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Review

The advances in brucellosis vaccines

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ABSTRACT

Brucellosis is a worldwide zoonosis affecting animal and human health. Till now, there is no effective vaccine licensed for brucellosis in humans. Although M5, H38 and 45/20 vaccines were used to prevent animal brucellosis in the early stages, the currently used animal vaccines are S19, Rev.1, S2, RB51 and SR82. However, these vaccines still have several drawbacks such as residual virulence and interfering conventional serological tests. With the development of DNA recombination technologies and the completion of the sequence of *Brucella* genome, much research focuses on the search for potential safer and more effective vaccines. Preliminary studies have demonstrated that new vaccines, including genetically engineered attenuated vaccines, subunit vaccines and other potential vaccines, have higher levels of protection, but there are still some problems. In this paper, we briefly review the main vaccines that have been used in controlling the brucellosis for decades and the progress in the development of new brucellosis vaccines.

Contents

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Introduction

Brucellosis is a zoonotic disease caused by *Brucella*, which spreads in more than 170 countries and regions around the world [1]. Brucellosis in human is characterized by a chronic debilitating disease including nonspecific influenza-like symptoms, diaphoresis, and bacteremia among others [2]. Since the late 1980s, the epidemic of brucellosis in some countries and regions are rapidly escalating, it infects more than 60 species of wild animals, forms a worldwide endemic disease and causes large economic losses in animal husbandry [3]. Preventing and treating this disease is imperative, but it has a long way to go. For animals with high economic value and losses after infection, developing countries are not suitable for brucellosis eradication programs [4–6].

Since the beginning of the twentieth century, the research work on the development of vaccines for brucellosis started [7]. The development of brucellosis vaccines has experienced inactivated vaccines, live attenuated vaccines, and rough attenuated vaccines. Inactivated vaccines were used in the early stages for the prevention and control of brucellosis, and have subsequently been replaced by attenuated live vaccines which are more immune-effective [8]. The attenuated live vaccines currently used include S19, Rev.1, S2, SR82 and RB51 [9,10]. RB51 is the only officially approved rough attenuated vaccine. These vaccines are widely used but still have some drawbacks. For example, some of these vaccines can cause human infection and possible abortion complications in pregnant female cattle [11]. Although the existing vaccines have some deficiencies, they have played an essential role in prevention of brucellosis worldwide. With the development of molecular biology techniques and the deep understanding of pathogenic mechanism of *Brucella*, new genetic engineering vaccines are expected to replace traditional vaccines for brucellosis control [12]. In this study, we briefly review the main vaccines that have been used in controlling the brucellosis for decades and the advance in new brucellosis vaccines.

