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REVIEW ARTICLE

Brucella-Host Interactions in Cattle: Zoonotic Transmission, Immunological Insights and Non-Antibiotic Strategies

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A B S T R A C T

Bovine brucellosis, caused by *Brucella* species, is a highly contagious disease of veterinary and medical importance. It poses significant effects on cattle health, reduces productivity, and poses serious threats to public health. The peculiar characteristic of *Brucella* to survive within the host cells and its ability to evade the immune responses cause major challenges in diagnosis, treatment, and control. Moreover, the zoonotic nature of *Brucella* is due to the close interaction among livestock animals and humans, especially those who work in proximity to the animals, such as veterinarians and farmers. Various antibiotics have been used over the years to treat brucellosis, but due to their frequent use, scientists have diverted their attention towards more suitable, eco-friendly, sustainable, and immunomodulatory non-antibiotic alternatives such as vaccines, phytochemicals, nanoparticles, probiotics, and most importantly, phage therapy. Vaccines stimulate host immune response and produce antibodies, while phytochemicals and nanoparticles, because of their active chemical constituents, have direct antibacterial effects against *Brucella*. Similarly, phage therapy causes precise lysis of *Brucella* while probiotics and prebiotics improve gut microbiota and reduce the burden of pathogens. Finally, the review article highlights future directions, including the use of advanced vaccines, omics-based diagnostics, and artificial intelligence-based systems to increase control measures and decrease zoonotic transmission.

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INTRODUCTION

Brucellosis is a contagious disease caused by facultative intracellular, non-motile, gram-negative coccobacillus bacteria that belong to the order Rhizobiales, family α-2 proteobacteriacea, and genus *Brucella* (Głowacka *et al*., 2018). Brucellosis affects almost all types of species, including cattle, sheep, goats, dogs, cats, and pigs (Khurana *et al*., 2021), but it can be transferred to humans, making it a zoonotic disease (Saxena, 2021). *Brucella* is divided into six classical species, including *Brucella abortus* (cattle), *B. suis* (pigs), *B. melitensis* (sheep and goats), *B. ovis* (sheep), *B. canis* (dogs), and *B. neotomae* (wildwood rats) (Rossetti *et al*., 2022). The most important *B. abortus* is further subdivided into 8 biovars, while biovar type 1 is the causative agent for cattle brucellosis or bovine brucellosis (Whatmore and Foster, 2021). It has been extensively studied in the USA, Latin America, Brazil, and India, where biovar prevalence has been studied more extensively (Khoshnood *et al*., 2022).

The most common symptoms of bovine brucellosis are strongly associated with abortion in the last trimester in female cattle (Modise-Tlotleng *et al*., 2024). At the same time, other signs include retained placenta, metritis (inflammation of the uterus), birth of premature calves, stillbirth, and a sudden drop in milk production (Hecker *et al*., 2023). In bulls, it may lead to orchitis, epididymitis, swelling of the scrotum, abnormal or poor-quality semen, and infertility (Polo *et al*., 2023). Due to its zoonotic potential, brucellosis necessitates precise differentiation from other etiologies that cause abortion in cattle. The *Brucella* infection in cattle is strongly influenced by various factors such as age, immunological and reproductive status, inherent genetic resistance, route of infection, the magnitude of the infectious dose, and the virulence of *Brucella* strains (Yanti *et al*., 2021; Tulu, 2022). These factors collectively contribute to modulating the pathogenesis, transmission, and spread of the disease, hence creating hurdles in the diagnosis and treatment of infected animals.

Various antibiotics such as tetracycline, streptomycin, and doxycycline are used as primary treatment to control the *Brucella* infection, but they are unable to completely eradicate the bacteria from infected cattle (Sancho *et al*., 2022). Antibiotics are ineffective against *Brucella* because of the bacterial intracellular nature. These antibiotics reduce the disease frequency, but cattle can become lifelong carriers (Elbehiry *et al*., 2022a). The animals cause the spreading of the disease by continuously shedding the bacteria in their milk, urine, vaginal, placental and uterine secretions, amniotic fluids, and lochia (Udainiya *et al*., 2025). However, the continuous and frequent use of antibiotics has led to the development of resistance, which further increases the treatment cost (Shahrabi *et al*., 2023). Most of the developed countries do not recommend antibiotics because they are concerned about drug residues in milk and meat. They also disturb the normal microflora of the animal and cause gastrointestinal issues and secondary bacterial infections (Dahiya and Nigam, 2023). Furthermore, antibiotics are unable to prevent reinfection, which makes them an unpredictable solution to control brucellosis. Additionally, when animals excrete antibiotic residues through urine or feces may enter the soil and water system and affect microbial diversity, the nutrient cycle, and aquatic and terrestrial food chains (Shahid *et al*., 2021).

