.2010.529976](https://doi.org/10.3109/08830185.2010.529976).
- Lalsiamthara, J., Won, G., Lee, J.H., 2018. Effect of immunization routes and protective efficacy of *Brucella* antigens delivered via *Salmonella* vector vaccine. *Journal of Veterinary Science* 19, 416–425. [10.4142/jvs.2018.19.3.416](https://doi.org/10.4142/jvs.2018.19.3.416).
- Lamontagne, J., Butler, H., Chaves-Olarte, E., Hunter, J., Schirm, M., Paquet, C., Tian, M., Kearney, P., Hamaidi, L., Chelsky, D., Moriyón, I., Moreno, E., Paramithiotis, E., 2007. Extensive cell envelope modulation is associated with virulence in *Brucella abortus*. *Journal of Proteome Research* 6, 1519–1529. [10.1021/pr060636a](https://doi.org/10.1021/pr060636a).
- Lapaque, N., Moriyon, I., Moreno, E., Gorvel, J.P., 2005. *Brucella* lipopolysaccharide acts as a virulence factor. *Current Opinion in Microbiology* 8, 60–66. [10.1016/j.mib.2004.12.003](https://doi.org/10.1016/j.mib.2004.12.003).
- Ledwaba, M.B., Ndumnego, O.C., Matle, I., Gelaw, A.K., Van Heerden, H., 2020. Investigating selective media for optimal isolation of *Brucella* spp. in South Africa. *The Onderstepoort Journal of Veterinary Research* 87, e1–e9. [10.4102/ojvr.v87i1.1792](https://doi.org/10.4102/ojvr.v87i1.1792).
- Li, G., Rong, Z., Wang, S., Zhao, H., Piao, D., Yang, X., Tian, G., Jiang, H., 2020. Rapid detection of brucellosis using a quantum dot-based immunochromatographic test strip. *PLoS Neglected Tropical Diseases* 14, e0008557. [10.1371/journal.pntd.0008557](https://doi.org/10.1371/journal.pntd.0008557).
- Li, J.Y., Liu, Y., Gao, X.X., Gao, X., Cai, H., 2014. TLR2 and TLR4 signaling pathways are required for recombinant *Brucella abortus* BCSP31-induced cytokine production, functional upregulation of mouse macrophages, and the th1 immune response *in-vivo* and *in-vitro*. *Cellular & Molecular Immunology* 11, 477–494. [10.1038/cmi.2014.28](https://doi.org/10.1038/cmi.2014.28).
- Li, R., Liu, W., Yin, X., Zheng, F., Wang, Z., Wu, X., Zhang, X., Du, Q., Huang, Y., Tong, D., 2021. *Brucella* spp. omp25 promotes proteasome-mediated cGAS degradation to attenuate IFN- β production. *Frontiers in Microbiology* 12, 702881. [10.3389/fmicb.2021.702881](https://doi.org/10.3389/fmicb.2021.702881).
- Lin, Y., Cao, C., Shi, W., Huang, C., Zeng, S., Sun, J., Wu, J., Hua, Q., 2020. Development of a triplex real-time PCR assay for detection and differentiation of gene-deleted and wild-type African Swine Fever virus. *Journal of Virological Methods* 280, 113875. [10.1016/j.jviromet.2020.113875](https://doi.org/10.1016/j.jviromet.2020.113875).
- Liu, D., 2015. *Brucella*, in: *Molecular Medical Microbiology*. Elsevier, pp. 1781–1788. [10.1016/B978-0-12-397169-2.00101-3](https://doi.org/10.1016/B978-0-12-397169-2.00101-3).
- Luo, D., Ni, B., Li, P., Shi, W., Zhang, S., Han, Y., Mao, L., He, Y., Wu, Y., Wang, X., 2006. Protective immunity elicited by a divalent DNA vaccine encoding both the L7/L12 and Omp16 genes of *Brucella abortus* in BALB/c mice. *Infection and Immunity* 74, 2734–2741. [10.1128/IAI.74.5.2734-2741.2006](https://doi.org/10.1128/IAI.74.5.2734-2741.2006).
- Luo, X., Zhang, X., Wu, X., Yang, X., Han, C., Wang, Z., Du, Q., Zhao, X., Liu, S.L., Tong, D., Huang, Y., 2017. *Brucella* downregulates tumor necrosis factor- α to promote intracellular SurvivalviaOmp25 regulation of different MicroRNAs in porcine and murine macrophages. *Frontiers in Immunology* 8, 2013. [10.3389/fimmu.2017.02013](https://doi.org/10.3389/fimmu.2017.02013).
