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
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].
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].
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, CD4⁺ and CD8⁺ [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].
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
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, InpB and Ndk as subunit vaccines induced high levels of IgG2a titer 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) titer 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 multi-epitope 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 CD8+ 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
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 in 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,
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.
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. abortus* 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 mediated 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
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 ΔznxA *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 + ΔnodW *B. abortus*; DIVA.  
Δ znxA + 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 + p39, chimeric protein from OMP19 and p39 domains, OMP25-ELS fusion protein, OMP25c protein mixed with freund's adjuvant, AsnC, Dps, InpB 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 Gl-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. |

(continued on next page)
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

| 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*. |

Table 1. (Continued)
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.

Declarations

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

**References**

[1] S.Y. Tan, C. Davis, David Bruce (1855–1931): discoverer of brucellosis, Singapore Med. J. 52 (2011) 138–139.

[2] J.M. Lewis, J. Folb, S. Kalra, S.B. Squire, M. Taegtmeyer, N.J. Beeching, *Brucella melitensis* prosthetic joint infection in a traveller returning to the
UK from Thailand: case report and review of the literature, Travel Med. Infect. Dis. 14 (5) (2016) 444–450.

[3] M. Suárez-Esquivel, N. Ruiz-Villalobos, A. Castillo-Zeledón, C. Jiménez-Rojas, R.M. Roop II, D.J. Comerci, E. Barquero-Calvo, C. Chacón-Díaz, C.C. Caswell, K.S. Baker, *Brucella abortus* strain 2308 Wisconsin genome: importance of the definition of reference strains, Front. Microbiol. 7 (2016).

[4] E.T. Thorne, Brucellosis, Bison, Elk, and Cattle in the Greater Yellowstone Area, National Symposium on Brucellosis in the Greater Yellowstone Area (1994: Jackson, Wyo.), Wyoming Game and Fish Dept.[for] Greater Yellowstone Interagency Brucellosis Committee, 1997.

[5] H.N. Muloki, J. Erume, D.O. Owiny, J.M. Kungu, J. Nakavuma, D. Ogeng, G.W. Nasinyama, Prevalence and risk factors for brucellosis in prolonged fever patients in post-conflict Northern Uganda, Afr. Health Sci. 18 (2018) 22–28.

[6] J.Y. Lee, Y. Jeon, M.Y. Ahn, H.W. Ann, I.Y. Jung, W. Jung, M.H. Kim, J.Y. Ahn, J.E. Song, Y.C. Kim, D.H. Oh, E.J. Kim, S.J. Jeong, N.S. Ku, H. Kim, K. Lee, J.M. Kim, J.Y. Choi, An imported case of *Brucella melitensis* infection in South Korea, Infect. Chemother. 50 (2018) 149–152.

[7] M. Zamri-Saad, M.I. Kamarudin, Control of animal brucellosis: the Malaysian experience, Asian Pac. J. Trop. Med. 9 (2016) 1136–1140.

[8] S.C. Olsen, W. Stoffregen, Essential role of vaccines in brucellosis control and eradication programs for livestock, Expert Rev. Vaccines 4 (6) (2005) 915–928.

[9] J. Xie, J. Wang, Z. Li, W. Wang, Y. Pang, Y. He, Ontology-based meta-analysis of animal and human adverse events associated with licensed brucellosis vaccines, Front. Pharmacol. 9 (2018) 503.

[10] U.T. Babaoglu, H. Ogutucu, G. Demir, D. Sanli, A.B. Babaoglu, S. Oymak, Prevalence of *Brucella* in raw milk: an example from Turkey, Niger. J. Clin. Pract. 21 (2018) 907–911.

[11] J. Pizarro-Cerda, E. Moreno, J.P. Gorvel, Invasion and intracellular trafficking of *Brucella abortus* in nonphagocytic cells, Microb. Infect. 2 (2000) 829–835.

[12] I. Lopez-Goni, C. Guzman-Verri, L. Manterola, A. Sola-Landa, I. Moriyon, E. Moreno, Regulation of *Brucella* virulence by the two-component system BvrR/BvrS, Vet. Microbiol. 90 (2002) 329–339.
[13] L.S. Guidolin, V. Arce-Gorvel, A.E. Ciocchini, D.J. Comerci, J.P. Gorvel, Cyclic beta-glucans at the bacteria-host cells interphase: one sugar ring to rule them all, Cell Microbiol. 20 (2018) e12850.

[14] L.S. Guidolin, S.M. Morrone Seijo, F.F. Guaimas, D.J. Comerci, A.E. Ciocchini, Interaction network and localization of Brucella abortus membrane proteins involved in the synthesis, transport, and succinylation of cyclic beta-1,2-glucans, J. Bacteriol. 197 (2015) 1640–1648.