Because of the limitations mentioned above, scientists and researchers have diverted their attention toward non-antibiotic strategies for the effective treatment of bovine brucellosis. The non-antibiotic strategies or alternatives under investigation include the use of effective vaccines, immunomodulators, probiotics, phage therapy, and plant-based herbal extracts (Kaleem *et al*., 2024). Among Vaccines, the most commonly used vaccines are live attenuated *Brucella abortus* strains S19 and RB51. S19 strains are typically administered to the young heifers, and they stimulate protective immunity and reduce the rate of infection. While RB51 strains are a rough mutant strain used in adults and allow differentiation between vaccinated and non-vaccinated animals, thus helping in disease surveillance and eradication programmes (Heidary *et al*., 2022). Both these vaccines are no doubt preventive, but they do not cure already infected or carrier animals. Their use is also limited due to the interference with the serological test because they are responsible for producing antibodies (Wu *et al*., 2023). Furthermore, their use is inhibited due to the risk of abortion in pregnant cows, short-term immunity, cold chain sensitivity, incomplete herd coverage, and strain specificity. In addition to the vaccine, immunomodulators and probiotics are being used to increase immunity and improve gut microbiota, respectively (Ötkün *et al*., 2023). Immunomodulators stimulate macrophages, which increase cytokine production such as interferon-gamma (IFN-γ) and tumor necrosis factor (TNF-α) that help in controlling intracellular pathogens, *Brucella* (Zamani *et al*., 2022). Another innovative approach known as phage therapy is also in process that uses bacteriophage for the lysis of *Brucella.* No doubt these are in the experimental phase, but these non-antibiotic therapies offer promising and sustainable solutions to control bovine brucellosis. So, this review article explores the complex interaction between *Brucella* and its bovine host and highlights the immunological mechanism involved in infection and disease progression. It also gives zoonotic significance to bovine brucellosis and evaluates emerging non-antibiotic strategies for its control and prevention.

Zoonotic transmission of *Brucella:* Humans can acquire brucellosis through direct contact with infected animals, their discharges, or by ingesting unpasteurized animal products (Qureshi *et al*., 2023). Mostly, transmission is common among those persons who work in proximity to animals, including veterinarians, farmers, and laboratory staff. Moreover, seroprevalence surveys show that transmission risk is greater in individuals living close to animals (Deka *et al*., 2021). Studies have also revealed that butchers all over the world experience a more frequent incidence of infection with the disease, which is also transmitted by inhalation of aerosols (Ali *et al*., 2018; Esmaeili *et al*., 2019). Furthermore, for a veterinarian, direct contact with the infected animals during the handling of aborted fetuses, placenta, and other reproductive discharges poses another significant risk. It is less likely to be transmitted to humans through the respiratory route and conjunctival route. Contaminated water, raw vegetables, and undercooked meat from infected cattle can also transmit the disease even when the *Brucella* load is minimal (Zenu and Bekele, 2024). *B. abortus* gains entry into the cells through the conjunctiva or through the mucus membrane. Once bacteria enter the cell, it gets attacked by the immune cells, including macrophages, dendritic cells, and polymorph nuclear cells, but due to the specificity, it not only survive but also replicate within these cells (Ali and Alsayeqh, 2022). These infected immune cells then transport the bacteria to the lymphatic system, especially to the lymph nodes. From here, the infection spread to other body organs, including the lungs, liver, spleen, and bone marrow, where *Brucella* persist and establish chronic infections. The zoonotic transmission cycle and pathogenesis of bacteria are shown in Fig. 1.

***Brucella* host interaction in cattle and pathogenesis:** *Brucella* host interaction is essential for understanding the mechanism of chronic bacterial infection (Huy *et al*., 2022). *Brucella*, unlike other bacteria, has developed defenses to live, survive, and proliferate inside the macrophages of the host (Oliveira, 2021). The intracellular nature of bacteria protects them from the host's immune response, whereas the host cells also provide space for their multiplication and enable the bacteria to survive when antibiotics have been used for their treatments (Jiao *et al*., 2021).

One of the most important phases of *B. abortus* pathogenesis is its ability to enter both phagocytic and non-phagocytic host cells. *B. abortus* has the ability to specifically infiltrate the intestinal mucosa through the M cells (Rungue *et al*., 2021). Intra-epithelial phagocytes might facilitate *B. abortus* migration across the epithelium and into the submucosa and lamina propria (Rainard *et al*., 2022). Opsonized *B. abortus* internalized in phagocytes through complement or Fc receptor but non-opsonized organisms seem to enter by binding with lectin and fibronectin receptors (Pérez *et al*., 2024). Opsonized bacteria that are phagocytosed by activated macrophages

