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Review Article

Vaccines and Vaccine Candidates against Brucellosis

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Abstract

Brucella is a facultative intracellular pathogen, and brucellosis is commonest zoonotic disease worldwide. *Brucella* species, isolated from domestic animals, are important pathogen for humans. Annually, more than 500,000 new cases of brucellosis are reported, and this figure is an underestimate due to extended under-reporting cases in several endemic countries. *Brucella* has a variety of virulence mechanisms that prevent detection and activation of innate immunity, but protection against intracellular pathogen is represented by cell-mediated immunity. As yet, much research has been performed to develop a safe *Brucella* vaccine to control the disease in human and animals. Despite the availability of several live attenuated vaccine for animals, currently, no effective human vaccine is available. Moreover, due to the potential use of *Brucella* in bioterrorism or biowarfare, development of an effective vaccine against brucellosis for human use is necessary. In this paper, we aimed to review and discuss the efforts of researchers to develop vaccines against Brucellosis.

Keywords:Brucella, Vaccine, Brucellosis, Zoonoses

1.Background

Brucella is a facultative intracellular pathogen causeing severe febrile illness in human, known as brucellosis (1). Brucellosis is the commonest zoonotic disease in the worldwide, and annually, more than 500,000 new cases of brucellosis are reported, and this figure is an underestimate due to extended under-reporting cases in several endemic countries (2). In many countries, Brucellosis is a serious public health problem, especially those around the Middle East, Mediterranean Sea, and South America as it is endemic in this areas (2,3).

Domestic and wild animals are the primary hosts for *Brucella*. Four species of *Brucella,* isolated from domestic animals, are important pathogens for humans. *Brucella* strains may either expressing smooth lipopolysaccharide (S-LPS) or rough lipopolysaccharide (R-LPS). This species are different in their pathogenicity and their host preference and include: *Brucella melitensis* (goatsandsheep), *B. abortus* (cattle), *B. suis* (swine), and *B. canis* (dogs), which cause abortion in ewes and goats, resulting in huge economic losses (4,5). Common routes of human infection are the ingestion of unpasteurized dairy products such as cheese or milk, contact with infected animals and inhalation of aerosols (6).

The disease has the tendency to affect several organs; then to cause chronic diseases such as arthritis, spondylitis, encephalitis, meningitis, orchitis, prostatitis, and endocarditis; and to persist for prolonged periods in the reticuloendothelial system of infected hosts (2,7). It has been reported that despite early diagnosis and treatment, chronic disease develops in 10-30% of the cases, and approximately 2% of untreated patients die from brucellosis (8,9). In the zoonotic hosts, *Brucella spp.* infect the reproductive tract, and can cause infertility or abortion (10,11).

Although brucellosis in most developed countries has been controlled in domestic animals, but it remains as a public and animal health problem in the developing countries. In order to prevent brucellosis, it is crucial that intervention strategies in animals and humans be improving. Over the last decades, most promising strategies have been conducted to control and eradicate the disease by developing safer and more effective vaccines for animals, but there is no licensed vaccine against human brucellosis yet (2,12).

Human vaccine would be applied to protect laboratory personnel, farmers, veterinarians, and general population living in brucellosis endemic areas (13). Moreover, *Brucella* bacteria can be used as a biological weapon due to their highly infectious nature and the potential use of the agents as a weapon for biowarfare or bioterrorism (14,15).

In this paper, we aimed to review and discuss the efforts of researchers to develop vaccines against Brucellosis.

2. Context

2.1. Immune system response

2.1.1.Innate immune system

Human immune system interaction with the *Brucella* is critical for the development of chronic disease or clearance of infection. Upon arrival, *Brucella* has a four-week latency period before becoming symptomatic (16,17). Detection of the bacteria inside of the body is mediated by the innate immune system with pattern recognition receptors, including the nucleotide binding and oligomerization domain-like receptors (NLRs), the toll-like receptors (TLR1, TLR2, TLR4, TLR5 and TLR6), and alternative complement pathway (18).