[15] M.I. Marchesini, S.M. Morrone Seijo, F.F. Guaimas, D.J. Comerci, A T4SS effector targets host cell alpha-enolase contributing to Brucella abortus intracellular lifestyle, Front. Cell. Infect. Microbiol. 6 (2016) 153.

[16] A.B. Den Hartigh, H.G. Rolán, M.F. De Jong, R.M. Tsolis, VirB3 to VirB6 and VirB8 to VirB11, but not VirB7, are essential for mediating persistence of Brucella in the reticuloendothelial system, J. Bacteriol. 190 (13) (2008) 4427–4436.

[17] N. Lapaque, I. Moriyon, E. Moreno, J.-P. Gorvel, Brucella lipopolysaccharide acts as a virulence factor, Curr. Opin. Microbiol. 8 (1) (2005) 60–66.

[18] E. Barquero-Calvo, E. Chaves-Olarte, D.S. Weiss, C. Guzmán-Verri, C. Chacón-Díaz, A. Rucavado, I. Moriyón, E. Moreno, Brucella abortus uses a stealthy strategy to avoid activation of the innate immune system during the onset of infection, PLoS One 2 (7) (2007) e631.

[19] M.C. Miraglia, A.M. Rodriguez, P. Barrionuevo, J. Rodriguez, K.S. Kim, V.A. Dennis, M.V. Delpino, G.H. Giambartolomei, Brucella abortus traverses brain microvascular endothelial cells using infected monocytes as a Trojan horse, Front. Cell. Infect. Microbiol. 8 (2018) 200.

[20] B.H. Bellaire, R.M. Roop 2nd, J.A. Cardelli, Opsonized virulent Brucella abortus replicates within nonacidic, endoplasmic reticulum-negative, LAMP-1-positive phagosomes in human monocytes, Infect. Immun. 73 (2005) 3702–3713.

[21] T. Starr, T.W. Ng, T.D. Wehrly, L.A. Knodler, J. Celli, Brucella intracellular replication requires trafficking through the late endosomal/lysosomal compartment, Traffic 9 (5) (2008) 678–694.

[22] J. Celli, C. de Chastellier, D.-M. Franchini, J. Pizarro-Cerda, E. Moreno, J.-P. Gorvel, Brucella evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum, J. Exp. Med. 198 (4) (2003) 545–556.
[23] P. de Figueiredo, T.A. Ficht, A. Rice-Ficht, C.A. Rossetti, L.G. Adams, Pathogenesis and immunobiology of brucellosis: review of Brucella-host interactions, Am. J. Pathol. 185 (2015) 1505–1517.

[24] J. Dornand, A. Gross, V. Lafont, J. Liautard, J. Oliaro, J.P. Liautard, The innate immune response against Brucella in humans, Vet. Microbiol. 90 (2002) 383–394.

[25] C.L. Baldwin, R. Goenka, Host immune responses to the intracellular bacteria Brucella: does the bacteria instruct the host to facilitate chronic infection? Crit. Rev. Immunol. 26 (2006) 407–442.

[26] C.L. Baldwin, M. Parent, Fundamentals of host immune response against Brucella abortus: what the mouse model has revealed about control of infection, Vet. Microbiol. 90 (2002) 367–382.

[27] E.A. Murphy, J. Sathiyaseelan, M.A. Parent, B. Zou, C.L. Baldwin, Interferon-gamma is crucial for surviving a Brucella abortus infection in both resistant C57BL/6 and susceptible BALB/c mice, Immunology 103 (2001) 511–518.

[28] S.C. Oliveira, G.A. Splitter, CD8+ type 1 CD44hi CD45 RBlo T lymphocytes control intracellular Brucella abortus infection as demonstrated in major histocompatibility complex class I- and class II-deficient mice, Eur. J. Immunol. 25 (1995) 2551–2557.

[29] A. Martirosyan, K. Von Bargen, V. Arce Gorvel, W. Zhao, S. Hannify, J. Bonnardel, S. Meresse, J.P. Gorvel, In vivo identification and characterization of CD4(+) cytotoxic T cells induced by virulent Brucella abortus infection, PLoS One 8 (2013) e82508.

[30] E.M. Dorneles, A. Teixeira-Carvalho, M.S. Araujo, G.K. Lima, O.A. Martins-Filho, N. Sriranganathan, A.P. Lage, T lymphocytes subsets and cytokine pattern induced by vaccination against bovine brucellosis employing S19 calfhood vaccination and adult RB51 revaccination, Vaccine 32 (2014) 6034–6038.

[31] Z.I. Goodwin, D.W. Pascual, Brucellosis vaccines for livestock, Vet. Immunol. Immunopathol. 181 (2016) 51–58.