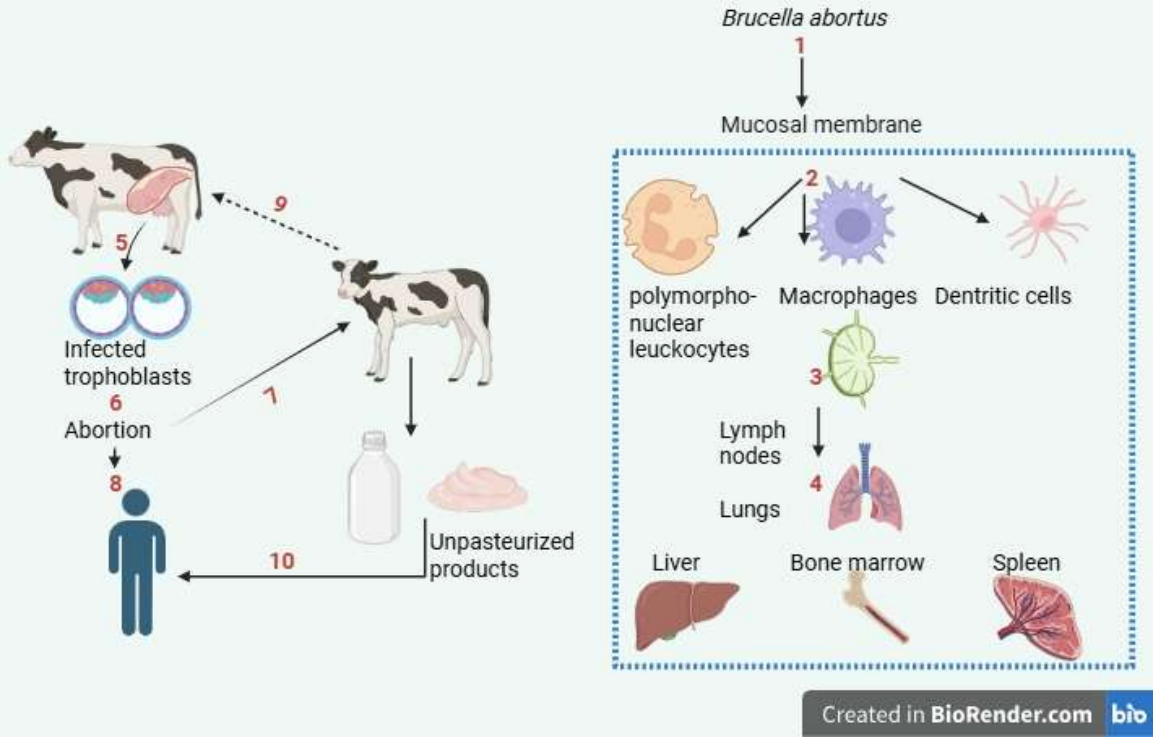

Fig. 1: Transmission and pathogenesis of *Brucella abortus* in cattle and humans showing bacterial spread, abortion, and zoonotic transmission through direct and unpasteurized dairy products. (www.biorender.com).

are usually destroyed in the phagolysosome before they can get to the intracellular replication sites (Palankar *et al*., 2022). Attenuated strains attach and infiltrate host cells despite not being able to survive intracellularly. Compared to other facultative intracellular bacterial infections, such as *Salmonella enterica*, *B. abortus* is far less invasive, even if it can infiltrate bovine trophoblastic cells and epithelial cell lines (Chauhan *et al*., 2024).

To survive intracellularly, *B. abortus* has to circumvent the acidic environment of the phagosome by inhibiting phagosome-lysosome fusion. By rerouting the *Brucella*-containing vacuole (BCV) into the endoplasmic reticulum (ER), where it multiplies, it inhibits killing and diverges from normal intracellular trafficking following internalization (Marchesini *et al*., 2024). Since it induces survival-enhancing alterations in gene expression, early infection acidification of the BCV is crucial. Surprisingly, *B. abortus* grows in macrophages, HeLa cells, and trophoblasts (particularly late in pregnancy), but grows poorly within early compartments and neutrophils (Roop *et al*., 2021; Xiao *et al*., 2022). Furthermore, the ability of *Brucella* to change surface antigens and biofilm formation enables it to evade antibiotic treatment. Besides antibiotic treatment, it limits the efficacy of the natural defenses of the host and vaccine-induced immunity (Yu *et al*., 2022). It provokes hormonal changes in trophoblastic cells, such as diminished progesterone and elevated cortisol and prostaglandin F2α, which mimic parturition and lead to abortion in infected animals (Monteiro *et al*., 2024). Fig. 2 shows the simple *Brucella* host interaction while detail of mechanism is explained above.

Immune host response against *Brucella:* Innate immunity is significant in the course of *B. abortus* infection as it reduces the initial bacterial population and can affect the induction of protective adaptive immunity (Priyanka *et al*., 2021). The initial neutrophil, macrophage, and dendritic cell (DC) recognition of *Brucella* engage Toll-like receptors (TLRs) (Yu *et al*., 2024). TLRs are activated by conserved microorganism components referred to as pathogen-associated molecular patterns (PAMPs) (Wicherska-Pawłowska *et al*., 2021). Bacterial PAMPs such as lipoproteins, LPS, flagellin, and DNA is detected by TLR2, TLR4, TLR5, and TLR9, respectively, *B. abortus* LPS is detected by CD14, which is attached to molecules with transmembrane domains essential for signaling, i.e., the TLR4 (Ciesielska *et al*., 2021). *Brucella* LPS although activates TLR4 is less immunostimulatory than other gram-negative bacteria such as *Salmonella enterica* serotype Typhimurium, which can cause a strong and intense inflammatory response (Hedges *et al*., 2023). TLR2 and TLR9 were found to be able to identify *B. abortus* antigens and induce an immune response (Alonso Paiva *et al*., 2023).

