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Review Article

Development of new generation of vaccines for *Brucella abortus*

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Abstract

Brucella abortus is a Gram-negative facultative and intracellular bacteria, it causes bovine brucellosis, a zoonotic disease that is responsible for considerable economic loss to owners of domesticated animals and can cause problems in otherwise healthy humans. There are a few available live attenuated vaccines for animal immunization against brucellosis; however, these have significant side effects and offer insufficient protective efficacy. Thus, the need for more research into the Molecular pathobiology and immunological properties of *B. abortus* that would lead to the development of better and safer vaccines. In this paper we have reviewed the main aspects of the pathology and the responsive immunological mechanisms, we have also covered current and new prospective vaccines against *B. abortus*.

Keywords: Veterinary medicine, Vaccines, Pharmaceutical science

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1. Introduction

Brucella is a genus of Gram-negative bacteria, named after Major-General Sir David Bruce who isolated *B. melitensis* from British soldiers that had died from Malta fever [1]. It covers at least ten species that genetically are very similar, although each has slightly different host specificity. *Brucella* spp. is the cause of brucellosis, which is the most common zoonotic disease, transmitted through close contact with blood, feces, urine, and placenta of infected host animals, or through inhalation of contaminated dust or aerosols. The economic loss as a result of such infections can be quite considerable to the owners of domesticated animals. In humans, the exposure may lead to acute inflammation in variety of organs and flu like symptoms [2, 3]. *Brucella abortus* is primarily associated with cattle; infections can however occur in sheep, goats, pigs, bison, buffalo, horses, elk, and many other animal species. It is possible for the infection to be spread from wild animals into cattle herds [4]. A potential concern also exists about the use of *Brucella* as a bioterrorism weapon [5, 6]. Vaccination of animals especially in endemic areas is the most economic and critical way for control and eradication of Brucellosis; this would also minimize potential human infections [7, 8]. Currently there are no approved vaccines against human infection and the use of animal vaccines in humans have serious drawbacks [9]. Much research has been focused in the development of effective vaccines for cattle; with a few using live attenuated vaccines. However, the efficacy and performance of these vaccines have been relatively poor due to various drawbacks, including: interference with diagnostic test by inducing anti − LPS antibodies, being virulent for humans, cause persistent infection in vaccinated animals, having risk of virulence reversion, secretion into milk, inducing abortions in pregnant animals even at a single dose, and not having long lasting protection [10]. For these reasons, there is an urgent need for the development of an effective and safe vaccine(s) for brucellosis that could also be applicable for humans. In order to develop safer and more effective vaccines, a full understanding of the pathogenesis, immunologic mechanisms, gene interaction networks, and determining protective markers of *Brucella* are necessary. Lately, new candidate vaccines have been designed and evaluated using a mouse model; these include, recombinant *B. abortus* subunit, DNA, and a live vector. In this review we discuss the main aspects of the immunological mechanisms, current and in-progress research for finding suitable candidate vaccines against *B. abortus*.

2. Main text

2.1. Pathogenesis and immunity of *Brucella*

Brucella organisms use smart mechanisms for invasion, survive for long periods of time, and replicate in the host cells without any classical virulence factors such as exotoxin, endotoxin lipopolysaccharide (LPS), capsule, pilus and cytolysin [11].

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BvrR and BvrS are two components of the regulatory system that *Brucella* requires for invasion by changing the host cell cytoskeleton upon internalization of *Brucella* [12]. Cyclic β-1,2-glucan synthase (Cgs) is a virulent factor for complex formation of host-cell interaction [13, 14]. Type IV secretion systems (T4SSs) help for intracellular survival of *Brucella* by avoiding fusion of the phagosome with lysosome [15, 16]. The integrity of LPS on the *Brucella* surface appears to be crucial for it's evasion of host immunity as it does not exhibit strong endotoxic activity [17]. *Brucella* can invade through mucosal barriers of respiratory or digestive tract without eliciting any inflammatory response, where it is engulfed by macrophages and dendritic cells [18]. However, *Brucella* is capable of infecting both phagocytic and non-phagocytic cells [19]. The majority of the uptaken *Brucella* are killed by the bactericidal activities of oxygen free radicals and nitric oxide. However, a small number of the bacteria do escape the killing mechanisms and interfere with intracellular trafficking by inhibiting phagosome − lysosome fusion, which leads to survival, intracellular replication, and chronic infection of the host cells [20, 21, 22]. *Brucella abortus* during intracellular trafficking, that is after uptake by the host cell, is initially localized inside early phagosomes, where it interacts with endosomes and lysosomes. Following residence in these compartments the majority of the bacteria are destroyed by bactericidal killing mechanisms and only a few of the *Brucella* avoid lysosomal degradation by blocking the phagosome-lysosome fusion that leads to the host cell survival of organism. Afterwards, the *Brucella* redirects it's trafficking to the endoplasmic reticulum derived vesicles and starts replication 12 h post infection, without any cytotoxic effects to the cells. In the final step of the *Brucella* lifecycle, replicative *Brucella* containing vacuole are converted into autophagic *Brucella* containing vacuole 48−72 h after internalization, allowing cell to cell transmission of *Brucella* [23].