[32] I. Moriyon, M.J. Grillo, D. Monreal, D. Gonzalez, C. Marin, I. Lopez-Goni, R.C. Mainar-Jaime, E. Moreno, J.M. Blasco, Rough vaccines in animal brucellosis: structural and genetic basis and present status, Vet. Res. 35 (2004) 1–38.
[33] G.G. Schurig, R.M. Roop II, T. Bagchi, S. Boyle, D. Buhrman, N. Sriranganathan, Biological properties of RB51; a stable rough strain of *Brucella abortus*, Vet. Microbiol. 28 (1991) 171–188.

[34] D.A. Ashford, J. di Pietra, J. Lingappa, C. Woods, H. Noll, B. Neville, R. Weyant, S.L. Bragg, R.A. Spiegel, J. Tappero, B.A. Perkins, Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51, Vaccine 22 (2004) 3435–3439.

[35] Q.L. Truong, Y. Cho, S. Park, B.K. Park, T.W. Hahn, *Brucella abortus* mutants lacking ATP-binding cassette transporter proteins are highly attenuated in virulence and confer protective immunity against virulent *B. abortus* challenge in BALB/c mice, Microb. Pathog. 95 (2016) 175–185.

[36] F.J. Sangari, J.M. García-Lobo, J. Agüero, The *Brucella abortus* vaccine strain B19 carries a deletion in the erythritol catabolic genes, FEMS Microbiol. Lett. 121 (3) (1994) 337–342.

[37] G.G. Schurig, N. Sriranganathan, M.J. Corbel, Brucellosis vaccines: past, present and future, Vet. Microbiol. 90 (2002) 479–496.

[38] D.H. Yu, X.D. Hu, H. Cai, A combined DNA vaccine encoding BCSP31, SOD, and L7/L12 confers high protection against *Brucella abortus* 2308 by inducing specific CTL responses, DNA Cell Biol. 26 (2007) 435–443.

[39] L.A. Corner, G.G. Alton, Persistence of *Brucella abortus* strain 19 infection in adult cattle vaccinated with reduced doses, Res. Vet. Sci. 31 (1981) 342–344.

[40] A.V. Ivanov, K.M. Salmakov, S.C. Olsen, G.E. Plumb, A live vaccine from *Brucella abortus* strain 82 for control of cattle brucellosis in the Russian Federation, Anim. Health Res. Rev. 12 (2011) 113–121.

[41] J. Lalsiamthara, G. Won, J.H. Lee, Effect of immunization routes and protective efficacy of Brucella antigens delivered via Salmonella vector vaccine, J. Vet. Sci. 19 (2018) 416–425.

[42] W.K. Kim, J.Y. Moon, S. Kim, J. Hur, Comparison between immunization routes of live attenuated *Salmonella typhimurium* strains expressing BCSP31, Omp3b, and SOD of *Brucella abortus* in murine model, Front. Microbiol. 7 (2016) 550.

[43] M. Abkar, A.S. Lotfi, J. Amani, K. Eskandari, M.F. Ramandi, J. Salimian, G.N. Brujeni, S. Alamian, M. Kamali, H. Koushki, Survey of Omp19 immunogenicity against *Brucella abortus* and *Brucella melitensis*: influence of...
nanoparticulation versus traditional immunization, Vet. Res. Commun. 39 (2015) 217–228.

[44] D. Singh, V.K. Somani, S. Aggarwal, R. Bhatnagar, PLGA (85:15) nanoparticle based delivery of rL7/L12 ribosomal protein in mice protects against Brucella abortus 544 infection: a promising alternate to traditional adjuvants, Mol. Immunol. 68 (2015) 272–279.

[45] G. Tadepalli, A.K. Singh, K. Balakrishna, H.S. Murali, H.V. Batra, Immunogenicity and protective efficacy of Brucella abortus recombinant protein cocktail (rOmp19+rP39) against B. abortus 544 and B. melitensis 16M infection in murine model, Mol. Immunol. 71 (2016) 34–41.

[46] K.A. Pasquevich, S.M. Estein, C.G. Samartino, A. Zwerdling, L.M. Coria, P. Barrionuevo, C.A. Fossati, G.H. Giambartolomei, J. Cassataro, Immunization with recombinant Brucella species outer membrane protein Omp16 or Omp19 in adjuvant induces specific CD4+ and CD8+ T cells as well as systemic and oral protection against Brucella abortus infection, Infect. Immun. 77 (1) (2009 Jan) 436–445.

[47] A. Al-Mariri, A. Tibor, P. Mertens, X. De Bolle, P. Michel, J. Godefroid, K. Walravens, J.J. Letesson, Protection of BALB/c mice against Brucella abortus 544 challenge by vaccination with bacterioferritin or P39 recombinant proteins with CpG oligodeoxynucleotides as adjuvant, Infect. Immun. 69 (2001) 4816–4822.