1. Common smooth vaccines

1.1. Brucella abortus vaccine strain S19

The S19 vaccine is the first and widely used vaccine in cattle, and it is the reference vaccine in many countries including India, Argentina, and Brazil [13]. S19 strain was introduced into China in 1958. The formulation of S19 vaccine is whole organism of *B. abortus* strain 19. Adjuvants are not used in S19 vaccine. It was utilized to vaccinate 6-month-old calves and achieved satisfactory results [14]. The S19 vaccine strain initially was a virulent strain isolated from milk of a Jersey cow in the early twentieth century, and the smooth mutant obtained by spontaneously attenuated at room temperature for 1 year [15]. The current used S19 is an erythritol-sensitive S19 strain selected by American scientists. Erythritol (ery) gene contains four open reading frames (ORFs): eryA, eryB, eryC and eryD. The new S19 has a nucleotide deletion of 703 bp which affects the coding regions of eryC (BAB2_0370) and eryD (BAB2_0369), and it has relatively higher safety compared with the old S19 vaccine strains [16]. S19 vaccine can provide better protection in adult bovines, prevent miscarriage, decrease the epidemic in the herds, but its protective effect depends on the age of inoculation, dose, immunization route, and immune status of cattle [9,11]. The report from National Animal Diseases Laboratory of the United States demonstrated that 65% of immunized with S19 were not infected with *Brucella* again, and clinical symptoms such as miscarriage were improved obviously in another 35% of immunized animals [17]. S19 is the smooth *Brucella* strain phenotype, and its LPS still contains O-polysaccharide that can continuously stimulate animal to produce anti-LPS antibodies, which interferes with conventional serological tests between immunized and naturally infected animals. For the improvement of S19 vaccine and maintain its good immunogenicity and genetic stability, it is possible to improve the efficiency and ease of differential diagnosis by adding marker genes. At present, multiple candidates can be used as marker genes, such as bp26 and P39 gene. Experiments showed that the absence of Bp26 or P39 protein does not affect the protection of S19 vaccine in mouse or pregnant cows [18,19]. Although S19 is an officially approved vaccine, which is widely used to prevent brucellosis worldwide, S19 vaccine can still cause orchitis in adult males, prolonged infection, and gravid females may miscarry [20]. Moreover, S19 has a certain degree of virulence to humans [21]. In recent years, there have been many human brucellosis reported cases caused by vaccine production or vaccination around the world [22,23]. In addition, there is little understanding of immune response induced by S19 vaccination in large animals. Definition of immune markers correlated protection will be helpful in the screening of more effective S19. In the future, the recombinant DNA technologies should be utilized to develop a better S19 in terms of safety, efficacy and other desirable characteristics.

1.2. B. melitensis Rev.1 vaccine

The Rev.1 vaccine is a type of streptomycin-resistant smooth *B. melitensis* strain [24]. The formulation of Rev.1 vaccine is whole organism of the live attenuated *B. melitensis* strain derived from a virulent *B. melitensis* isolate. Adjuvants are not used in Rev.1 vaccine. This vaccine was used to prevent animal brucellosis in the 1960s in many countries such as Greece, Jordan, Tajikistan, Mongolia and Spain [25]. In 1990s, Rev.1 vaccine was introduced into China to prevent and control the infection with *B. ovis* [14]. Rev.1 vaccine is innocuous when administered to rams [26]. Rev.1 also seems to be safe in camels since immunized animals did not abort, and no Rev.1 was recovered from udder secretion samples collected from all vaccinated lactating camels [27]. Conjunctival immunization with this vaccine can provide a good level of protection and reduce the probability of miscarriage of ewes. Moreover, vaccine via conjunctival immunization is mainly replicated in the cranial lymph nodes which will little effect on conventional serological diagnosis; therefore, it is helpful for implementing brucellosis Quarantine-Slaughter eradication program [26]. However, Rev.1 vaccine can completely restore virulence which can cause abortions in pregnant animals under appropriate conditions [28,29]. Moreover, Rev.1 strain has a long survival period in animals and may spread horizontally through secretions and milk [30]. Although scientists suggest that the above drawbacks can be overcome via reducing the dose of Rev.1 vaccine, its use is not allowed in those countries where the infection by *B. melitensis* has been eradicated.

1.3. B. melitensis H38 vaccine

B. melitensis H38 Vaccine was originally derived from *B. melitensis* in 1965. The vaccine is an emulsion made by blending with

inactivated H38 strain with emulsifier. Freunds emulsified oil adjuvant was used in H38 vaccine. Although stimulating goats to produce antibody, the vaccine provides poor protection for host and causes local purulence after vaccination [31]. Because it belongs to smooth phenotype, immunization with H38 vaccine interferes with the serological diagnosis to distinguish between vaccinated animals and natural infection [32]. The vaccine is no longer allowed to use for goat vaccination.