Based on our current knowledge, immune response against *B. abortus* involves both innate and adaptive immunity [24]. However, *Brucella* have multiple different mechanisms that they use to evade detection by the innate immune system and minimize protective response of adaptive immunity [25]. After entering the body, *Brucella* are recognized by pattern recognition receptors of the macrophage and dendritic cells. These activated cells present *Brucella* antigenic structures to lymphocytes and produce tumor necrosis factor-alpha (TNF-α) and interleukin12 (IL-12), that enhance bactericidal activity of macrophage and cause differentiation of T helper cells to type 1 (Th1) leading to the control of the *Brucella* infection [25]. Moreover, multiple immune subsets such as CD4+, CD8+ T and Natural killer cells also contribute to immunity against brucellosis [26]. Both CD4+ and CD8+ T cells as adaptive immunity have important role against *Brucella*. Mouse model studies suggest that CD8+ T cells producing IL2 play a critical role in controlling brucellosis. However, CD4+ T cells through production of interferon-gamma (IFN-γ) are another T cell population that have an essential role against brucellosis [27, 28].

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In addition Th1 type immune response mediated by IFN-γ has an essential role in protection against *Brucella* infection. Also, Th1 type antibodies opsonize the blood *Brucella* cells and promote their phagocytosis [29, 30]. On the whole, an understanding of the *Brucella* trafficking pathway and the human immune system interaction with this pathogen could go a long way in improving our ability to design protective vaccines that are devoid of drawbacks.

2.2. Classic attenuated *B. abortus* vaccines

A few approved live attenuated vaccines against *B. abortus* are available for immunization in cattle. These strains are derived spontaneously from primary strains. RB51, S19, 45/20 and SR82 are live attenuated vaccine [8, 31]. Past research has demonstrated that live attenuated vaccines provide the desirable protection over other types of *Brucella* strains because they have all the immunogenic components of replication and cell invasion, and can induce diverse immunity in the host. In addition, they can prevent abortion and transmission of brucellosis, but may cause abortion in pregnant animals and are virulent for humans. Other drawbacks include residual virulence and interference with serodiagnostic tests thus underscoring the need for much needed research towards development of new safe and potent vaccines for human application [32].

B. abortus strain RB51 which is a rifampicin resistant mutant, was derived from a virulent smooth strain of *B. abortus* 2308, by serial subculture on medium containing penicillin and rifampicin and was isolated as a single rough colony. The wbo A gene which encodes a glycosyl transferase that is necessary for *O*-side chain synthesis was deleted from *B. abortus* 2308 through serial subculturing [33]. Although *B. abortus* RB51 prevents abortion in vaccinated cattle, its full dose during pregnancy can cause abortion in the cow. However, this strain exhibited low protective efficacy in cattle. RB51 is stable without residual virulence, or serodiagnostic test interference. In addition despite it being more attenuated, this vaccine is infectious to humans and of course a major disadvantage of this vaccine is its resistance to rifampicin, which is used for treatment of brucellosis in humans [34, 35].

B. abortus strain 19 (S19) was isolated accidentally when the virulent strain was left out at room temperature for a long period of time leading to a 720 bp deletion in the erythritol catabolic genes. It has lower virulence compared with the primary strain [36]. Vaccination by this attenuated strain induces relatively high immunogenicity and protects animals against *Brucella* for a long time, reaching almost the whole productive lifetime of the animal [37]. Mouse animal model studies have shown high production levels of IFN-γ, CD_4^+ and CD_8^+ [38]. Unfortunately, S19 has many side effects including: interference with serodiagnostic brucellosis test, causing abortions in some vaccinated and pregnant animals, reduced milk production, and being completely virulent for humans [39, 40].