DC activation during *B. abortus* infection induces a profound regulatory process by eliciting T-cell-induced interferon-gamma (IFN-γ) production (Tyler *et al*., 2024). Likewise, smooth *Brucella* LPS activates DCs to produce IL-12, thereby activating CD4+ T-cells (Xu *et al*., 2024). Natural immunity to brucellosis in cattle is another critical element of innate immunity (Maurizio *et al*., 2021). The resistant phenotype is associated with the ability of bovine macrophages to inhibit intracellular development of *B. abortus*. Research links this resistant phenotype to polymorphisms of the gene natural resistance-associated macrophage protein (NRAMP1). Polymorphisms of the 3′untranslated region (3′UTR) of bovine NRAMP1 were thought to be associated with the activity of macrophages to inhibit intracellular development of *B. abortus* (Suwannawong *et al*., 2022).

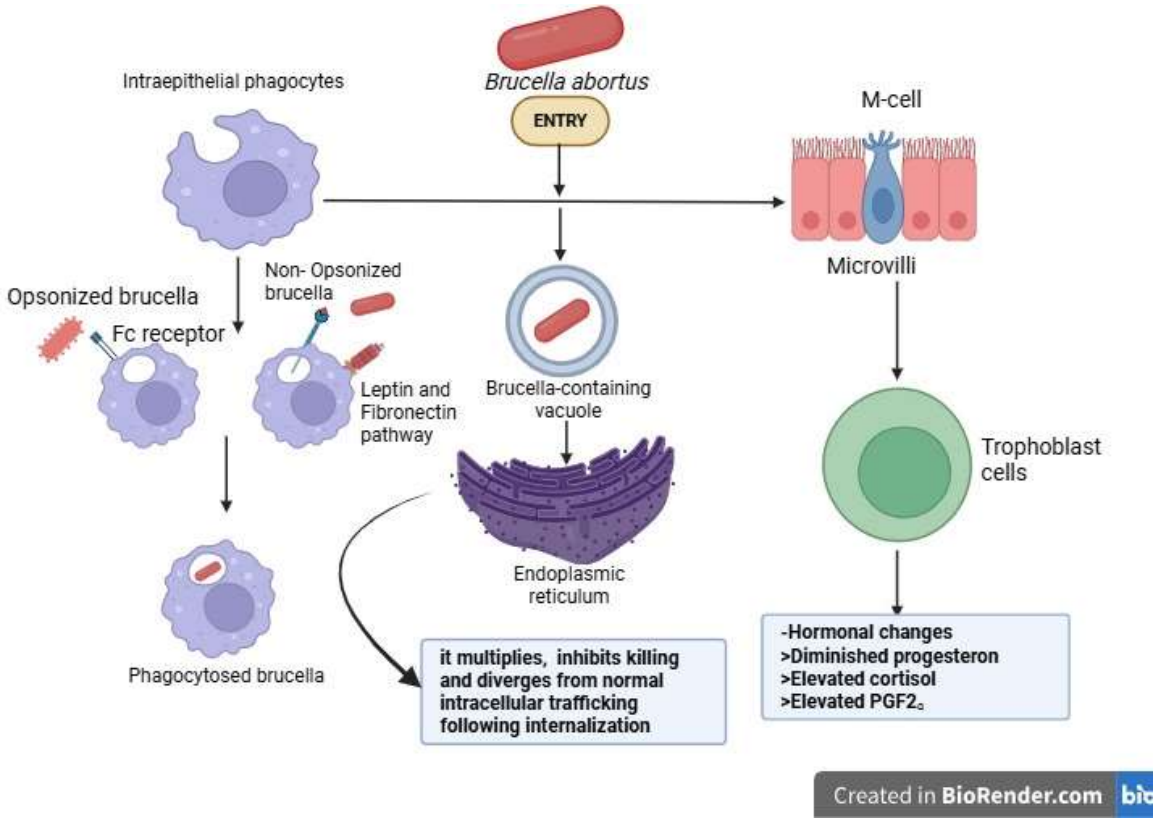

Fig. 2: Interaction of *Brucella* with the host cell receptors and its multiplication within the cell (www.biorender.com).