On the other hand, *Brucella* has a variety of virulence mechanisms that prevent detection and activation of innate immunity such as producing poorly recognizable Lipid A and flagellin, which lack the TLR5 agonist domain and molecules, which suppress innate immune signalling (19,20).

2.1.2.Cell-mediated immunity

Protection against intracellular pathogens represented by *Brucella*, depends on cell-mediated immunity involving activated macrophages, dendritic cells and, T-lymphocytes ($CD4^+$, $CD8^+$ and γδ T cells), whereas humoral immunity has a minor role in the control of infection (16,21).

Activated macrophage and dendritic cells present *Brucella* immunogenic antigens to T-lymphocytes and induce differentiation of T-helper 1 (Th1). Then Th1 produces cytokines, and this mechanism has an essential role in clearance of infection (16,22). After entering the host, *Brucella* is taken up by macrophage and dendritic cells. *Brucella* can survive and replicate in this immune cells and evad adaptive immune system (21,23). Macrophages are key elements in the cellular immune response against intracellular bacteria like *Brucella* (17). Infected macrophages produce critical cytokines such as TNF-α, enhancing the bactericidal activity of phagocytes, and IL-12, driving the Th1 immune response. IL-12 induces the production of IFN-γ from $CD4^+$, $CD8^+$, and γδ T lymphocytes, resulting in the Th1 immune response and bactericidal activity of macrophages, which in turn lead to the prevention of the intracellular survival of *Brucella* (17,24). In addition, cytotoxic activity of the $CD8^+$ and $\gamma\delta$ T cells are significant for killing the infected macrophages (25). In the mouse model, IgG2a isotype antibody, opsonises the bacteria and facilitates effective phagocytosis (14,16).

Despite the mechanisms mentioned above, *Brucella* produces various virulence factors that modify those mechanisms, then it can survive and replicate for many years in hosts reticuloendothelial system, afterwards it can produce chronic and persistent infection (26).

2.2. Prevention against brucellosis

As mentioned, brucellosis is transmitted through contact with infected animals or dairy products, so the disease control programmes in countries with a high prevalence mainly have been focused on vaccination of animals with killed and live attenuated strains (14).

2.2.1. Killed vaccines

Over the years, a wide variety of killed vaccines such as *B. abortus* strain 45/20 and *B. melitensis* H38 have been developed to protect animals against brucellosis, but they have had limited success because they induce persistent antibody titters that can interfere with common serological tests; in addition, protection after challenge by this strains are insufficient (27).

2.2.2. Live attenuated vaccines

Live attenuated vaccines carry several advantages over killed vaccines. They are less expensive, as live vaccines are administered, the organism is allowed to replicate within the host and to permanently induce cellular immunity (27,28).

B. abortus strain19 (S19)

B. abortus S19 is live smooth attenuated vaccine licensed for control of bovine brucellosis. This strain was isolated in the early twentieth century and naturally attenuated when a virulent culture of *B. abortus* was left at room temperature for one year (29). After vaccination, the animal will be protected against brucellosis for several years, which can be extended by revaccination (4,29). Despite of being attenuated, it is serologically indistinguishable from virulent strains due to its smooth nature. It induces strong antibody response against the LPS O-side chain (30).

However, *B. abortus* S19 is not completely avirulent, significant reduction in milk production and low rate of abortion in cows have been reported with this vaccine (31,32). Side effects associated with using live attenuated vaccines, prevent their widespread use in humans. In 1952, a derivative vaccine of S19, *B. abortus* VA 19, was used in the former USSR (Union of Soviet Socialist Republics) as a live vaccine for human but unfortunately some of those people were diseased with vaccine strain because vaccine was found to be insufficiently attenuated (33,34).

Some studies focused on the development of live attenuated *Brucella* vaccines for human by deletion of important genes required for survival. A *vjbR*, quorum sensing-related transcriptional regulator, knockout was generated in the S19 vaccine and investigated for its potential as a vaccine on mice model. To enhance vaccination efficacy, the live S19 Δ*vjbR* was encapsulated in alginate microspheres containing the parasite *Fasciola hepatica* nonimmunogenic eggshell precursor protein. Vaccine candidate was able to elicit an anti-*Brucella*-specific IgG response and to protect mice in challenge with virulent *B. abortus* strain 2308 (35).