- López-Goñi, I., García-Yoldi, D., Marín, C.M., de Miguel, M.J., Barquero-Calvo, E., Guzmán-Verri, C., Albert, D., Garin-Bastuji, B., 2011. New bruce-ladder multiplex PCR assay for the biovar typing of *Brucella suis* and the discrimination of *Brucella suis* and *Brucella canis*. *Veterinary Microbiology* 154, 152–155. [10.1016/j.vetmic.2011.06.035](https://doi.org/10.1016/j.vetmic.2011.06.035).
- López-Santiago, R., Sánchez-Argáez, A.B., De Alba-Núñez, L.G., Baltierra-Urbe, S.L., Moreno-Lafont, M.C., 2019. Immune response to mucosal *Brucella* infection. *Frontiers in Immunology* 10, 1759. [10.3389/fimmu.2019.01759](https://doi.org/10.3389/fimmu.2019.01759).
- M. Roushdy, C., M. Moustafa, A.M., G. Abdelwahab, M., K. Ibrahim, F., M. El-bauomy, E., 2021. Latex agglutination: A rapid, specific immunoassay for diagnosis of ruminant brucellosis. *Advances in Animal and Veterinary Sciences* 9. [10.17582/journal.aavs/2021/9.9.1292.1301](https://doi.org/10.17582/journal.aavs/2021/9.9.1292.1301).
- Mancilla, M., 2015. Smooth to rough dissociation in *Brucella*: the missing link to virulence. *Frontiers in Cellular and Infection Microbiology* 5, 98. [10.3389/fcimb.2015.00098](https://doi.org/10.3389/fcimb.2015.00098).
- Mantur, B.G., Mulimani, M.S., Bidari, L.H., Akki, A.S., Tikare, N.V., 2008. Bacteremia is as unpredictable as clinical manifestations in human brucellosis. *International Journal of Infectious Diseases* 12, 303–307. [10.1016/j.ijid.2007.09.004](https://doi.org/10.1016/j.ijid.2007.09.004).
- Martirosyan, A., Moreno, E., Gorvel, J.P., 2011. An evolutionary strategy for a stealthy intracellular *Brucella* pathogen. *Immunological Reviews* 240, 211–234. [10.1111/j.1600-065X.2010.00982.x](https://doi.org/10.1111/j.1600-065X.2010.00982.x).
- Martirosyan, A., Pérez-Gutierrez, C., Banchereau, R., Dutartre, H., Lecine, P., Dullaers, M., Mello, M., Salcedo, S.P., Muller, A., Leserman, L., Levy, Y., Zurawski, G., Zurawski, S., Moreno, E., Moriyón, I., Klechevsky, E., Banchereau, J., Oh, S., Gorvel, J.P., 2012. *Brucella* β 1,2 cyclic glucan is an activator of human and mouse dendritic cells. *PLoS Pathogens* 8, e1002983. [10.1371/journal.ppat.1002983](https://doi.org/10.1371/journal.ppat.1002983).
- Meyle, J., Dommisch, H., Groeger, S., Giacaman, R.A., Costalonga, M., Herzberg, M., 2017. The innate host response in caries and periodontitis. *Journal of Clinical Periodontology* 44, 1215–1225. [10.1111/jcpe.12781](https://doi.org/10.1111/jcpe.12781).
- Moeini-Zanjani, A., Pournajaf, A., Ferdosi-Shahandashti, E., Gholami, M., Masjedjan, F., Khafri, S., Rajabnia, R.,

2020. Comparison of loop-mediated isothermal amplification and conventional PCR tests for diagnosis of common *Brucella* species. *BMC Research Notes* 13, 533. [10.1186/s13104-020-05377-8](https://doi.org/10.1186/s13104-020-05377-8).
- Mohan, T., Verma, P., Rao, D.N., 2013. Novel adjuvants & delivery vehicles for vaccines development: a road ahead. *The Indian Journal of Medical Research* 138, 779–795. URL: <https://www.ncbi.nlm.nih.gov/pubmed/24434331>.