[48] D. Goel, R. Bhatnagar, Intradermal immunization with outer membrane protein 25 protects Balb/c mice from virulent B. abortus 544, Mol. Immunol. 51 (2012) 159–168.

[49] D. Goel, V. Rajendran, P.C. Ghosh, R. Bhatnagar, Cell mediated immune response after challenge in Omp25 liposome immunized mice contributes to protection against virulent Brucella abortus 544, Vaccine 31 (2013) 1231–1237.

[50] A.I. Mallick, H. Singha, P. Chaudhuri, A. Nadeem, S.A. Khan, K.A. Dar, M. Owais, Liposomised recombinant ribosomal L7/L12 protein protects BALB/c mice against Brucella abortus 544 infection, Vaccine 25 (2007) 3692–3704.

[51] S.K. Verma, S. Jain, S. Kumar, Immunogenicity and protective potential of a bacterially expressed recombinant dihydrolipoamide succinyltransferase (rE2o) of Brucella abortus in BALB/c mice, World J. Microbiol. Biotechnol. 28 (2012) 2487–2495.
[52] S. Jain, P. Afley, S. Kumar, Immunological responses to recombinant cysteine synthase A of Brucella abortus in BALB/c mice, World J. Microbiol. Biotechnol. 29 (2013) 907–913.

[53] M.V. Delpino, S.M. Estein, C.A. Fossati, P.C. Baldi, J. Cassataro, Vaccination with Brucella recombinant DnaK and SurA proteins induces protection against Brucella abortus infection in BALB/c mice, Vaccine 25 (2007) 6721–6729.

[54] G. Tadepalli, B. Konduru, H.S. Murali, H.V. Batra, Intraperitoneal administration of a novel chimeric immunogen (rOP) elicits IFN-gamma and IL-12p70 protective immune response in BALB/c mice against virulent Brucella, Immunol. Lett. 192 (2017) 79–87.

[55] S. Yousefi, T. Abbassi-Daloii, M.H. Sekhavati, M. Tahmoorespur, Evaluation of immune responses induced by polymeric OMP25-BLS Brucella antigen, Microb. Pathog. 115 (2018) 50–56.

[56] S. Paul, B.V. Peddayelachagiri, S. Nagaraj, J.J. Kingston, H.V. Batra, Recombinant outer membrane protein 25c from Brucella abortus induces Th1 and Th2 mediated protection against Brucella abortus infection in mouse model, Mol. Immunol. 99 (2018) 9–18.

[57] H.T. Hop, L.T. Arayan, T.X.N. Huy, A.W.B. Reyes, W. Min, H.J. Lee, S.J. Park, H.H. Chang, S. Kim, Immunization of BALB/c mice with a combination of four recombinant Brucella abortus proteins, AspC, Dps, InpB and Ndk, confers a marked protection against a virulent strain of Brucella abortus, Vaccine 36 (2018) 3027–3033.

[58] H.T. Hop, A.W. Reyes, H.L. Simborio, L.T. Arayan, W.G. Min, H.J. Lee, J.J. Lee, H.H. Chang, S. Kim, Immunization of mice with recombinant Brucella abortus organic hydroperoxide resistance (ohr) protein protects against a virulent Brucella abortus 544 infection, J. Microbiol. Biotechnol. 26 (2016) 190–196.

[59] B. Clapp, N. Walters, T. Thornburg, T. Hoyt, X. Yang, D.W. Pascual, DNA vaccination of bison to brucellar antigens elicits elevated antibody and IFN-responses, J. Wildl. Dis. 47 (3) (2011) 501–510.

[60] L. Gomez, F. Alvarez, D. Betancur, A. Onate, Brucellosis vaccines based on the open reading frames from genomic island 3 of Brucella abortus, Vaccine 36 (2018) 2928–2936.

[61] F. Sislema-Egas, S. Cespedes, P. Fernandez, A. Retamal-Diaz, D. Saez, A. Onate, Evaluation of protective effect of DNA vaccines encoding the
BAB1_0263 and BAB1_0278 open reading frames of *Brucella abortus* in BALB/c mice, Vaccine 30 (2012) 7286–7291.

[62] R. Riquelme-Neira, A. Retamal-Diaz, F. Acuna, P. Riquelme, A. Rivera, D. Saez, A. Onate, Protective effect of a DNA vaccine containing an open reading frame with homology to an ABC-type transporter present in the genomic island 3 of *Brucella abortus* in BALB/c mice, Vaccine 31 (2013) 3663–3667.