However, *B. abortus* S19 is not entirely avirulent in humans, cases have been reported that in which veterinarians were infected with the vaccine strain (4).

B. abortus RB51

B. abortus RB51 is R-LPS mutant that is spontaneously attenuated and obtained by subculturing the virulent strain of *B. abortus* 2308 on medium containing penicillin and rifampicin (36). *B. abortus* RB51 is very stable and in some countries introduced instead of *B. abortus* S19 as a vaccine for cattle. RB51strain has low virulence and does not interfere with diagnostic serology tests but can induce very low level of abortion (32,37).

RB51 carries an IS771 insertion disrupting the *wbo*A gene, a gene encoding a glycosyl transferase that is responsible for O-side chain synthesis. It is thought that this strain has several unknown mutations (38). Vaccine strain RB51 can infect humans, but it is less virulent than S19 strain(39). On the other hand, it is resistant to rifampicin which is used in the groups of brucellosis patients who cannot be treated with routine drugs; for example, children, pregnant women, endocarditis and neurobrucellosis cases; therefore, it is considered unsuitable as human vaccine (32,34).

B. melitensis Rev.1

B. melitensis Rev.1 is live smooth attenuated vaccine used for immunization of sheep and goats. This strain was derived from a virulent strain, it is resistant to 2.5 $\mu g.mL^{-1}$ streptomycin and susceptible to 5 IU penicillin G. Having S-LPS phenotype, *B. melitensis* Rev.1 raises antibody response in serological tests, so that is difficult to distinguish between vaccinated and infected animals (37). *B. Melitensis* Rev.1 retains some virulence, leading to abortions in pregnant animals. In some cases, it has been reported that *B. melitensis* Rev.1 was excreted into the milk of animals, which enhance concerns about the vaccine strain infect other animal and humans (37,40). It has also been reported that veterinarians vaccinating sheep, were infected with this organism (33).

A number of genetically attenuated mutants have been developed, but their suitability for human use has not been evaluated (28,41). A genetically defined, attenuated *pur*EK mutant of *B. melitensis* strain 16M was developed, and it was found that it protects mice against disseminated infection of spleens and livers caused by virulent strain of *B. Melitensis* (42).

2.3. Subunit vaccines against Brucellosis

The live attenuated strains are good choice for vaccination due to induction high level of protection and being less expensive, but they produce some unpleasant side effects such as abortion in pregnant animals and infection in humans (4,29,36). Currently, there is no immunization strategy for human; thus, the development of an effective subunit vaccine is necessary.

There have been many studies showing the protective effect of subunit vaccines, formulated either as DNA, purified proteins, and antigenic fractions[e.g. LPS, ribosomal L7/L12 protein, P39, 31 kDa outer membrane protein (Omp31) and Outer membrane vesicles (OMVs), as mentioned below], which are extracted from *Brucella* and tested as vaccine candidates on the animal models. Some of mentioned antigens tend to poorly stimulate immune system and require to coadministration with an adjuvant (43).

Brucella LPS has been shown to be 268-fold less pyrogenic than *Escherichia coli*, but it has been shown that adjuvant protein could increase expression of costimulatory molecules on murine B cells and enhance humoral responses to polysaccharide antigens (44).

Subcutaneous immunization of mice by *Brucella* LPS in conjugation with *Helicobacter pylori*'s recombinant CagA protein, significantly increased immune system response when challenged with *B. abortus* strain 544 intraperitoneally (44). Ribosomal vaccines have been proposed against different disease. In one model, immunization of mice with recombinant *B. abortus* ribosomal L7/L12 protein has been provide reduction of *Brucella* in spleen and to elicite some levels of protection (45). The 39-kDa protein (P39) is one of the most immunodominant proteins detected in *Brucella* infections; P39 elicits production of IFN- γ from mononuclear cell (45).