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B. abortus strain 45/20 and SR82 are another classic attenuated vaccines that are used in some countries for bovine brucellosis prevention. Variable protection efficacy has been reported for 45/20 from different studies, and there are several drawbacks in its use, which limit its application as a viable vaccine. Based on some limited studies, the protective efficacy of SR82 has been reported to be similar to that of S19 [37, 40].

2.3. *B. abortus* subunit vaccines

Currently numerous fragments of *Brucella* including recombinant peptide, protein, DNA, lipopolysaccharide (LPS), and outer membrane vesicles (OMVs) have been evaluated as subunit vaccines against *B. abortus*. These have several advantages over the classic live attenuated vaccines, which includes, high safety without residual virulence, and possible use in humans and pregnant animals. For these reasons, subunit vaccines are considered to be an interesting area for research and further development. However, it should be pointed out that while they offer attractive alternatives to the classic live attenuated vaccines, they do have considerable hurdles that need to be overcome. These include low protection efficacy and the need for adjuvant and booster shots. Using powerful T-cell antigens, which induce Th1 immune response as dominant immunity against brucellosis, can enhance protection levels of subunit vaccines. Vaccination strategies such as using adjuvant, suitable delivery vehicle, and immunization route are other options that lend themselves for development of effective subunit vaccine [41, 42, 43, 44].

In this regard, many antigens as protein or DNA vaccines have been evaluated in mice, with each offering different levels of protection. *Brucella* protein subunit vaccines are OMP16, OMP19, liposomized protein L7/L12, OMP25, p39 (a putative periplasmic binding protein), and AsnC. In general these promote Th1 type immunity and impart protection levels that are comparable to the commercial S19 live vaccine [45, 46, 47, 48, 49, 50]. In contrast, dihydrolipoamide succinyltransferase (rE2o) and cysteine synthase A (rCysK) subunit vaccines elicited Th2 type immunity, with relatively low levels of protection [51, 52]. Administration of cytosolic protein SurA and DnaK (chaperons from heat shock protein 70 family) as proteins subunit vaccines in mice induced lower levels of protection against *B. abortus* compared with the classic live vaccine S19 [53]. Vaccination of mice with a recombinant protein cocktail (rOMP19 + rp39) induced Th1 mediated isotype antibodies and cellular immunity responses that protected mice against *B. abortus* 544 strain [45]. In addition, *B. abortus* chimeric subunit protein from OMP19 and p39 domains, exhibited IgG 2a and cytokines associated with Th1 type immune response in mice after a second boosting [54]. The immunogenic effects of OMP25-BLS fusion protein, formulated with chitosan nanoparticles for delivery were evaluated alone or in combination with heat shock protein 60 kDa. Using combination type

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of these subunit candidate vaccines induced higher cellular immune response than rOMP25 or heat shock protein 60 kDa when they were used individually [55]. Conservative *Brucella* OMP25c recombinant protein mixed with freund's adjuvant induced both Th1 and Th2 type immune response with protection levels equivalent with that of S19 strain in mice [56]. Mixture of several recombinant *B. abortus* proteins including: AspC, Dps, lnpB and Ndk as subunit vaccines induced high levels of IgG2a titter and offered similar protection efficacy to that of RB51 strain when challenged against *Brucella* infection [57]. Vaccination of mice by recombinant organic hydroperoxide resistance protein elicited both humoral and cellular immunity [58].

DNA based *Brucella* vaccines as another type of subunit vaccine that have been capable to induce both humoral and cellular immune response after several administrations [59]. *B. abortus* genomic island 3 (GI-3) region encodes several open reading frames (ORFs) which express antigens that play important role in intracellular survival and pathogenesis of the organism. Designing a *Brucella* DNA vaccine based on GI-3 region may be an effective vaccine candidate against *B. abortus* infection [60]. DNA vaccines encoding BAB1-0263 or BAB1-0278 genes from ORFs of GI-3, stimulated both humoral and cellular immunity with a high level of IFN-γ production. In addition a DNA vaccine expressing BAB1-0278 exhibited protection in mice when challenged with *B. abortus* 2308 strain [61].