[63] L. Gomez, J. Llanos, E. Escalona, D. Saez, F. Alvarez, R. Molina, M. Flores, A. Onate, Multivalent fusion DNA vaccine against *Brucella abortus*, Bio-Med Res. Int. 2017 (2017) 6535479.

[64] E. Escalona, D. Saez, A. Onate, Immunogenicity of a multi-epitope DNA vaccine encoding epitopes from Cu-Zn superoxide dismutase and open reading frames of *Brucella abortus* in mice, Front. Immunol. 8 (2017) 125.

[65] A. Gonzalez-Smith, R. Vemulapalli, E. Andrews, A. Onate, Evaluation of *Brucella abortus* DNA vaccine by expression of Cu-Zn superoxide dismutase antigen fused to IL-2, Immunobiology 211 (2006) 65–74.

[66] D.H. Yu, M. Li, X.D. Hu, H. Cai, A combined DNA vaccine enhances protective immunity against *Mycobacterium tuberculosis* and *Brucella abortus* in the presence of an IL-12 expression vector, Vaccine 25 (2007) 6744–6754.

[67] X.D. Hu, D.H. Yu, S.T. Chen, S.X. Li, H. Cai, A combined DNA vaccine provides protective immunity against *Mycobacterium bovis* and *Brucella abortus* in cattle, DNA Cell Biol. 28 (2009) 191–199.

[68] D. Luo, B. Ni, P. Li, W. Shi, S. Zhang, Y. Han, L. Mao, Y. He, Y. Wu, X. Wang, Protective immunity elicited by a divalent DNA vaccine encoding both the L7/L12 and Omp16 genes of *Brucella abortus* in BALB/c mice, Infect. Immun. 74 (2006) 2734–2741.

[69] C.A. Velikovsky, J. Cassataro, G.H. Giambartolomei, F.A. Goldbaum, S. Estein, R.A. Bowden, L. Bruno, C.A. Fossati, M. Spitz, A DNA vaccine encoding lumazine synthase from *Brucella abortus* induces protective immunity in BALB/c mice, Infect. Immun. 70 (2002) 2507–2511.

[70] A. Al-Mariri, A. Tibor, P. Mertens, X. De Bolle, P. Michel, J. Godefroid, K. Walravens, J.J. Letesson, Induction of immune response in 332 BALB/c mice with a DNA vaccine encoding bacterial ferritin or P39 of 333 *Brucella* spp, Infect. Immun. 69 (2001) 6264–6270.
[71] R. Conde-Alvarez, V. Arce-Gorvel, Y. Gil-Ramirez, M. Iriarte, M.J. Grillo, J.P. Gorvel, I. Moriyon, Lipopolysaccharide as a target for brucellosis vaccine design, Microb. Pathog. 58 (2013) 29–34.

[72] R.B. Alcantara, R.D. Read, M.W. Valderas, T.D. Brown, R.M. Roop, Intact purine biosynthesis pathways are required for wild-type virulence of Brucella abortus 2308 in the BALB/c mouse model, Infect. Immun. 72 (8) (2004) 4911–4917.

[73] C.G. Trant, T.L. Lacerda, N.B. Carvalho, V. Azevedo, G.M. Rosinha, S.P. Salcedo, J.-P. Gorvel, S.C. Oliveira, The Brucella abortus phosphoglycerate kinase mutant is highly attenuated and induces protection superior to that of vaccine strain 19 in immunocompromised and immunocompetent mice, Infect. Immun. 78 (5) (2010 May) 2283–2291.

[74] A.B. Den Hartigh, Y.-H. Sun, D. Sondervan, N. Heuvelmans, M.O. Reinders, T.A. Ficht, R.M. Tsolis, Differential requirements for VirB1 and VirB2 during Brucella abortus infection, Infect. Immun. 72 (9) (2004 Sep) 5143–5149.

[75] G.P. Ferguson, A. Datta, J. Baumgartner, R.M. Roop 2nd, R.W. Carlson, G.C. Walker, Similarity to peroxisomal-membrane protein family reveals that Sinorhizobium and Brucella BacA affect lipid-A fatty acids, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 5012–5017.

[76] M. Almiron, M. Martinez, N. Sanjuan, R.A. Ugalde, Ferrochelatase is present in Brucella abortus and is critical for its intracellular survival and virulence, Infect. Immun. 69 (2001) 6225–6230.

[77] X. Yang, B. Clapp, T. Thornburg, C. Hoffman, D.W. Pascual, Vaccination with a DeltanorD DeltaznuA Brucella abortus mutant confers potent protection against virulent challenge, Vaccine 34 (2016) 5290–5297.