The *Brucella spp*. major outer membrane proteins (OMPs) were identified and characterised as immunogenic and protective antigens. Mice immunized with recombinant Omp31 (rOmp31) or rOmp31 plus R-LPS, provide the best protection level against *Brucella ovis* (46).

OMVs are bilayer membrane vesicles haveing the outer membrane and periplasmic components, released during the growth of *Brucella* by a mechanism involving cell wall turnover (47). Proteins present in OMVs from *B. melitensis* are SOD, co-chaperonin GroES, Omp19, Omp25, Omp31, bp26, and Omp16. A group of researchers purified OMVs from both *B. melitensis* strain 16M (smooth strain) and VTRM1 (rough strain) and used them for mice immunization. OMVs from a rough *B. melitensis* VTRM1 induced significantly higher expression of IL-12, TNF-α and IFN-γ genes. Mice immunized intramuscularly with rough OMVs shown protection against challenge with virulent *B. melitensis* strain 16M. It is possible that the absence of the O-side chain inpurified OMVs from rough strain, could allow to higher exposure of bacterial surface molecules, such as OMPs, to immune receptors (48).

In addition to fractions mentioned above, number of vaccine candidates such as; Omp25 (49), rOmp28 (50), rOmp31 (51), rOmp16 and rOmp19 (52), recombinant S-adenosyl-lhomocysteine hydrolase (53), rDnaK and rSurA (54), SodCprotein (55), *sod*C gene(56), Lumazine synthase (57), Bp26(58),Heat shock protein (59), recombinant superoxide dismutase (rSOD) proteins (60) were identified and examined by different research teams.

3. Conclusion

Brucellosis is a highly contagious zoonotic disease that is an important economic and sanitary problem affecting millions of people worldwide. *Brucella* was classified as biosafety Level 3 agent and the Centers for Disease Control and Prevention designated it as Class B bioterrorist threat agent because of being potential abiological weapon (61,62).

The best effective alternative approach to control animal brucellosis is the use of vaccination programs (63). S19, Rev.1and RB51 vaccines have been used successfully in eradication and control programs against animal brucellosis in many countries (64). Side effects associated with live attenuated strains of *Brucella*, prevent widespread use of this type of vaccines in human. Therefore, they are considered unsuitable as human vaccine.

Subunit vaccines are good choice for vaccination due to induction good level of protection in animal model. In compare to other subunit vaccines, OMPs and OMVs are characterised as immunogenic and protective antigens and therefore considered suitable candidates as human vaccine. OMVs have considerable advantages; they are multicomplex antigens that strongly activate the host innate and acquired immune response pathways and are less expensive in terms of purification. However, further research's are required to fully evaluate the benefits and risks of Subunit vaccines.

At present, there is no licensed vaccine for prevention of human brucellosis, and current animal vaccines are both virulent in humans and lack clinical efficiency (37).

Vaccination of human beings could be considered as an alternative approach for the prevention of naturally acquired disease and also as a defence strategy against bioterrorism or biowarfare (65). Subunit vaccines could avoid the drawbacks of live attenuated vaccines because of being avirulent, noninfectious, nonviable, and we can select and provide good protective immunodominant antigens, differe from those used for immunodiagnosis.

Conflict of Interests

Authors declare they have no conflict of interests.

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Authors' Contribution

All authors contribute in writing different parts of this manuscript.