1.4. B. melitensis M5 vaccine

The *B. melitensis* M5 Vaccine was developed by Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences in 1962 [33]. This live attenuated vaccine originated from the virulent smooth *B. melitensis* M28 ovine isolate by serial passage in chicken, acriflavine treatment and further passage for 90 generations in chicken embryo fibroblasts. The formulation of M5 vaccine is whole organism of the live attenuated *B. melitensis* M5. Adjuvants are not used in M5 vaccine. M5 can provide good protection for both sheep and goats almost one year via aerosol immunization at a dose of five billion bacteria per animal [33]. It was first used in 1964 on a trial basis. In 1970s, the M5 vaccine was used in large scale vaccination in China to control brucellosis of sheep and goats [33]. Compared with *B. melitensis* M28 and M16, the virulence of M5 vaccine is markedly decreased [34]. However, it still is virulent to humans, and can induce high seroreaction. In China's current vaccines, it is the strongest virulent strain. Moreover, this vaccine is unstable; the mutation of genome often occurs during the stage of cultivation. Another drawback of this vaccine is that it is not able to provide differential diagnosis between infected and vaccinated goat. Therefore, in consideration of the safety, inoculation method, and seroreaction that are induced, M5 vaccine is seldom used in brucellosis epidemic area of China [35]. At present, the related genes that are responsible for virulence and instability of M5 are identified through genomic sequence [36]. We believe that these genes will provide clues to design more effective M5 vaccines in the future.

1.5. B. suis vaccine strain S2

The *B. suis* strain S2 was isolated from the swine aborted fetus by China Institute of Veterinary Drug Control in 1952. After serial passage on media, this smooth strain was naturally attenuated to *B. suis* S2 vaccine strain [37]. The formulation of S2 vaccine is whole organism of the live attenuated *B. suis* strain S2. Adjuvants are not used in S2 vaccine. S2 vaccine can efficaciously prevent swine from *Brucella* infection via oral immunization (drinking water containing S2 vaccine). For susceptible animals, such as cows, sheep and goats, oral immunization with S2 also can provide a satisfactory protection rate [14,38]. Compared with S19 and Rev.1, S2 has the following merits: lower residual virulence, lower cost of production and relative easy oral immunization. Therefore, it has been widely used in China to vaccinate cows, goats, sheep and swine since 1971 [39,40]. However, some studies demonstrated that the protection rate of S2 is lower than that of S19 and Rev.1 in large animals [38,41]. In the mice model of evaluation, scientists found that S2 has less persistence time and short-term immunity vaccines [42]. Another weakness of S2 is that its oral vaccination via drinking water cannot ensure reproducible vaccine doses.

2. Common rough vaccines

2.1. B. abortus RB51 vaccine

The *B. abortus* RB51 vaccine is a spontaneous rough attenuated mutant of *B. abortus* 2308 [43]. The formulation of RB51 vaccine is whole organism of *B. abortus* strain RB51. Adjuvants are not used in RB51 vaccine. The rough characteristic is due to IS711 element insertion disrupting the whoA gene encoding a glycosyl transferase in RB51 strain [44]. Because of the rough feature, immunization of animals with RB51 can be easily differentiated from naturally infected animals, allowing effective vaccination policies. The protection against abortion and infection induced by RB51 is similar to that induced by S19 vaccine [45,46]. Moreover, RB51 is very stable and less virulent for humans than strain S19. Therefore, it is currently used as reference vaccine in many countries instead of S19 [43]. However, RB51 is resistant to rifampicin, which is one of antibiotics in treatment of human brucellosis, and is not able to be detected by routine serological tests [47]. RB51 can infect humans especially for individuals associated with accidental exposure to RB51 by needle stick injury [47]. Thus these phenotypes should be aware during diagnosis and treatment of human brucellosis. Although RB51 can reduce the rate of abortion and infection of cattle, it is not safe for pregnant cattle when immunized with full dose (3.4×10^{10} CFU) [43,45,48]. It seems that there are ongoing extensive efforts focused on the development of better RB51 vaccine.