[78] J.E. Ugalde, D.J. Comerci, M.S. Leguizamón, R.A. Ugalde, Evaluation of Brucella abortus phosphoglucomutase (pgm) mutant as a new live rough-phenotype vaccine, Infect. Immun. 71 (2003) 6264–6269.

[79] Z.Q. Li, J.L. Zhang, L. Xi, G.L. Yang, S.L. Wang, X.G. Zhang, J.B. Zhang, H. Zhang, Deletion of the transcriptional regulator GntR down regulated the expression of genes related to virulence and conferred protection against wild-type Brucella challenge in BALB/c mice, Mol. Immunol. 92 (2017) 99–105.

[80] Z. Li, S. Wang, J. Zhang, G. Yang, B. Yuan, J. Huang, J. Han, L. Xi, Y. Xiao, C. Chen, H. Zhang, Brucella abortus 2308DeltaNodVDeltaNodW
double-mutant is highly attenuated and confers protection against wild-type challenge in BALB/c mice, Microb. Pathog. 106 (2017) 30–39.

[81] X. Yang, T. Thornburg, N. Walters, D.W. Pascual, znuA purE *Brucella abortus* 2308 mutant as a live vaccine candidate, Vaccine 28 (2010) 1069–1074.

[82] G. Briones, N.I. de Iannino, M. Roset, A. Vigliocco, P.S. Paulo, R.A. Ugalde, *Brucella abortus* cyclic -1, 2-glucan mutants have reduced virulence in mice and are defective in intracellular replication in HeLa cells, Infect. Immun. 69 (2001) 4528–4535.

[83] A. Arenas-Gamboa, T. Ficht, M. Kahl-McDonagh, G. Gomez, A. Rice-Ficht, The *Brucella abortus* S19 vjbR live vaccine candidate is safer than S19 and confers protection against wild-type challenge in BALB/c mice when delivered in a sustained-release vehicle, Infect. Immun. 77 (2) (2009 Feb) 877–884.

[84] Q.L. Truong, Y. Cho, K. Kim, B.K. Park, T.W. Hahn, Booster vaccination with safe, modified, live-attenuated mutants of *Brucella abortus* strain RB51 vaccine confers protective immunity against virulent strains of *B. abortus* and *Brucella canis* in BALB/c mice, Microbiology 161 (2015) 2137–2148.

[85] Q.L. Truong, Y. Cho, S. Park, K. Kim, T.W. Hahn, *Brucella abortus* Delta-cydCDeltacydD and DeltacydCDeltapurD double-mutants are highly attenuated and confer long-term protective immunity against virulent *Brucella abortus*, Vaccine 34 (2016) 237–244.

[86] Q.L. Truong, Y. Cho, A.K. Barate, S. Kim, T.W. Hahn, Characterization and protective property of *Brucella abortus* cydC and looP mutants, Clin. Vaccine Immunol. 21 (2014) 1573–1580.

[87] J.A. de Souza Filho, V. de Paulo Martins, P.C. Campos, J. Alves-Silva, N.V. Santos, F.S. de Oliveira, G.B. Menezes, V. Azevedo, S.L. Cravero, S.C. Oliveira, Mutant *Brucella abortus* membrane fusogenic protein induces protection against challenge infection in mice, Infect. Immun. 83 (2015) 1458–1464.

[88] Y. Bao, M. Tian, P. Li, J. Liu, C. Ding, S. Yu, Characterization of *Brucella abortus* mutant strain Delta22915, a potential vaccine candidate, Vet. Res. 48 (2017) 17.

[89] M. Edmonds, N. Booth, S. Hagijs, J. Walker, F. Enright, R.M. Roop 2nd, P. Elzer, Attenuation and immunogenicity of a *Brucella abortus* htrA cycL double mutant in cattle, Vet. Microbiol. 76 (2000) 81–90.
[90] T.L. Lacerda, P.G. Cardoso, L. Augusto de Almeida, I.L. Camargo, D.A. Afonso, C.C. Trant, G.C. Macedo, E. Campos, S.L. Cravero, S.P. Salcedo, J.P. Gorvel, S.C. Oliveira, Inactivation of formyltransferase (wbkC) gene generates a Brucella abortus rough strain that is attenuated in macrophages and in mice, Vaccine 28 (2010) 5627–5634.

[91] A.W.B. Reyes, H.L.T. Simborio, H.T. Hop, L.T. Arayan, T.X.N. Huy, W. Min, S. Kim, The two highly immunogenic antigens of Brucella: lipopolysaccharide (LPS) and outer membrane proteins (OMPs), J. Prevent. Vet. Med. 39 (2015) 198–206.

[92] X.R. Wang, G.M. Yan, R. Zhang, X.L. Lang, Y.L. Yang, X.Y. Li, S. Chen, J. Qian, X.L. Wang, Immunogenic response induced by wzm and wzt gene deletion mutants from Brucella abortus S19, Mol. Med. Rep. 9 (2014) 653–658.