Evaluation of a DNA vaccine that contains ABC-type transporter (pv278a) cassette from ORFs of GI-3 in mice showed a significant increase in immunoglobulin G2a (IgG2a) titter and Th1 immune response [62]. Gomez et al. constructed multivalent fusion DNA vaccines containing BAB1 0273 and/or BAB1 0278 and SOD C gene from *B. abortus* 2308 and reported both cellular and humoral immune responses and production of IFN-γ, antibodies and Th1 type response in mice. However, the protection efficacy was low [63]. Escalona et al. used in silico tools to design a multiepitope DNA vaccine encoding 21 epitopes from ORFs of GI-3 and SOD of *B. abortus*. The immunized mice exhibited Th1 type immunity and high levels of protection against *B. abortus* 2308 strain [64].

DNA vaccine encoding SOD Cu/Zn superoxide dismutase- IL-2 fusion protein, induced IgG2a and TNF-α in mice that lead to effective protection against *B. abortus* 2308 strain in comparison with *B. abortus* RB51 vaccine [65]. Also, combination of SOD by L7/L12 and BCSP31 stimulated a robust cytotoxic CD_8^+ T cell and specific IgG that induced a higher protection level compared to *B. abortus* S19 [38]. In another study, *Brucella* genes (SOD, BCSP31, and L7/L12) were combined with multiple genes from *Mycobacterium bovis* or *Mycobacterium tuberculosis* yielding effective DNA vaccines applicable for both diseases. The results have shown promising protection levels better than *B. abortus* S19 and Bacillus- Calmette- Guerin (BCG) vaccines [66, 67]. Another divalent DNA vaccine encoding both the *B. abortus* L7/L12 and OMP16 genes evaluated by Luo et al. has promoted cellular and

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humoral immunity by IFN-γ and IgG2a production in mice. Also this divalent DNA vaccine induced higher protection levels compared to univalent OMP16 or L7/L12 DNA vaccines; although protection efficacy of the divalent OMP16 and L7/L12 was lower than conventional *B. abortus* RB51 [68]. A mouse model study of the DNA vaccine encoding *B. abortus* BLS has shown promotion of both humoral and cellular immunity and protection [69]. Administration of DNA vaccines encoding Bp 26 and trigger factor (TF) in bison induced cellular immunity and high levels of IFN-γ response [59]. DNA vaccine encoding p39, groEL, and numerous other *B. abortus* DNA vaccine candidates are under development; these appear to need several booster vaccinations and have low protection levels which are major disadvantages [70]. So further studies are needed to overcome these drawbacks in the DNA vaccine field.

2.4. Genetically engineered live attenuated vaccines for *B. abortus*

Characterization of genes associated with virulence or survival of organism can help in the development of safe and protective new vaccines. Currently engineered live attenuated vaccines that induces high protection levels compared with classical live attenuated vaccines but without mentioned their disadvantages is the best option for development of new vaccines with minimal residual virulence and high level protection.

A number of vaccines based on various deletions in *B. abortus* virulence genes that ultimately lead to significant attenuation, are under development including: the purine biosynthesis pathway genes, Ferrochelatase hem H mutant, lipid A fatty acid transporting gene, phosphoglycerate kinase encoding gene, the Type IV secretion virB genes, and the LPS biosynthesis pathway genes [71, 72, 73, 74, 75, 76]. Protection levels of these mutants are similar to that of classical live attenuated vaccines. Deletion of *B. abortus* 2308 norD and high affinity zinc uptake system (znuA) genes cause sufficient attenuation of the strain in mouse and human cell studies. Moreover, in contrast to classic RB51 vaccinated groups, this live recombinant strain efficiently increased T cells and pro-inflammatory cytokines [77]. Based on these observations, further evaluation of this candidate vaccine in cattle is need for highlighted potency and safety. Ugalde et al., prepared a recombinant strain without serodiagnostic interference and protective Th1 immune responses equivalent to S19 strain, by deletion of phosphoglucomutase (pgm) gene of *B. abortus* 2308 that exchanged smooth phenotype to rough morphology [78]. Deletion of *B. abortus* 2308 GntR, a transcriptional regulator of several virulence molecules, resulted is an attenuated mutant with high protection levels in mice against parental *B. abortus* 2308 challenge [79]. Double deletion of NodV and NodW genes from *B. abortus* 2308, led to a attenuated live vaccine that reduced survival in cell lines and a mouse model, without interference in serological diagnosis test [80]. In another study reported by Yang et al in 2010,