2.2. B. abortus 45/20 vaccine

The live *B. abortus* 45/20 strain is derived from *B. abortus* smooth virulent strain 45/0. The rough characteristics of *B. abortus* 45/20 were obtained after 20 passages of 45/0 strain in guinea pigs. The formulation of 45/20 vaccine is whole organism of live *B. abortus* strain 45/20. Adjuvants are not used in 45/20 vaccine. Because 45/20 strain is not stable and can revert to the smooth virulent strain when used as a live vaccine [49,50]. *B. abortus* 45/20 vaccine is prepared with heat-killed *B. abortus* 45/20 strain combined with oil adjuvant [51]. The purpose of developing *B. abortus* 45/20 vaccine was to replace S19 because S19 has strong virulence and cannot been easily differentiated from naturally infected animals. However, the immunologic effect of 45/20 vaccine was not steady under field or experimental conditions, and studies show that S19 vaccination is more efficacious than 45/20 vaccine [52,53]. Furthermore, 45/20 vaccine can induce anti-OPS antibodies detectable by routine serologic tests employed in the diagnosis of infected animals [54]. In addition, the oil adjuvant in 45/20 vaccine can lead to severe local reactions at the site of vaccine injection after repeat vaccination [7,55]. The above drawbacks eventually prompted the discontinuation of the use of 45/20 vaccine.

2.3. B. abortus SR82 vaccine

B. abortus SR82 strain (in the smooth-rough form) is a biovar 6 live attenuated vaccine, which was isolated from an aborted bovine fetus in 1961 [10]. The formulation of SR82 vaccine is whole organism of *B. abortus* SR82 strain. Adjuvants are not used in SR82 vaccine. SR82 vaccine has been used by the former Union of Soviet Socialist Republics for bovine brucellosis control since 1974 [10,56]. This strain combined the weak agglutination test responses with comparatively higher efficacy against brucellosis under field conditions [10,56]. Currently SR82 is the most widely and successfully used vaccine in the Russian Federation, Azerbaijan, Tejikistan and other countries in the region [10].Thus far, there are no concerning reports about its safety (Table1).

3. Genetically engineered attenuated vaccines

With the development of homologous recombination (HR) technology, some engineered attenuated strains were made as vaccine candidates. For example, disruption of *per*, *pgm*, *wboA*, and *wbkA*

Table 1
Classical and commercial vaccines.

Vaccine name	Advantages	Disadvantages	Refs.
S19	Higher levels of protection;the reference vaccine (OIE)	Residual virulence; interferes conventional serological tests	[9,11,13,20,21]
Rev.1	Higher levels of protection	Reversion to virulent strain; longer survival period in natural hosts	[26–30]
H38	Stimulating goats to produce antibody	Interferes conventional serological tests; poor protection for goat	[31,32]
M5	Good protection for goats and sheep	Unstable mutation occurs during the stage of cultivation; interferes conventional serological tests	[33,34,36]
S2	Lower residual virulence; easy oral immunization	Lower levels of protection and short-term immunity	[38–42]
RB51	Comparatively safe; good protection; differentiating infected from vaccinated animals (DIVA).	Rifampin resistance	[45–48]
45/20	Better immune protection	Instability; severe local reactions; sero-inteference	[49,50,54,55]
SR82	DIVA; higher levels of protection	No obvious disadvantages were reported till now.	[10,56]

(genes involved in the LPS biosynthesis pathway) results in rough mutants [57–59], which are not only helpful for routine serological diagnosis but for enhancement of protection efficiency. Other mutants, such as disruption of *purL*, *purD*, *purE, bacA, hemH, virB or pgk*, have shown encouraging results by exhibiting a protection level similar to or even higher than RB51 [60–65]. Despite several promising results, trials have not been performed to analyze the protection efficiency of mutants in large animal model. In general, the formulation of these vaccines is whole organism of genetically engineered attenuated *Brucella* spp. Adjuvants are not used in vaccines. Therefore, the definite protective capabilities have not been validated in the target host.