[93] A. Al-Mariri, N.H. Mahmoud, R. Hammoud, Efficacy evaluation of live Escherichia coli expression Brucella P39 protein combined with CpG oligodeoxynucleotides vaccine against Brucella melitensis 16M, in BALB/c mice, Biologicals 40 (2012) 140–145.

[94] A. Al-Mariri, A. Tibor, P. Lestrate, P. Mertens, X. De Bolle, J.J. Letesson, Yersinia enterocolitica as a vehicle for a naked DNA vaccine encoding Brucella abortus bacterioferritin or P39 antigen, Infect. Immun. 70 (4) (2002) 1915–1923.

[95] Y. He, R. Vemulapalli, G.G. Schurig, Recombinant Ochrobactrum anthropi expressing Brucella abortus Cu,Zn superoxide dismutase protects mice against B. abortus infection only after switching of immune responses to Th1 type, Infect. Immun. 70 (2002) 2535–2543.

[96] D. Saez, P. Fernandez, A. Rivera, E. Andrews, A. Onate, Oral immunization of mice with recombinant Lactococcus lactis expressing Cu, Zn superoxide dismutase of Brucella abortus triggers protective immunity, Vaccine 30 (2012) 1283–1290.

[97] W.K. Kim, J.Y. Moon, J.S. Cho, J. Hur, Protective efficacy by various doses of a new brucellosis vaccine candidate based on Salmonella strains expressing Brucella abortus BSCP31, Omp3b and superoxide dismutase against brucellosis in murine model, Pathog. Dis. 75 (2017).

[98] Z. Zhao, M. Li, D. Luo, L. Xing, S. Wu, Y. Duan, P. Yang, X. Wang, Protection of mice from Brucella infection by immunization with attenuated Salmonella enterica serovar typhimurium expressing A L7/L12 and BLS fusion antigen of Brucella, Vaccine 27 (2009) 5214–5219.
[99] M. Leya, W.K. Kim, J.S. Cho, E.C. Yu, Y.J. Kim, Y. Yeo, K.S. Lyoo, M.S. Yang, S.S. Han, J.H. Lee, D. Tark, J. Hur, B. Kim, Vaccination of goats with a combination of Salmonella vector expressing Brucella antigens (BLS, PrpA, Omp19, SOD) confers a protection against Brucella abortus infection, J. Vet. Sci. 19 (5) (2018 Sep 30) 643–652.

[100] J. Lalsiamthara, J.H. Lee, Brucella lipopolysaccharide reinforced Salmonella delivering Brucella immunogens protects mice against virulent challenge, Vet. Microbiol. 205 (2017) 84–91.

[101] J. Lalsiamthara, J.H. Lee, Immunization of Guinea pigs with Salmonella delivered anti-Brucella formulation reduces organs bacterial load and mitigates histopathological consequences of Brucella abortus 544 challenge, Vet. Immunol. Immunopathol. 195 (2018) 40–45.

[102] S. Baloglu, T. Toth, G. Schurig, N. Sriranganathan, S. Boyle, Humoral immune response of BALB/c mice to a vaccinia virus recombinant expressing Brucella abortus GroEL does not correlate with protection against a B. abortus challenge, Vet. Microbiol. 76 (2000) 193–199.

[103] S. Baloglu, S.M. Boyle, R. Vemulapalli, N. Sriranganathan, G.G. Schurig, T.E. Toth, Immune responses of mice to vaccinia virus recombinants expressing either Listeria monocytogenes partial listeriolysin or Brucella abortus ribosomal L7/L12 protein, Vet. Microbiol. 109 (2005) 11–17.

[104] R. Vemulapalli, S. Cravero, C.L. Calvert, T.E. Toth, N. Sriranganathan, S.M. Boyle, O.L. Rossetti, G.G. Schurig, Characterization of specific immune responses of mice inoculated with recombinant vaccinia virus expressing an 18-kilodalton outer membrane protein of Brucella abortus, Clin. Diagn. Lab. Immunol. 7 (2000) 114–118.

[105] A. Cabrera, D. Sáez, S. Céspedes, E. Andrews, A. Oñate, Vaccination with recombinant Semliki Forest virus particles expressing translation initiation factor 3 of Brucella abortus induces protective immunity in BALB/c mice, Immunobiology 214 (2009) 467–474.

[106] A.A. Onate, G. Donoso, G. Moraga-Cid, H. Folch, S. Céspedes, E. Andrews, An RNA vaccine based on recombinant Semliki Forest virus particles expressing the Cu, Zn superoxide dismutase protein of Brucella abortus induces protective immunity in BALB/c mice, Infect. Immun. 73 (6) (2005) 3294–3300.