- Mora-Cartín, R., Gutiérrez-Jiménez, C., Alfaro-Alarcón, A., Chaves-Olarte, E., Chacón-Díaz, C., Barquero-Calvo, E., Moreno, E., 2019. Neutrophils dampen adaptive immunity in brucellosis. *Infection and Immunity* 87. [10.1128/IAI.00118-19](https://doi.org/10.1128/IAI.00118-19).
- Moreno, E., 2014. Retrospective and prospective perspectives on zoonotic brucellosis. *Frontiers in Microbiology* 5, 213. [10.3389/fmicb.2014.00213](https://doi.org/10.3389/fmicb.2014.00213).
- Moreno, E., Barquero-Calvo, E., 2020. The role of neutrophils in brucellosis. *Microbiology and Molecular Biology Reviews* 84. [10.1128/MMBR.00048-20](https://doi.org/10.1128/MMBR.00048-20).
- Nagalingam, M., Basheer, T.J., Balamurugan, V., Shome, R., Kumari, S.S., Reddy, G.B.M., Shome, B.R., Rahman, H., Roy, P., Kingston, J.J., Gandham, R.K., 2021. Comparative evaluation of the immunodominant proteins of *Brucella abortus* for the diagnosis of cattle brucellosis. *Veterinary World* 14, 803–812. [10.14202/vetworld.2021.803-812](https://doi.org/10.14202/vetworld.2021.803-812).
- Nakamura, Y., Kimura, S., Hase, K., 2018. M cell-dependent antigen uptake on follicle-associated epithelium for mucosal immune surveillance. *Inflammation and Regeneration* 38, 15. [10.1186/s41232-018-0072-y](https://doi.org/10.1186/s41232-018-0072-y).
- Nyerere, N., Luboobi, L.S., Mpeshe, S.C., Shirima, G.M., 2020. Optimal control strategies for the infectiology of brucellosis. *International Journal of Mathematics and Mathematical Sciences* 2020, 1–17. [10.1155/2020/1214391](https://doi.org/10.1155/2020/1214391).
- Oliveira, S.C., de Oliveira, F.S., Macedo, G.C., de Almeida, L.A., Carvalho, N.B., 2008. The role of innate immune receptors in the control of *Brucella abortus* infection: toll-like receptors and beyond. *Microbes and Infection* 10, 1005–1009. [10.1016/j.micinf.2008.07.005](https://doi.org/10.1016/j.micinf.2008.07.005).
- Paixão, T.A., Roux, C.M., den Hartigh, A.B., Sankaran-Walters, S., Dandekar, S., Santos, R.L., Tsois, R.M., 2009. Establishment of systemic *Brucella melitensis* infection through the digestive tract requires urease, the type IV secretion system, and lipopolysaccharide o antigen. *Infection and Immunity* 77, 4197–4208. [10.1128/IAI.00417-09](https://doi.org/10.1128/IAI.00417-09).
- Perkins, S.D., Smither, S.J., Atkins, H.S., 2010. Towards a *Brucella* vaccine for humans. *FEMS Microbiology Reviews* 34, 379–394. [10.1111/j.1574-6976.2010.00211.x](https://doi.org/10.1111/j.1574-6976.2010.00211.x).
- Pratt, A.J., DiDonato, M., Shin, D.S., Cabelli, D.E., Bruns, C.K., Belzer, C.A., Gorringer, A.R., Langford, P.R., Tabatabai, L.B., Kroll, J.S., Tainer, J.A., Getzoff, E.D., 2015. Structural, functional, and immunogenic insights on Cu,Zn superoxide dismutase pathogenic virulence factors from *Neisseria meningitidis* and *Brucella abortus*. *Journal of Bacteriology* 197, 3834–3847. [10.1128/JB.00343-15](https://doi.org/10.1128/JB.00343-15).
- Priyanka, Shringi, B.N., Choudhary, O.P., Kashyap, S.K., 2021. Cytokines in brucellosis: biological rhythm at the interface of innate and adaptive immunity. *Biological Rhythm Research* 52, 1031–1043. [10.1080/09291016.2019.1613794](https://doi.org/10.1080/09291016.2019.1613794).
- Qiu, L., Bi, Y., Wang, C., Li, J., Guo, P., Li, J., He, W., Wang, J., Jiang, P., 2014. Protein a detection based on quantum dots-antibody bioprobe using fluorescence coupled capillary electrophoresis. *International Journal of Molecular Sciences* 15, 1804–1811. [10.3390/ijms15021804](https://doi.org/10.3390/ijms15021804).