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References

1. Ghasemi A, Salari MH, Zarnani AH, Pourmand MR, Ahmadi H, Shirazi MH, et al. Optimization and efficient purification in production of *Brucellamelitensis*recombinant HSP and TF proteins with low endotoxin contents. Jundishapur J Microbiol. 2013; 6(7): e6875.
2. Bahador A, Mansoori N, Esmaeili D, Amini Sabri R. Brucellosis: prevalence and retrospective evaluation of risk factors in western cities of Tehran province, Iran J Bacteriol. 2012; 4(3): 33-7.
3. Al-Majali AM, Shorman M. Childhood brucellosis in Jordan: prevalence and analysis of risk factors. Int J Infect Dis. 2009;13(2):196-200.
4. Haag AF, Myka KK, Arnold MF, Caro-Hernandes P, Ferguson GP. Importance of lipopolysaccharide and cyclic β-1, 2-glucans in *Brucella*-mammalian infections. Int J Microbiol. 2010; 2010; 124509.
5. Soler-Lloréns P, Gil-Ramírez Y, Zabalza-Baranguá A, Iriarte M, Conde-Alvarez R, Zunipa-Riga A, et al. Mutants in the lipopolysaccharide of*Brucellaovis* are attenuated and protect against *B. ovis* infection in mice. Vet Res. 2014; 45(1):72.
6. Atluri VL, Xavier MN, de Jong MF, den Hartigh AB, Tsolis RM Interactions of the human pathogenic *Brucella* species with their hosts. Annu Rev Microbiol. 2011;65:523-41.
7. Pappas G. The changing *Brucella* ecology: novel reservoirs, new threats. Int J Antimicrob Agents. 2010; 36(Suppl 1): S8-11.
8. Skendros P, Boura P, Kamaria F, Raptopoulou-Gigi M. CD80/CD28 co-stimulation in human brucellosis. ClinExpImmunol. 2006; 146(3):400-8.
9. Silva TM, Paixão TA, Costa ÉA, Xavier MN, Cortez Sa J, Moustacas VS, et al. Putative ATP-binding cassette transporter is essential for *Brucellaovis* pathogenesis in mice. Infect Immun. 2011; 79(4):1706-17.