[107] K. Tabynov, A. Sansyzbay, Z. Kydyrbayev, B. Yespembetov, S. Ryskeldinova, N. Zinina, N. Assanzhanova, K. Sultankulova, N. Sandybayev, B. Khairullin, I. Kuznetsova, B. Ferko, A. Egorov, Influenza
viral vectors expressing the Brucella OMP16 or L7/L12 proteins as vaccines against *B. abortus* infection, Virol. J. 11 (2014) 69.

[108] K. Tabynov, Z. Kydyrbayev, S. Ryskeldinova, B. Yespembetov, N. Zinina, N. Assanzhanova, Y. Kozhamkulov, D. Inkarbekov, T. Gotskina, A. Sansyzbay, Novel influenza virus vectors expressing Brucella L7/L12 or Omp16 proteins in cattle induced a strong T-cell immune response, as well as high protectiveness against *B. abortus* infection, Vaccine 32 (2014) 2034–2041.

[109] K. Tabynov, B. Yespembetov, S. Ryskeldinova, N. Zinina, Z. Kydyrbayev, Y. Kozhamkulov, D. Inkarbekov, A. Sansyzbay, Prime-booster vaccination of cattle with an influenza viral vector *Brucella abortus* vaccine induces a long-term protective immune response against *Brucella abortus* infection, Vaccine 34 (2016) 438–444.

[110] A. Mailybayeva, B. Yespembetov, S. Ryskeldinova, N. Zinina, A. Sansyzbay, G.J. Renukaradhya, N. Petrovsky, K. Tabynov, Improved influenza viral vector based *Brucella abortus* vaccine induces robust B and T-cell responses and protection against *Brucella melitensis* infection in pregnant sheep and goats, PLoS One 12 (2017) e0186484.

[111] G.Z. Lin, J.T. Yang, S.C. Wei, S.E. Chen, S.D. Huo, Z.R. Ma, Immunogenicity of adenovirus and DNA vaccines co-expressing P39 and lumazine synthase proteins of *Brucella abortus* in BALB/c mice, Trop. Anim. Health Prod. 50 (2018) 957–963.

[112] R. Vemulapalli, Y. He, S. Cravero, N. Sriranganathan, S.M. Boyle, G.G. Schurig, Overexpression of protective antigen as a novel approach to enhance vaccine efficacy of *Brucella abortus* strain RB51, Infect. Immun. 68 (2000) 3286–3289.

[113] S.C. Olsen, S.M. Boyle, G.G. Schurig, N.N. Sriranganathan, Immune responses and protection against experimental challenge after vaccination of bison with *Brucella abortus* strain RB51 or RB51 overexpressing superoxide dismutase and glycosyltransferase genes, Clin. Vaccine Immunol. 16 (2009) 535–540.

[114] P. Rajasekaran, N. Surendran, M.N. Seleem, N. Sriranganathan, G.G. Schurig, S.M. Boyle, Over-expression of homologous antigens in a leucine auxotroph of *Brucella abortus* strain RB51 protects mice against a virulent *B. suis* challenge, Vaccine 29 (2011) 3106–3110.

[115] R. Vemulapalli, Y. He, S.M. Boyle, N. Sriranganathan, G.G. Schurig, *Brucella abortus* strain RB51 as a vector for heterologous protein expression
and induction of specific Th1 type immune responses, Infect. Immun. 68 (2000) 3290–3296.

[116] M. Rezaei, M. Rabbani-Khorasgani, S.H. Zarkesh-Esfahani, R. Emamzadeh, H. Abtahi, Prediction of the Omp16 epitopes for development of an epitope based vaccine against brucellosis, Infect. Disord. Drug Targets (2018).

[117] C.N. Pollak, M.V. Delpino, C.A. Fossati, P.C. Baldi, Outer membrane vesicles from Brucella abortus promote bacterial internalization by human monocytes and modulate their innate immune response, PLoS One 7 (2012) e50214.

[118] N. Jain-Gupta, A. Contreras-Rodriguez, R. Vemulapalli, S.G. Witonsky, S.M. Boyle, N. Sriranganathan, Pluronic P85 enhances the efficacy of outer membrane vesicles as a subunit vaccine against Brucella melitensis challenge in mice, FEMS Immunol. Med. Microbiol. 66 (2012) 436–444.

[119] E.D. Avila-Calderon, M.G. Araiza-Villanueva, J.C. Cancino-Diaz, E.O. Lopez-Villegas, N. Sriranganathan, S.M. Boyle, A. Contreras-Rodriguez, Roles of bacterial membrane vesicles, Arch. Microbiol. 197 (2015) 1–10.