- Retamal-Díaz, A., Riquelme-Neira, R., Sáez, D., Rivera, A., Fernández, P., Cabrera, A., Guzmán, C.A., Oñate, A., 2014. Use of s-[2,3-bispalmitoyloxy-(2R)-propyl]-r-cysteinyl-amidomonomethoxy polyethylene glycol as an adjuvant improved protective immunity associated with a DNA vaccine encoding Cu,Zn superoxide dismutase of *Brucella abortus* in mice. *Clinical and Vaccine Immunology* 21, 1474–1480. [10.1128/CVI.00554-14](https://doi.org/10.1128/CVI.00554-14).
- Rey, E.G., O'Dell, D., Mehta, S., Erickson, D., 2017. Mitigating the hook effect in lateral flow sandwich immunoassays using real-time reaction kinetics. *Analytical Chemistry* 89, 5095–5100. [10.1021/acs.analchem.7b00638](https://doi.org/10.1021/acs.analchem.7b00638).
- Roset, M.S., Ibañez, A.E., de Souza Filho, J.A., Spera, J.M., Minatel, L., Oliveira, S.C., Giambartolomei, G.H., Cassataro, J., Briones, G., 2014. *Brucella* cyclic β -1,2-glucan plays a critical role in the induction of splenomegaly in mice. *Plos One* 9, e101279. [10.1371/journal.pone.0101279](https://doi.org/10.1371/journal.pone.0101279).
- Salcedo, S.P., Marchesini, M.I., Lelouard, H., Fugier, E., Jolly, G., Balor, S., Muller, A., Lapaque, N., Demaria, O., Alexopoulou, L., Comerci, D.J., Ugalde, R.A., Pierre, P., Gorvel, J.P., 2008. *Brucella* control of dendritic cell maturation is dependent on the TIR-containing protein btp1. *PLoS Pathogens* 4, e21. [10.1371/journal.ppat.0040021](https://doi.org/10.1371/journal.ppat.0040021).
- Santos, C.P.d., Herrera, H.M., Campos, J.B.V., Trindade, C.S.P.C., Silva, T.F., Gonçalves, E.R.A., Santos, F.M., Ramos, C.A.d.N., Llario, P.F., Taveira, A.A.R., Oliveira, C.E.d., 2021. Use of postbiotics as an immunomodulatory of the immune response against brucellosis in Nelore calves immunized with S 19 vaccine. *Microbiology Research Journal International* , 52–61. [10.9734/mrji/2021/v3i1430312](https://doi.org/10.9734/mrji/2021/v3i1430312).
- Santos, R., Campos, P.C., Rungue, M., Rocha, V., Santos, D., Mendes, V., Marinho, F.V., Martins, F., Ricci, M.F., Reis, D.C.D., Cassali, G.D., Alves-Filho, J.C., Vieira, A.T., Oliveira, S.C., 2020. The role of ST2 receptor in the regulation of *Brucella abortus* oral infection. *Pathogens* 9. [10.3390/pathogens9050328](https://doi.org/10.3390/pathogens9050328).
- Schmidt, F., Völker, U., 2011. Proteome analysis of host-pathogen interactions: Investigation of pathogen responses to the host cell environment. *Proteomics* 11, 3203–3211. [10.1002/pmic.201100158](https://doi.org/10.1002/pmic.201100158).
- Seimenis, A., Araj, G.F., Moriyon, I., Tabbaa, D., 2019. Brucellosis prevention and control in the Mediterranean and Middle East regions : A guidance towards approaching the targets. *Lebanese Medical Journal* 67, 52–61. [10.12816/0053749](https://doi.org/10.12816/0053749).
- Skendros, P., Boura, P., 2013. Immunity to brucellosis. *Revue Scientifique et Technique (International Office of Epizootics)* 32, 137–147. [10.20506/rst.32.1.2190](https://doi.org/10.20506/rst.32.1.2190).
- Smith, J.A., 2018. *Brucella* lipopolysaccharide and pathogenicity: The core of the matter. *Virulence* 9, 379–382. [10.1080/21505594.2017.1395544](https://doi.org/10.1080/21505594.2017.1395544).