10. Ghasemi A, Salari MH, Zarnani AH, Pourmand MR, Ahmadi H, Mirshafiey A, et al. Immunereactivity of *Brucellamelitensis* vaccinated rabbit serum with recombinant Omp31 and DnaK proteins. Iran JMicrobiol. 2013; 5(1):19-23.
11. Xavier MN, Paixão TA, Poester FP, Lage AP, Santos RL. Pathological, immunohistochemical and bacteriological study of tissues and milk of cows and fetusesexperimentally infected with*Brucellaabortus*. J Comp Pathol. 2009; 140(2):149-57.
12. Blasco JM, Molina-Flores B. Control and eradication of *Brucellamelitensis*infection in sheep and goats. VetClin North Am FoodAnimPract. 2011; 27(1): 95-104.
13. Luna-Martínez JE, Mejía-Terán C. Brucellosis in Mexico: current status and trends. VetMicrobiol. 2002; 90(1): 19-30.
14. Fugier E, Pappas G, Gorvel J-P. Virulence factors in brucellosis: implications for aetiopathogenesis and treatment. Expert Rev Mol Med. 2007; 9(35):1-10.
15. Guillemin J. Scientists and the history of biological weapons. EMBO Rep. 2006; 7(1S): S45-9.
16. Martirosyan A, Von Bargen K, Gorvel VA, Zhao W, Hanniffy S, Bonnardel J, et al. In Vivo identification and characterization of CD4+ Cytotoxic T cells induced by virulent *Brucellaabortus*infection. PloSOne. 2013; 8(12):e82508.
17. Baldwin CL, Goenka R. Host immune responses to the intracellular bacteria *Brucella*: does the bacteriainstruct the host to facilitate chronic infection? Crit Rev Immunol. 2006; 26(5):407-42.
18. Franchi L, Park JH, Shaw MH, Marina-Garsia N, Chen G, Kim YG, et al. Intracellular NOD-like receptors in innate immunity, infection and disease. Cell Microbiol. 2008; 10(1):1-8.
19. Radhakrishnan GK, Yu Q, Harms JS, Splitter GA. *Brucella* TIR domain-containing protein mimics properties of the Toll-like receptor adaptor protein TIRAP. J BiolChem. 2009; 284(15):9892-8.
20. Sengupta D, Koblansky A, Gaines J, Brown T, West AP, Zhang D, et al. Subversion of innate immune responses by *Brucella* through the targeted degradation of the TLR signaling adapter. MAL. J Immunol. 2010;184(2):956-64.
21. Gorvel JP. *Brucella*: a Mr "Hide" converted into Dr Jekyll. Microbes Infect. 2008; 10(9):1010-3.
22. Dorneles EM, Teixeira-Carvalho A, Araújo MS, Lima GK, Martins-Filho OA, Sriranganathan N, et al. T lymphocytes subsets and cytokine pattern induced by vaccination against bovine brucellosis employing S19 calfhood vaccination and adult RB51 revaccination. Vaccine. 2014; 32(46):6034-8.
23. Maria-PilarJdB, Dudal S, Dornand J, Lafont V, Loisel S, Liautard J, et al. Cellular bioterrorism: how *Brucella* corrupts macrophage physiology to promote invasion and proliferation. ClinImmunol. 2005; 114(3):227-38.
24. Feldman KE, Loriaux PM, Saito M, Luero I, Villaverde H, Siva T, et al. Ex vivo innate immune cytokine signature of enhanced risk of relapsing Brucellosis. PLoSNegl Trop Dis. 2013; 7(9):e2424.
25. Ariza J, Bosilkovski M, Cascio A, Colmenero JD, Corbel MJ, Falagas ME, et al. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations, PLoS Med. 2007; 4(12):e317.
26. Vrioni G, Pappas G, Priavali E, Gartzonika C, Levidiotou S. An eternal microbe: *Brucella* DNA load persists for years after clinical cure. Clin Infect Dis. 2008; 46(12):e131-6.
27. Avila-Calderón ED, Lopez-Merino A, Sriranganathan N, Boyle SM, Contreras-Rodriguez A, et al. A history of the development of *Brucella* vaccines. Biomed Res Int. 2013;2013: 743509.
28. Edmonds MD, Cloeckaert A, Elzer PH. *Brucella* species lacking the major outer membrane protein Omp25 are attenuated in mice and protect against *Brucellamelitensis* and *Brucellaovis*. VetMicrobiol. 2002; 88(3):205-21.
29. Weinhold M, Eisenblätter M, Jasny E, Fehlings M, Finke A, Gayum H, et al. The attenuated *Brucellaabortus*strain 19 invades, persists in, and activates human dendritic cells, and induces the secretion of IL-12p70 but not IL-23. PloSOne. 2013; 8(6): e65934.
30. Poester FP, Gonçalves VS, Paixao TA, Santos RL, Olsen SC, Schurig GG, et al. Efficacy of strain RB51 vaccine in heifers against experimental brucellosis. Vaccine. 2006; 24(25):5327-34.
31. Olsen SC, Stoffregen WS. Essential role of vaccines in brucellosis control and eradication programs for livestock.Expert Rev Vaccines. 2005; 4(6):915–28
32. Moriyón I, Grilló MJ, Monreal D, Grillo MG, Gonzalez D, Marin C, et al. Rough vaccines in animal brucellosis: structural and genetic basis and present status. Vet Res. 2004; 35(1):1-38.
33. Bhattacharjee AK, Izadjoo MJ, Zollinger WD, Nikolich MP, Hoover DL. Comparison of protective efficacy of subcutaneous versus intranasal immunization of mice with a *Brucellamelitensis* lipopolysaccharide subunit vaccine. Infect Immun. 2006; 74(10):5820-5.
34. Perkins SD, Smither SJ, Atkins HS. Towards a *Brucella* vaccine for humans. FEMS Microbiol Rev. 2010; 34(3):379-94.
35. Arenas-GamboaAM, Ficht T, Kahl-McDonagh M, Rice-Ficht AC. The *Brucellaabortus* S19 ΔvjbR 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. 2009; 77(2):877-84.