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deletion of both znuA and purE in *B. abortus*, caused more live attenuated mutant, which needed two doses for administration to induce suitable immune responses in mice [81]. Deletion in cgs gene of *B. abortus* S19 caused more attenuation of S19 without affecting protective efficacy against a challenge with *B. abortus* 2308 [82]. Also, deletion of vjbR gene, which is required for intracellular *Brucella* surveillance in S19, developed a recombinant mutant with high levels of protection and decreased inflammation [83]. Truong et al developed significant attenuated mutants by growth deficiency in cell lines through single deletions in virulent *B. abortus* cydD and cydC genes that encode ATP- binding cassette transporter protein. Moreover, mice evaluation exhibited Th1 type immune response and high protection efficacy against *B. abortus* 2308 strain infection when compared with RB51 strain [35]. Single and double deletion study of cyd C cyd D and cyd C pur D genes in *B. abortus* RB51 have shown significant attenuation by rapid clearance of the organism from the spleen in mice. Single dose administration of these mutants showed low-level protection in mice compared with RB51 strain. However, booster dose vaccination of these mutants induced both humoral and cellular immune responses with improved protection against a challenge with *B. abortus* 2308 compared with classical *B. abortus* RB51 vaccine. Further evaluations in bovine are needed to verify efficacy [84]. Also, same double deletion in *B. abortus* biovar 1 field isolate (BA15) provided similar protective results but without the need for further booster vaccination [85]. Deletion of ATP/GDP-binding protein motif A (p-loop) and ATP-binding/permease protein (cyd C) in *B. abortus* biovar 1 strain IVKB 9007, produced attenuated mutants which could not replicated intracellularly in a cell line model. Protective efficacy of these mutants was suitable against a challenge with 544 strain [86]. *B. abortus* targeted mutant by deletion in membrane fusogenic protein (Mfp) or OMP19 genes have been reported to reduce persistence in mouse study because of attenuation. However challenge evaluations have shown similar protection level to classical attenuation vaccines such as S19 and RB51 strains [87]. Cell and mouse model studies of *B. abortus* 2308 mutant produced through deletion within the putative lytic transglycosylase gene BAB_RS22915 has shown rapid clearance of the mutant with minimal pathological damage and effective immunity [88]. Studies in cattle with *B. abortus* 2308 mutant comprising a double deletion of htrA. cycL genes, has shown to have sufficient attenuation compared to the parental 2308 strain [89]. Deletion of formyltransferase (wbkC) gene that plays a critical role in LPS biosynthesis caused *B. abortus* rough strain with more attenuation and lower protective immunity in mice compared with smooth S19 strain [90]. Glycosyltransferase Wad C gene is involved in synthesis of core oligosaccharide section of *B. abortus* LPS and is required for evading efficient recognition by the innate immunity [91]. Deletion of glycosyltransferase Wad C gene produced a mutant that could not evade effectively from detection by host immunity, and vaccination of mice with this mutant induced protection level similar to that given by S19

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strain [71]. The disruption of wzm and wzt genes of *Brucella* caused decreased immune response of mutant compared with S19 strain in a mouse model [92].