Natural killer cell (NK) cytotoxicity is also an innate immunity to *B. abortus*, while NK cells may act directly by releasing IFN-γ (Fu *et al*., 2021). Unlike the comprehensive study of *Brucella's* interaction with macrophages, DCs, and non-phagocytic cells, there is minimal understanding of how it interacts with trophoblastic cells, which are the target cells of bovine brucellosis. Experimental *in vivo* and *in vitro* studies of bovine placenta have proved the capacity of *B. abortus* to infect trophoblastic cells (Xiao *et al*., 2022; Collantes-Fernández *et al*., 2024). Recently, researchers investigated the bovine trophoblast gene expression profile during the initial phases of infection with *B. abortus* using a chorioallantoic membrane explant model (Mol *et al*., 2014; Fernández *et al*., 2017). Notably, *B. abortus* suppressed the pro-inflammatory genes early in the course of infection, which was followed by an attenuated and delayed expression of pro-inflammatory chemokines, specifically CXCL6 (GCP-2) and CXCL8 (IL-8) in trophoblastic cells *in vitro* (Zhao *et al*., 2023).

A successful adaptive immune response to *B. abortus* involves cell-mediated immunity that is facilitated by the activation of certain T-cells (Pellegrini *et al*., 2022). T-cells identify *B. abortus* by way of α and β receptors bound to co-receptor molecules CD4+ for T helper cells or CD8+ for T cytotoxic cells. Then, bacterial antigens are processed and presented to the major histocompatibility complex (MHC), MHC-II or I-specific antigen (Yu *et al*., 2022).

The primary cytokine produced by T helper (Th1) cells is IFN-γ, which stimulates macrophages and restricts *Brucella* infection *in vivo* and *in vitro* (Khatun *et al*., 2021). IL-2 produced by Th1 cells also supports T-cell clonal expansion and is involved in regulating the growth of *B. abortus* in macrophages (Faliti *et al*., 2024). However, cytokines playing a part in a Th2 response, such as IL-10, can act against *B. abortus* infection by limiting the inflammatory process and favoring the development of persistent infection in mice (Priyanka *et al*., 2021). CD8+ T-cells are also crucial in defense against *B. abortus*. Indeed, CD8+ knockout mice are more susceptible to infection with *Brucella* (Pellegrini *et al*., 2022). In addition, CD8+ T-cells secrete IFNγ and increase the cytotoxic activity of *B. abortus*-infected macrophages. The simple immune response generation in response to bacteria is shown in Fig. 3.

Non-antibiotic treatment of bovine brucellosis: Non-antibiotic strategies, including vaccination, plant extracts, nanoparticles, phage therapy, and probiotics against *Brucella* infections primarily focus on prevention, pathogen reduction, immunomodulation, and control (Muhammad *et al*., 2021; Elbehiry *et al*., 2022b; Heidary *et al*., 2022). Vaccination helps in the reduction of infection and limits the spread of the disease. Plant extracts and their derived nanoparticles have been explored against *Brucella* infections because they are more antimicrobials, immunomodulators, eco-friendly, less toxic, and more sustainable. Similarly, probiotics and prebiotics have also been studied because of their immunomodulatory effects (Zamani *et al*., 2022). Also, phage therapy is yet another promising and innovative approach that employs bacteriophages to infect and destroy *Brucella* (Ötkün *et al*., 2023). The specifics of some of the significant non-antibiotic measures for controlling and dealing with brucellosis are explained step by step.

Vaccination: In order to avoid brucellosis, a zoonotic disease that affects both animals and humans, *Brucella* vaccination is required. The live attenuated strain B*rucella melitensis* Rev-1 is the most commonly used vaccine to vaccinate sheep and goats against brucellosis (Naseer *et al*., 2023). It has been effective in decreasing the incidence of the disease, it has some limitations, such as the possibility of infecting those who give the vaccine or come into contact with vaccinated animals (Elbehiry *et al*., 2023). Moreover, it would be hard to differentiate between the animals vaccinated from those really infected due to serological cross-reactions with naturally occurring infections elicited by Rev-1 (Aruna, 2023). *Brucella suis* 2 and *Brucella abortus* strain 19 are two other live attenuated vaccines that have been widely employed in pigs and cattle, respectively. These vaccines immunize but usually produce adverse effects, such as localized infection or abortion in pregnant animals (Li *et al*., 2023c).

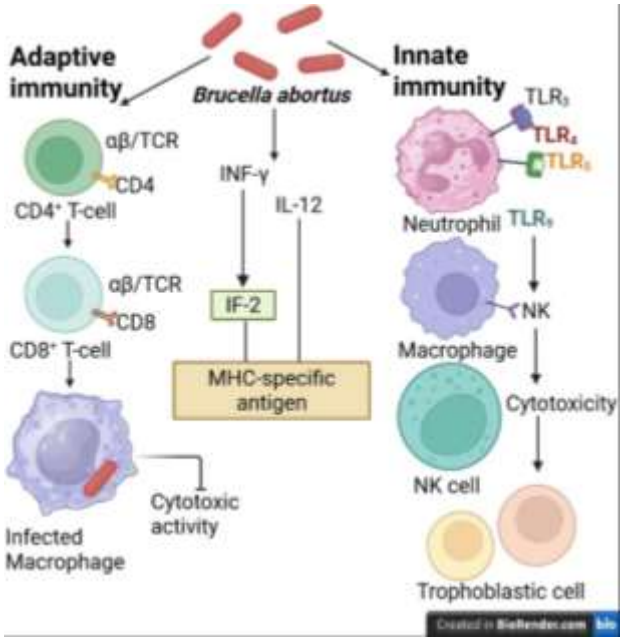

Fig. 3: Immune response generated by the host cell in response to *Brucella* invasion. (www.biorender.com)