36. Adone R, Muscillo M, La Rosa G, Francia M, Tarantino M. Antigenic, immunologic and genetic characterization of rough strains *B. abortus* RB51, *B. melitensis* B115, and *B. melitensis* B18. PloS One. 2011; 6(10):e24073.
37. Schurig GG, Sriranganathan N, Corbel MJ. Brucellosis vaccines: past, present, and future. VetMicrobiol. 2002; 90(1):479-96.
38. Vemulapalli R, McQuiston JR, Schurig GG, Sriranganathan N, Halling SM, Boyle SM. Identification of an IS711 element interrupting the wboAgene of *Brucellaabortus*vaccine strain RB51 and a PCR assay to distinguish strain RB51 from other *Brucella*species and strains. ClinDiagn Lab Immunol. 1999; 6(5):760-4.
39. Ashford DA, di Pietra J, Lingappa J, Woods C, Noll H, Neville B, et al. Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51. Vaccine. 2004; 22(25):3435-9.
40. Banai M. Control of small ruminant brucellosis by use of *Brucellamelitensis* Rev. 1 vaccine: laboratory aspects and field observations. VetMicrobiol. 2002; 90(1):497-519.
41. McQuiston JR, Vemulapalli R, Inzana TJ, Schurig GG, Sriranganathan N, Fritzinger D, et al. Genetic characterization of a Tn5-disrupted glycosyltransferase gene homolog in*Brucellaabortus* and its effect on lipopolysaccharide composition and virulence. Infect Immun. 1999; 67(8):3830-5.
42. Crawford RM, Van De Verg L, Yuan L, Hadfield TL, Warren RL, Drazek ES, et al. Deletion of *pur*E attenuates *Brucellamelitensis* infection in mice. Infect Immun. 1996; 64(6):2188-92.
43. Pasquevich KA, Samartino CG, Coria LM, Estein SM, Zwerdling A, Ibanez AE, et al. The protein moiety of *Brucellaabortus* outer membrane protein 16 is a new bacterial pathogen-associated molecular pattern that activates dendritic cells in vivo, induces a Th1 immune response, and is a promising self-adjuvanting vaccine against systemic and oral acquired brucellosis. J Immunol. 2010; 184(9):5200-12.
44. Bahador A, Esmaeili D, Mansoori N, Mahdavi M. Protection against *Brucellaabortus* 544 strain infection in BALB/c mice by subcutaneouse administration of multicomponent vaccine of rCagA conjugated with LPS plus CpG. J PureApplMicrobiol. 2013; 7(3):1809-19.
45. Oliveira S, Zhu Y, Splitter G. Recombinant L7/L12 ribosomal protein and gamma-irradiated *Brucellaabortus* induce a T-helper 1 subset response from murine CD4+ T cells. Immunol. 1994; 83(4):659-65.
46. Estein SM, Cassataro J, Vizcaíno N, Zigmunt MS, Cloeckaert A, Bowden RA. The recombinant Omp31 from *Brucellamelitensis* alone or associated with rough lipopolysaccharide induces protection against *Brucellaovis* infection in BALB/c mice. Microb Infect. 2003; 5(2):85-93.
47. Amano A, Takeuchi H, Furuta N. Outer membrane vesicles function as offensive weapons in host–parasite interactions. Microb Infect. 2010; 12(11):791-8.
48. Avila-Calderón ED, Lopez-Merino A, Jain N, Peralta H, Lopez-Villages EO, Sriranganathan N, et al. Characterization of outer membrane vesicles from *Brucellamelitensis* and protection induced in mice. ClinDevImmunol. 2011; 2012:1-13.
49. Commander NJ, Spencer SA, Wren BW, MacMillan AP. The identification of two protective DNA vaccines from a panel of five plasmid constructs encoding *Brucellamelitensis* 16M genes. Vaccine. 2007; 25(1):43-54.
50. Kaushik P, Singh DK, Kumar SV, Tiwari AK, Shukla G, Dayal S, et al. Protection of mice against*Brucellaabortus* 544 challenge by vaccination with recombinant OMP28 adjuvanted with CpG oligonucleotides. Vet Res Commun. 2010; 34(2):119-32.
51. Cassataro J, Estein SM, Pasquevich KA, Velikovsky CA, de la Barrera S, Bowden R, et al. Vaccination with the recombinant *Brucella* outer membrane protein 31 or a derived 27-amino-acid synthetic peptide elicits a CD4+ T helper 1 response that protects against *Brucellamelitensis* infection. Infect Immun. 2005; 73(12):8079-88.
52. Pasquevich KA, Estein SM, Samartino CG, Coria LM, Zwerdling A, Ibanez AE, et al. 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 *Brucellaabortus* infection. Infect Immun. 2009; 77(1):436-45.
53. Yang Y, Yin J, Guo D, Lang X, Wang X. Immunization of mice with recombinant S-adenosyl-l-homocysteine hydrolase protein confers protection against*Brucellamelitensis* infection. FEMS Immunol Med Microbiol. 2011; 61(2):159-67.
54. Delpino MV, Estein SM, Fossati CA, Baldi PC, Cassataro J. Vaccination with *Brucella* recombinant DnaK and SurA proteins induces protection against *Brucellaabortus* infection in BALB/c mice. Vaccine. 2007; 25(37):6721-9.
55. Muñoz-Montesino C, Andrews E, Rivers R, Gonzalez-Smith A, Moraga-Cid G, Folch H, et al. Intraspleen delivery of a DNA vaccine coding for superoxide dismutase (SOD) of *Brucellaabortus* induces SOD-specific CD4+ and CD8+ T cells. Infect Immun. 2004; 72(4):2081-7.
56. Chaudhuri P, Singha H, Goswami TK, Jana C, Shukla G. DNA prime and protein boost immunization with combined SOD–L7/L12 antigen confers protection to mice against *Brucellaabortus* 544 Challenge. AdvAnimVet Sci. 2013;1(5):143 -147.
57. Velikovsky CA, Cassataro J, Giambartolomei GH, Goldbaum FA, Estein S, Bowden RA, et al. A DNA vaccine encoding lumazine synthase from