4. Vector-delivered *Brucella* vaccines

Because of the general similarity in infection, some nonpathogenic pathogens can be employed as a vector to deliver Brucella antigen at immunologically critical sites, such as the attenuated Salmonella strain, *Lactococcus lactis* strains, *E. coli* (K12) and Semliki Forest Virus (SFV) [66–69]. The system can provide a platform for the development of safer-effective vaccine candidates. Several *Brucella* proteins, including Omp19, PrpA, BLS or SOD, have been expressed in the *Salmonella* strain [70]. K12 expressing *B. melitensis* BP26 can induce IFN-r production, lymphocyte proliferation and protection against a *B. melitensis* challenge in mice [66]. *Lactococcus lactis* expressing Cu-Zn SOD has also been used as a Vector-delivered *Brucella* vaccine and can induce protection in mice [71]. SFV packed with RNA of *B. abortus* Cu-Zn SOD can induce protection against *B. abortus* challenge in mouse model [67]. However, the levels of protection induced by recombinant vaccinia viruses were not compared with bacteria-derived vectors [72]. The formulation of these vaccines is whole organism of nonpathogenic pathogens, which express the antigen of Brucella spp. Complete Freunds adjuvant, Montanide Gel01 adjuvant or CpG DNA vaccine adjuvant is often used in vaccines. Despite some of vector-delivered *Brucella* vaccine candidates showing very promising results in mice, the duration of the protective immune response and the ideal immunization dose for the vaccines in natural hosts after vaccination have not been explored [73].

5. Subunit vaccines

5.1. Recombinant protein

Recombinant protein can be made at high yield and purity, and not revert to virulent strains like live attenuated vaccines; therefore, the recombinant protein is a promising vaccine candidate. The formulation of these vaccines is recombinant *Brucella* spp. protein. Complete Freunds adjuvant (CFA) or incomplete Freunds adjuvant (IFA) is often used in vaccines. Numerous *Brucella* membrane and cytosolic proteins have been evaluated as protective antigens in mouse models, including the L7/L12 ribosomal protein [74], outer membrane protein 2b (Omp2b) [75], Cu-Zn superoxide dismutase [76], a 22.9-kDa protein [77], lumazine synthase [78], outer membrane protein Omp31 [79], outer membrane lipoproteins Omp16 and Omp19 [80], and nucleoside diphosphate [81]. However, some of recombinant proteins tend to be poor effect of vaccination in vivo compared to S19 vaccine [82]. Although Omp16 and Omp19 have self-adjuvant properties, most of recombinant proteins require the co-administration of adjuvants to enhance the immune response [83,84]. The usage of adjuvants often induces adverse reactions such as local inflammation at the injection site, the formation of granuloma or sterile abscess. Moreover, combining several recombinant proteins has no synergistic effect compared to the use of a single protein except for Omp31-BSL chimera [82]. Therefore, in the future, more extensive effort should focus on the development of new recombinant vaccines, which are composed of more than one *Brucella* antigen and have self-adjuvant properties.

5.2. DNA vaccine

DNA vaccine, namely vaccination with a plasmid expressing a gene coding for a specific antigen, has become an important strategy to develop new vaccines. The formulation of these vaccines is plasmids encoding *Brucella* spp. genes. Adjuvants are not used in vaccines. DNA vaccines encoding the following genes: L7/L12, BLS, BCSP31, Cu/Zn SOD, OMP16, P39 and BAB1_0278, have been identified to confer protection against *Brucella* challenge in mice model [85–88]. Two methods are used to enhance the efficacy of DNA vaccine or to maximize immune response. One method is that multiple antigens are expressed in a DNA vaccine. For example, gene fusion of the L7/L12 and Omp16 induced higher level of protection in mice than DNA vaccines expressing individual antigens [87]. The other method is that cytokines are co-expressed as adjuvants in DNA vaccines to improve immune response. When IL-2 or IL-18 gene was co-expressed with SOD in a DNA vaccine, the protection level was significantly improved compared to a SOD DNA vaccine alone [89,90]. Although DNA vaccines have demonstrated promising results in mouse models, most of DNA vaccines have not been investigated further in natural hosts. The main reason is that DNA vaccines rely on intramuscular immunizations that require large amounts of DNA. However, the use of gene-gun vaccination and nanoparticles, which require less DNA and enhance cellular DNA uptake or the half-life of DNA, could solve this problem [91,92]. Considering the good merits of *Brucella* DNA vaccines: longer expression, better stability, safer vaccination and easier production, the government should encourage scientists to translate

the mouse studies to large animals so that a better, safer and more efficacious subunit vaccine will be manufactured in the future.