Subunit vaccines commonly utilize *Brucella* recombinant proteins, which can stimulate an immune response without the use of live bacteria (Heidary *et al*., 2022). Preclinical trials have achieved positive outcomes with these vaccines in infection prevention without inducing side effects or affecting diagnostic tests. Another safer candidate in development is DNA vaccines, wherein *Brucella* genetic material is administered to confer immunity. They have to be studied to ensure optimum efficiency, even though they are likely to cause specific protection through the induction of humoral as well as cellular immunity (Heidary *et al*., 2022; Pascual *et al*., 2022).

Plant extracts: Plant extracts and their bioactive compounds are also being used more and more as effective alternatives or adjunct therapies for bovine brucellosis, a zoonotic disease caused mostly by *B. abortus* (Kaleem *et al*., 2024). The bioactive compounds, like tannins, terpenoids, alkaloids, flavonoids, and volatile oils contain inherent antibacterial activity with great potency for inhibiting bacterial growth of *B. abortus*, the causative bacterium of brucellosis in cattle (Khurana *et al*., 2021). For instance, allicin, a very active antibiotic compound of garlic (*Allium sativum*), is potent in interfering with bacterial cell walls and metabolism, resulting in the death of *Brucella* species (Bhatwalkar *et al*., 2021). Likewise, by the action of survival pathways in the majority of intracellular pathogens, one of the bioactive molecules of *Curcuma longa* or turmeric, curcumin, has managed to prove effective in inhibiting their growth (Fuloria *et al*., 2022). To trigger cellular immunity, plant compounds like *Azadirachta indica* (neem) and *Tinospora cordifolia* activate phagocytic cells and trigger the release of cytokines like interleukin-12 (IL-12) and interferon-gamma (IFN-γ) (Kaur and Ghorai, 2022; Ogwuche *et al*., 2025).

The use of plant extracts to control bovine brucellosis is in experimental stages now, despite being very promising. Few *in-vivo* studies are currently being carried out; most have been done on laboratory animals or *in vitro* systems (Kar *et al*., 2021). All the same, the hopeful findings are that plant drugs could be used either in combination with or as an adjuvant to more traditional treatments like immunization, antibiotic therapy, and strict biosecurity (Fayazi *et al*., 2024). Various plant extracts with their mechanism of action with minimum inhibitory concentrations (MIC) against *Brucella* are shown in Table 1.

Nanoparticles: Several NPs, including PLGA, calcium phosphate, and gold NPs, transport *Brucella* antigens in vaccine synthesis with ease, activating extensive and prolonged immune reactions, which could contribute to greater stability and broader protection (Elrashedy *et al*., 2022). Nanobiosensors prove invaluable for instant and sensitive identification. Therapeutic use of NPs, such as solid lipid and chitosan formulations, facilitates increased delivery of antibiotics into infected cells, enhancing efficacy and reducing side effects, two key considerations in resistance battles (Hemdan *et al*., 2024).

AgNPs kill bacteria directly by disrupting bacterial cell membranes and generating reactive oxygen species with minimal inhibitory concentrations as low as 4 ppm (Bruna *et al*., 2021; Tripathi and Goshisht, 2022). When used in combination with conventional antibiotics, ZnO-NPs exhibit a synergistic effect that leads to enhanced inhibition zones against *B. melitensis* (Masadeh *et al*., 2025). In addition, doxycycline-loaded solid lipid nanoparticles (SLNs) have been proven to deliver the drug more efficiently to macrophages and host cells infected with Brucella (Hosseini *et al*., 2022). This has resulted in a noteworthy decrease in bacterial load and therapeutic results in the long term owing to the controlled release of drug encapsulated in. pH sensitivity of certain formulations of nanoparticles maximizes the efficacy of the antimicrobial payload by maximally releasing the drug in the acidic phagolysosome compartment where *Brucella* exists (Alavi and Nokhodchi, 2023).