2.5. Vector based *B. abrtus* vaccines

Recently, numerous viral or bacterial vector based *Brucella* vaccines have been developed that offer an effective approach in delivering various heterologous or homologous antigens [93]. Intracellular organisms' induced cell mediate immunity response could potentially represent the best option for presenting the *Brucella* antigens to the target host immune system. These types of vaccine are live and replicate in the host cells, thus they produce multiple copies of the *Brucella* antigens. These properties are all very attractive, however, currently there are no effective vector based *Brucella* vaccine with optimal protection, even in a mouse model. Attenuated *Yersinia enterocolitica* encoding bacterioferritin (BFR) or P39 proteins were used for immunization of mice that induced Th1 type immune response [94]. He et al., in 2002 reported that Th1 based response and protective immunity against *Brucella* in mice using Ochrobactrum anthropic expressing superoxide dismutase (SOD) of *Brucella* [95]. Likewise, *Lactococcus lactis* expressing SOD has been shown to have similar results [96]. Attenuated salmonella strains expressing a variety of *Brucella* antigens were used as vaccine vectors in several research projects, including: 31 kDa, BCSP31, SOD, OMP3b, OMP19, L7/L12, BLS, and prp A. Using mixture of salmonella vectors expressing BCSP31, SOD, and OMP3b, promoted Th1 response and improved protection in a mouse model [97]. Oral administration of salmonella as vector expressing ribosomal protein L7/L12 and the lumazine synthase enzyme (BLS) resulted in a Th1 type response but failed to protect mice against *B. abortus* challenge [98]. Immunization of goats with attenuated salmonella vector based vaccine expressing heterologous *Brucella* antigens (SOD, BLS, prpA and OMP19) induced high levels of IFN-γ and afforded suitable protection efficacy that was comparable with the classic RB51 vaccine [99]. Formulation of this vector with purified *Brucella* LPS, promoted the efficacy of the delivery when injected *ip* in mice [100]. Evaluation of the said salmonella vector based vaccine in combination with LPS in guinea pigs, showed suitable safety and protection levels compared to non-immunized animals [101]. Numerous recombinant vaccinia viruses expressing *Brucella* proteins such as L7/L12, OMP18, and GroEL have been evaluated in various mouse models, however, protection against *Brucella* was not effective [102, 103, 104]. Replication deficient Semliki Forest virus is another viral vector that carry *Brucella* antigens such as translation initiation factor 3 (IF3) and Sod C. Treatment of mice with these vectors induced Th1 type response and gave some protection in mice which was less than that seen with RB51 [105, 106]. Influenza viruses expressing *Brucella* ribosomal proteins L7/L12 and OMP16 as vector-based vaccines have been developed [107]. Administration of this recombinant vector to cattle presented high safety characteristics compared with the S19 strain and

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Table 1. Comparison and properties of *B. abortus* vaccines.

Vaccines type	Properties
Current classical live attenuated vaccines	RB51; rough phenotype (does not induce anti LPS antibody and differentiating infected from vaccinated animals (DIVA)), stable, less virulent than S19, low level of abortion, varying level of protection, infectious to humans, rifampin resistant. S19; smooth phenotype (interference with diagnostic test), residual virulence, causes abortion, high levels of protection, fully virulent for humans, reduction of milk production. 45/20; rough strain, residual virulence, varying level of protection, local reaction, require adjuvant, need for repeat vaccination. SR82; limit application in some countries, similar protection to S19.
Genetically engineered live *B. abortus* vaccines studied in mouse models	Similar protection to classical live attenuated vaccines without the disadvantages. Δ norD or ΔznuA *B. abortus*; sufficient attenuation, increased T cell response. Δ pgm *B. abortus*; rough phenotype, DIVA, Th1 type immunity similar to S19. Δ GntR *B. abortus*; sufficient attenuation, high protection levels. Δ NodV + NodW *B. abortus*; DIVA. Δ znuA + purE *B. abortus*; highly attenuation, two doses need for administration. Δ cgs of S19 strain; DIVA, similar protection and more attenuated compare to S19. Δ vjbR of S19 strain; high level protection, less inflammatory response. Δ cydC or Δ cydD *B. abortus*; Th1 type immunity, high protection efficacy compare with RB51 strain. Δ cydC + cydD or Δ cydC + purD of RB51 strain, significant attenuation and low protection efficacy compare with RB51 strain. Δ p- loop or Δ cydC *B. abortus*; suitable protection level against *Brucella* 544 strain challenge. Δ Mfp or Δ OMP19 *B. abortus*; similar protection level compare to S19 and RB51. Δ BAB _ RS22915 *B. abortus*; minimal pathological damage, effective immune response. Δ htrA + cydL *B. abortus*; sufficient attenuation of 2308 strain. Δ wbkC *B. abortus*; rough mutant, more attenuated, low level protection compare to S19. Δ WadC *B. abortus*; similar protection level to S19. Δ wzm or Δ wzt *B. abortus*; less immune response compare to S19.
Protein vaccines	Nonviable, no residual virulent, DIVA, avirulent, suitable for human use, low level of protection, adjuvant requirement, requires multiple booster, high cost. *Brucella* protein vaccines are OMP16, OMP19, liposomized protein L7/L12, OMP25, p39, AsnC, rE2o, rCysK, SurA, DnaK, rOMP19 + rp39, chimeric protein from OMP19 and p39 domains, OMP25-BLS fusion protein, OMP25c protein mixed with freund's adjuvant, AspC, Dps, lnpB and Ndk.
DNA vaccines	Safe, induce both humoral and cellular immune response, low level of protection compare to protein vaccines, no residual virulent, requires prime boosting. DNA vaccines encoding BAB1-0263, BAB1-0278, BAB1-0278, BAB1 0273, BAB1 0278 + SOD C, 21 epitopes from ORFs of GI-3 and SOD, SOD Cu/Zn and IL-2 fusion protein, (SOD, BCSP31, and L7/L12) combined with multiple genes from Mycobacterium bovis or Mycobacterium tuberculosis, L7/L12 + OMP16, Bp 26 + TF, p39, groEL.