- Snyder, G.A., Deredge, D., Waldhuber, A., Fresquez, T., Wilkins, D.Z., Smith, P.T., Durr, S., Cirl, C., Jiang, J., Jennings, W., Luchetti, T., Snyder, N., Sundberg, E.J., Wintrade, P., Miethke, T., Xiao, T.S., 2014. Crystal structures of

- the toll/interleukin-1 receptor (TIR) domains from the *Brucella* protein TcpB and host adaptor TIRAP reveal mechanisms of molecular mimicry. *The Journal of Biological Chemistry* 289, 669–679. [10.1074/jbc.M113.523407](https://doi.org/10.1074/jbc.M113.523407).
- Spera, J.M., Comerci, D.J., Ugalde, J.E., 2014. *Brucella* alters the immune response in a prpA-dependent manner. *Microbial Pathogenesis* 67–68, 8–13. [10.1016/j.micpath.2014.01.003](https://doi.org/10.1016/j.micpath.2014.01.003).
- Stear, M.J., 2005. The OIE manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees) 5th Ed. volumes 1 & 2. world organization for animal health 2004. ISBN 92 9044 622 6. EUR140. *Parasitology* 130, 727–727. [10.1017/S0031182005007699](https://doi.org/10.1017/S0031182005007699).
- Stranahan, L.W., Arenas-Gamboa, A.M., 2021. When the going gets rough: The significance of *Brucella* lipopolysaccharide phenotype in host-pathogen interactions. *Frontiers in Microbiology* 12, 713157. [10.3389/fmicb.2021.713157](https://doi.org/10.3389/fmicb.2021.713157).
- Sylla, S., Sidimé, Y., Sun, Y., Doumbouya, S., Cong, Y., 2014. Seroprevalence investigation of bovine brucellosis in Macenta and Yomou, Guinea. *Tropical Animal Health and Production* 46, 1185–1191. [10.1007/s11250-014-0625-2](https://doi.org/10.1007/s11250-014-0625-2).
- Tatum, F.M., Detilleux, P.G., Sacks, J.M., Halling, S.M., 1992. Construction of cu-zn superoxide dismutase deletion mutants of *Brucella abortus*: analysis of survival *in-vitro* in epithelial and phagocytic cells and *in-vivo* in mice. *Infection and Immunity* 60, 2863–2869. [10.1128/iai.60.7.2863-2869.1992](https://doi.org/10.1128/iai.60.7.2863-2869.1992).
- Tesfaye, G., Tsegaye, W., Chanie, M., Abinet, F., 2011. Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms. *Tropical Animal Health and Production* 43, 1001–1005. [10.1007/s11250-011-9798-0](https://doi.org/10.1007/s11250-011-9798-0).
- Tian, M., Song, M., Yin, Y., Lian, Z., Li, Z., Hu, H., Guan, X., Cai, Y., Ding, C., Wang, S., Li, T., Qi, J., Yu, S., 2020. Characterization of the main immunogenic proteins in *Brucella* infection for their application in diagnosis of brucellosis. *Comparative Immunology, Microbiology and Infectious Diseases* 70, 101462. [10.1016/j.cimid.2020.101462](https://doi.org/10.1016/j.cimid.2020.101462).
- Tomar, S., Anand, S., Sharma, P., Sangwan, V., Mandal, S., 2015. Role of probiotic, prebiotics, synbiotics and postbiotics in inhibition of pathogens. *The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs*. Mendez-vilas Ed , 717–732.
- Ulu Kilic, A., Metan, G., Alp, E., 2013. Clinical presentations and diagnosis of brucellosis. *Recent Patents on Anti-infective Drug Discovery* 8, 34–41. [10.2174/157489113805290746](https://doi.org/10.2174/157489113805290746).
- Vatankhah, M., Beheshti, N., Mirkalantari, S., Khoramabadi, N., Aghababa, H., Mahdavi, M., 2019. Recombinant Omp2b antigen-based ELISA is an efficient tool for specific serodiagnosis of animal brucellosis. *Brazilian Journal of Microbiology* 50, 979–984.
- Velikovskiy, C.A., Cassataro, J., Giambartolomei, G.H., Goldbaum, F.A., Estein, S., Bowden, R.A., Bruno, L., Fossati, C.A., Spitz, M., 2002. A DNA vaccine encoding lumazine synthase from *Brucella abortus* induces protective immunity in BALB/c mice. *Infection and Immunity* 70, 2507–2511. [10.1128/IAI.70.5.2507-2511.2002](https://doi.org/10.1128/IAI.70.5.2507-2511.2002).