Despite such progress, issues such as potential toxicity and the requirement for extensive clinical evidence continue to exist. Additionally, *Brucella's* intricate immune evasion mechanisms also necessitate further research to

Table 1: Mechanism of action of various plants extracts against *Brucella* species

Plant extract	Active compound	Target specie	Mechanism of Action	In vitro efficacy (MIC)	In vivo efficacy	References
Oregano oil	Thymol	*Brucella abortus*	Disrupts bacterial cell membrane integrity	0.2-1.5 µg/mL	40% reduction in bacterial load within 14 days	(Kaleem *et al*., 2024)
Garlic extract	Allicin	*B. melitensis*	Inhibits the synthesis of RNA/DNA	0.8-2.0 µg/mL	Improved antibody response in 21 days	(Kaleem *et al*., 2024)
Neem leaf	Azadirachtin	*B. suis*	Blocks the replication enzyme of bacteria	1.0-3.5 µg/mL	35% reduction in milk shedding	(Kaleem *et al*., 2024)
Turmeric	Carcumin	*B. abortus*	Enhances macrophage activity	5.0-10.0 µg/mL	Reduced placental infection in pregnant cow	(Kaleem *et al*., 2024)
Echinaceae	Alkylamides	*B. melitensis*	Immune modulation, cytokine stimulation	Not tested in vitro	Improved lymphocyte proliferation	(Kaleem *et al*., 2024)
Thyme oil	Thymol	*B. abortus*	Lysis of membrane and biofilm inhibition	0.5-2.0 µg/mL	About 50% reduction in abortion rate	(Kaleem *et al*., 2024)
Green Tea extract	Epigallocatechin gallate	*B. melitensis*	Suppresses bacterial adhesion proteins	3.0-6.0 µg/mL	Enhanced quality of milk	(Kaleem *et al*., 2024)

further improve the advancement of nanoparticle preparations towards longer duration and increased therapeutic efficacy (Elrashedy *et al*., 2022; Li *et al*., 2023a). The mechanism of various NPs is discussed in the Table 2.

Phage therapy: The viruses have a highly developed mechanism of action that begins with specific adsorption to target bacterial surface receptors, a critical step mediated by proteins on their capsid or tail fibers (Leprince and Mahillon, 2023). When the phage infects the host bacteria, the phage genome harnesses the cell machinery to copy itself, and it produces a tremendous amount of new phage components (Wang *et al*., 2024). Lysis, or killing of the bacterial cell, is the consequence of this internal coup and releases a fresh wave of phages ready to infect neighboring bacteria. A benefit of phages over broad-spectrum antibiotics is that they possess natural specificity, which minimizes interference with the host's healthy microbiome (Emencheta *et al*., 2023). Furthermore, phages also show therapeutic potential in addition to mere bacterial lysis. Despite the sub-inhibitory concentrations, antibiotics have already been shown to enhance phage replication and overall activity, as seen through the phage-antibiotic synergy (PAS) phenomenon (Yarahmadi *et al*., 2025). This presents a solution to combat multidrug resistance and will reduce the selective pressure for either drug resistance. By promoting the entry of other antimicrobial agents, their ability to breakdown the biofilms, comprising complex populations of bacteria imbedded in a protective matrix, renders them that much more useful on a clinical level (Li *et al*., 2023b).

Phage therapy represents an extremely prospective approach in the case of *B. abortus*, the causative bacterium for brucellosis. Phages directed at *B. abortus*-killing are discriminatory killing with lower off-target activity, and the ability to undergo development within the host guarantees long-term antimicrobial efficacy (Rahman *et al*., 2024). Besides, effective and specific therapy is further supplemented by the potential of phage therapy that can be adjusted to a specific strain of *B. abortus* infecting a single individual. Methods such as rotation treatment and phage blends must be employed where bacteria become phage resistant. Additionally, more extensive clinical data are needed to definitively establish the safety and efficacy of phage therapy for *B. abortus* infecting a single individual. Methods such as rotation treatment and phage blends must be employed where bacteria become phage resistant. Additionally, more extensive clinical data are needed to definitively establish the safety and efficacy of phage therapy for *B. abortus* infections, despite preclinical studies appearing promising (Kumar *et al*., 2024). Against bacterial infections, such as brucellosis, the development of a bank of phages would make possible an immediate defense against newly emerging resistant strains, and combination drugs are being studied with promise to improve therapeutic effect (Pal *et al*., 2024).

Probiotics and prebiotics: Probiotics act in several different ways. Through the use of agents that inhibit infectious organisms and competition for substrates and adhesion sites on the host epithelial cells, they act against the bacteria directly and block the colonization of pathogens (Savitri *et al*., 2021). In addition, they enhance the integrity of the gut epithelial barrier, the first line of defense of the body against microorganisms (Zhou *et al*., 2024). Probiotics have the unique characteristic of being able to modulate immune responses by affecting immune cells such as natural killer cells, intraepithelial lymphocytes, and macrophages.

They influence cytokine production, which comprises signal molecules to regulate immunological and inflammatory reactions. Probiotics can modify the composition and activity of the gut microbiota and enable the beneficial bacteria to occupy a greater proportion of the gut and develop a healthy gut environment (Zhou *et al*., 2024). These traits allow them to train in the prevention and treatment of mostly immune-related diseases. It would become easier to characterize mechanisms and optimum practices in the clinical environment with more research in this area (Keerthi *et al*., 2023).