Brucellaabortus induces protective immunity in BALB/c mice. Infect Immun. 2002; 70(5):2507-11.

58. Gupta V, Radhakrishnan G, Harms J, Splitter G. Invasive *Escherichia coli* vaccines expressing *Brucellamelitensis* outer membrane proteins 31 or 16 or periplasmic protein BP26 confer protection in mice challenged with *B. melitensis*. Vaccine. 2012; 30(27):4017-22.
59. Ghasemi A, Salari MH, Zarnani AH, Pourmand MR, Ahmadi H, Shirazi MH, et al. Immunogenicity assessment of *Brucellamelitensis* HSP and TF proteins by immunized rabbit serum. Iran J Allergy Asthma Immunol. 2013; 12(2):192-4.
60. Singha H, Mallick AI, Jana C, Fatima N, Owais M, Chaudhuri P. Co-immunization with interlukin-18 enhances the protective efficacy of liposomes encapsulated recombinant Cu–Zn superoxide dismutase protein against *Brucellaabortus*. Vaccine. 2011; 29(29):4720-7.
61. Chang M-h, Glynn MK, Groseclose SL. Endemic, notifiable bioterrorism-related diseases, United States, 1992–1999. Emerg Infect Dis. 2003; 9(5):556-64.
62. Da Costa Martins R, Irache JM, Blasco JM, Gamgzo C. Evaluation of particulate acellular vaccines against *Brucellaovis* infection in rams. Vaccine. 2010; 28(17):3038-46.
63. Al-Mariri A, Mahmoud NH, Hammoud R. Efficacy evaluation of live *Escherichia coli* expression *Brucella* P39 protein combined with CpGoligodeoxynucleotides vaccine against *Brucellamelitensis* 16M, in BALB/c mice. Biol. 2012; 40(2):140-5.
64. Grilló MJ, Manterola L, De Miguel MJ, Munoz PM, Blasco JM, Moriyon I, et al. Increases of efficacy as vaccine against *Brucellaabortus* infection in mice by simultaneous inoculation with avirulent smooth *bvr*S/ *bvr*R and rough *wbk*A mutants. Vaccine. 2006; 24(15):2910-6.

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