- Virji, M., 2005. Bacterial adherence and tropism in the human respiratory tract, in: Nataro, J.P., Cohen, P.S., Mobley, H.L., Weiser, J.N. (Eds.), *Colonization of mucosal surfaces*. ASM Press, Washington, DC, USA, pp. 97–117. [10.1128/9781555817619.ch8](https://doi.org/10.1128/9781555817619.ch8).
- Wang, Z., Wu, Q., 2014. Research progress in live attenuated *Brucella* vaccine development. *Current Pharmaceutical Biotechnology* 14, 887–896. [10.2174/1389201014666131226123016](https://doi.org/10.2174/1389201014666131226123016).
- Wareth, G., Pletz, M.W., Neubauer, H., Murugaiyan, J., 2020. Proteomics of *Brucella*: technologies and their applications for basic research and medical microbiology. *Microorganisms* 8. [10.3390/microorganisms8050766](https://doi.org/10.3390/microorganisms8050766).
- Watarai, M., Kim, S., Erdenebaatar, J., Makino, S.i., Horiuchi, M., Shirahata, T., Sakaguchi, S., Katamine, S., 2003. Cellular prion protein promotes *Brucella* infection into macrophages. *The Journal of Experimental Medicine* 198, 5–17. [10.1084/jem.20021980](https://doi.org/10.1084/jem.20021980).
- Yagupsky, P., Morata, P., Colmenero, J.D., 2019. Laboratory diagnosis of human brucellosis. *Clinical Microbiology Reviews* 33. [10.1128/CMR.00073-19](https://doi.org/10.1128/CMR.00073-19).
- Yang, J., He, C., Zhang, H., Liu, M., Zhao, H., Ren, L., Wu, D., Du, F., Liu, B., Han, X., He, S., Chen, Z., 2021. Evaluation and differential diagnosis of a genetic marked *Brucella* vaccine a1912 for cattle. *Frontiers in Immunology* 12, 679560. [10.3389/fimmu.2021.679560](https://doi.org/10.3389/fimmu.2021.679560).
- Yang, X., He, Z., Zhang, G., Lu, J., Zhang, H., Ren, H., Tian, Y., Yang, H., Chen, C., Li, L., Fu, Y., Allain, J.P., Li, C., Wang, W., 2020. Evaluation of reactivity of monoclonal antibodies against Omp25 of *Brucella* spp. *Frontiers in Cellular and Infection Microbiology* 10, 145. [10.3389/fcimb.2020.00145](https://doi.org/10.3389/fcimb.2020.00145).
- Yin, D., Bai, Q., Li, L., Xu, K., Zhang, J., 2021. Study on immunogenicity and antigenicity of a novel *Brucella* multi-epitope recombinant protein. *Biochemical and Biophysical Research Communications* 540, 37–41. [10.1016/j.bbrc.2020.12.098](https://doi.org/10.1016/j.bbrc.2020.12.098).
- Zai, X., Yin, Y., Guo, F., Yang, Q., Li, R., Li, Y., Zhang, J., Xu, J., Chen, W., 2021. Screening of potential vaccine candidates against pathogenic *Brucella* spp. using composite reverse vaccinology. *Veterinary Research* 52, 75. [10.1186/s13567-021-00939-5](https://doi.org/10.1186/s13567-021-00939-5).
- Zameer Durrani, A., Usman, M., Kazmi, Z., Husnain, M., 2020. Evaluation of therapeutic trials in bovines, in: Ranjbar, M., Nojomi, M., T. Mascellino, M. (Eds.), *New Insight into Brucella Infection and Foodborne Diseases*. IntechOpen. [10.5772/intechopen.86324](https://doi.org/10.5772/intechopen.86324).
- Zamri-Saad, M., Kamarudin, M.I., 2016. Control of animal brucellosis: The Malaysian experience. *Asian Pacific Journal of Tropical Medicine* 9, 1136–1140. [10.1016/j.apjtm.2016.11.007](https://doi.org/10.1016/j.apjtm.2016.11.007).
- Zhu, L., Wang, Q., Wang, Y., Xu, Y., Peng, D., Huang, H., Hu, L., Wei, K., Zhu, R., 2020. Comparison of immune effects between *Brucella* recombinant Omp10-Omp28-L7/L12 proteins expressed in eukaryotic and prokaryotic systems. *Frontiers in Veterinary Science* 7, 576. [10.3389/fvets.2020.00576](https://doi.org/10.3389/fvets.2020.00576).