Brucella is often encountered on mucosal surfaces and displays a range of probiotic mechanisms of action to promote health (Latif *et al*., 2023). Thus, the development of vaccinations administered via the mucosa can be more desirable than the treatment of brucellosis at the entry points of microbes into the body.

Future directions: The research in the future about the *Brucella*-host interaction should focus on unveiling the intricate immune mechanism of the host and *Brucella* that directs the course of infection. Understanding the genetic basis of host susceptibility mechanisms, cellular immune responses, and the molecular strategies that *Brucella* employs to evade the host defense mechanisms is crucial. With the advancements in the fields of transcriptomics,

Table 2: Mechanism of action of various metallic nanoparticles against *Brucella* bacteria

Nanoparticles	Anti-microbial effect	Mechanism of action	Resistance potential	Toxicity profile	Reference
Silver (AgNPs)	Disrupts bacterial cell membranes	Release Ag+ ions, which cause oxidative stress, enzyme inhibition, and DNA damage	*Brucella* lacks a common resistance mechanism to AgNPs	Safe at low concentration equivalent to 0.1 mg/ml	(Elbehiry *et al*., 2022)
Gold (AuNPs)	Synergizes with antibiotics to maximize the efficiency	Generation of reactive oxygen species (ROS), which prevent biofilm formation, inhibit the replication process	physical damage to the bacterial structure,	Low toxicity in cattle is exhibited at a tested concentration of 0.05 mg/ml	(Elbehiry *et al*., 2022)
Calcium phosphate (CaPNPs)	Induce cross cross-protective immune response against *Brucella*	Adsorption on the surface of antigen (e.g., Omp31, Somp2) enhances antigen presentation, activates dendritic cells, and also promotes Th1/Th2 immune responses	By promoting the responses of the key antigen	Still no adverse effect reported	Sadeghi *et al*., 2020
Rifampicin loaded poly (lactic-*co*-glycolic acid) PLGA	Enhanced efficiency against *B. canis*	Targeted release of rifampicin, improving bioavailability and material uptake	Reduce (due to optimized delivery and dose)	Lower systemic toxicity	(Hernández-Giottonini *et al*., 2022)
Polymeric NP	Improved efficacy vs. free drug	Enhanced intracellular delivery, sustained release of antibiotics (e.g., doxycycline)	Low (dose-dependent)	Reduced due to dose sparing	(Lueth *et al*., 2019)
PLGA-Dox NPs	Dose sparing, bactericidal	Prolonged drug release, penetrate host cell to kill intracellular B*rucella*	Minimal	Lower systemic toxicity	(Lueth *et al*., 2019)
Chitosan NPs	Synergistic antimicrobial	Disrupts bacterial membrane, enhances antibiotic uptake	Low	Biocompatible, low toxicity	(Lueth *et al*., 2019)
AuNPs	Enhanced immune clearance of *Brucella*	Boost immune response, carrier for antigen (e.g., OMP19, L7/L12, and activate DCs and macrophages	Likely low due to vaccine-mediated immunity, and no potential studies explicitly	Biocompatible with no significant adverse effect reported in cattle	(Staroverov *et al*., 2024)

proteomics, and immunogenomics will provide novel biomarkers for disease prognosis, early diagnosis of the infection, and vaccine efficiency. Additionally, there is a need to elaborate on the vaccination strategies by incorporating a mucosal delivery system, effective adjuvants, and novel subunit designs that should be long-lasting and induce protective immunity that has no drawbacks.

On the other hand, exploring non-antibiotic treatment will be beneficial to deal with the growing antibiotic resistance in bacteria. Natural products and compounds, plant material, plant extracts, essential oils, and probiotics should be tested for anti-*Brucella* and immune-boosting effects, possibly using nanotechnology or other targeted delivery approaches. Integrating the one health surveillance across livestock, wildlife, and humans is key for early outbreak detection and risk prevention. Advanced sciences like AI and Machine learning may prove to be helpful for the prediction and control of *Brucella* transmissions between animals and humans. By integrating these sciences, brucellosis can be controlled, providing sustainable and antibiotic-free livestock management.

Conclusions: Brucellosis, caused by the *Brucella* pathogen, is a zoonotic disease and poses significant threats to animal health, food safety, and public health. The unique behavior of bacteria to interact with the host, their ability to survive within the host cell, and complex immunological responses are very important mechanisms and necessary to understand for their effective control. No doubt, antibiotics have been used for their complete removal, but due to their frequent use and other limitations, alternative treatments such as vaccines, botanicals, nanoparticles, probiotics, and phage therapy have been used, and they offer a promising approach to reducing infection and transmission. All these approaches are not only effective for in vitro studies but also have in vivo effects. By using an integrative approach, both scientifically and through practical interventions, we can achieve more sustainable control of bovine brucellosis, reduce its zoonotic transmission, and improve livestock production.

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