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Table 1. (*Continued*)

Vaccines type	Properties
Vector based vaccines	Live and replicative in host cell, induce cell mediate immunity, best presented to the immune system, varying level of protection. Yersinia encoding BFR or P39, Ochrobactrum anthropic expressing SOD, Lactococcus lactis expressing SOD, salmonella expressing 31 kDa, BCSP31, SOD, OMP3b, OMP19, L7/L12, BLS, and prp A, vaccinia viruses expressing L7/L12, OMP18, and GroEL, Semliki Forest virus encoding IF3 and Sod C, Influenza viruses expressing L7/L12 and OMP16, adenovirus expressing both p39 and lumazin synthase proteins, RB51 overexpressing SOD, wboA, L7/L12, β-galactosidase of Escherichia Coli and 65 kDa heat shock protein of *Mycobacterium bovis*.

prime-booster vaccination provided humoral and cellular immunity with long-term protection especially in pregnant heifers against *B. abortus* infection [108, 109]. Also, improvement of this vaccine formulation by addition of OMP19 and SOD proteins and Montanide Gel as adjuvant, resulted in effective protection in sheep and goats when challenged with *B. melitensis* [110]. Recently, Lin et al., (2018) designed an adenovirus vector based vaccine expressing both p39 and lumazin synthase proteins of *B. abortus* and applied this combined immunization strategy for *Brucella* vaccine development. A mouse model study indicated that this vaccine elicited significant humoral and cellular immune responses [111]. It is clear that overexpression of *Brucella* immunodominant antigens can promote protection efficacy against brucellosis. Accordingly, *B. abortus* RB51 strain has been used as vector vaccine. Mouse model studies have shown overexpression of *Brucella* homologous antigen SOD in RB51 led to an increase in Th1 type immune response [112]. In addition co-overexpression of SOD and glycosyl-transferase (wboA) significantly increased protection against *Brucella* infection compared with the parent RB51 [113]. Overexpression of L7/L12 ribosomal protein, SOD, and WboA genes in RB51 protected mice against *B. suis* infection challenge [114]. Moreover, RB51 strain has been used for delivery of heterologous antigens β-galactosidase of *Escherichia coli* and 65 kDa heat shock protein of *Mycobacterium bovis*. Vaccination of mice indicated production of IgG2a and IFN-γ [115]. Table 1 gives a list of vaccines in development and available for *Brucella*.

2.6. Potential candidate vaccines for *B. abortus*

Despite many studies and advances in the field of *Brucella* vaccine development, due to insufficient protection efficacy of conventional vaccines, there is an urgent need for further research. Identification of powerful T cell epitopes and their combination(s) for immunization can induce significant cellular immune responses leading to high levels of protection against *Brucella* infection [116]. OMVs are bilayer membrane vesicles derived from outer membranes of gram-negative bacteria and consist

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of periplasmic components. Due to existence of multiple antigens in the OMVs' structure, these vesicles are capable of stimulating strong immune responses and project effective protection in the target host [117]. OMVs derived from *B. melitensis* used in mice immunization gave similar protection compared with *B. melitensis* REV 1 against *B. melitensis* [118]. Moreover, licensed OMVs based vaccine against *Neisseria meningitides* are used in some countries [119]. Also, due to ease of separation and purification, OMVs may be developed as potential subunit vaccines against bovine brucellosis.

3. Conclusions

Development of a new generation of vaccines for brucellosis is an urgent need due to economic costs and potential bioterrorism. Further research for identification of immune-pathologic pathways, new immunodominant and protective *Brucella* antigens, and development of genomic and recombinant DNA technology could lead to more efficient, protective, and safe vaccines to prevent human brucellosis.

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