5.3. Synthetic peptide vaccines

The highly selective epitope of antigens capable of activating the immune system can be selected as vaccine candidates. The formulation of these vaccines is synthetic peptide. Complete Freunds adjuvant (CFA) or incomplete Freunds adjuvant (IFA) is used in vaccines. It was reported that synthetic peptide (GGAPGEKDGKIVPAG) of *B. abortus* Cu-Zn SOD possessed protective biological activity, which was able to modulate both splenomegaly and the extent of *Brucella* infection in mouse spleen [76,93]. However, in general, this class of vaccine displays relatively low protection. This drawback eventually prompted the interruption of development of the synthetic peptide vaccine.

6. Other potential vaccines

6.1. Brucella ghost vaccine

Brucella ghost vaccine is produced via expression of lysis gene E of X174 phage in bacterial cell, which results in the loss of cytoplasmic contents [94]. The formulation of ghost vaccine is whole organism of *Brucella* spp. without cytoplasmic contents. Adjuvants are not used in this vaccine. It was recently reported that *Brucella suis* S2 ghost vaccine can induce markedly the increase of the production of IgG and T cell responses compared with inactivated bacteria. However, this type of vaccine have obvious defects, such as horizontal transfer of antibiotics resistance gene to wild *Brucella* and the expensive cost use of antibiotics in large scale production [95].

6.2. Nanoparticle vaccine

Nanoparticle vaccine is made through vaccine antigens encapsulated within the nanocarriers or decorated on their surface. The formulation of these vaccines is *Brucella* spp. antigens. Lipopolysaccharide is often used as vaccine adjuvant. Nanocarriers provide a suitable route of administration of antigens and enhance cellular uptake thereby resulting in higher immune responses compared with unconjugated antigens [96]. In addition, nanocarrier can work as adjuvants to generate immune responses in lymphoid organs [96]. There are some studies related to the development of nanoparticle vaccines for brucellosis. The antigens in nanoparticle vaccines mainly come from *Brucella* outer membrane proteins (omp19, 25 and 31), rL7/L12 ribosomal protein, LPS or cytosolic proteins. Preliminary studies have shown that these nanoparticle vaccines could be used for vaccination against brucellosis [97–101]. However, the antibiotics of nanoparticle vaccine for the treatment of brucellosis still need deeply research.

6.3. Vaccine candidates identified via a reverse vaccinology approach

The reverse vaccinology (RV) approach was initially developed by Pizza et al to identify potential pathogenic microbial vaccine based on genomic data [102]. In general, the development of vaccine using RV approach contains the following steps: (1) The selected genomes through computational tools and online databases are analyzed to identify potential immunogenic epitopes that can induce an immune response. (2) Gene cloning, antigenic peptides or proteins of interest are synthesized or produced. (3) The immunogenicity and the protective efficacy of the recombinant antigens are tested in laboratory animal models [103]. The formulation of these vaccines is *Brucella* spp. antigens. ISCOMATRIX is often used as vaccine adjuvant. Three studies have applied RV to discover *Brucella* protective antigens through bioinformatics analysis of genome sequences and proteome [104–106]. Studies to assess immunogenicity and protective efficacy of individual vaccine candidates in the mouse are currently underway. Although much progress in identification of *Brucella* vaccines has been made through RV approach, many challenges still exist. For example, while different *Brucella* gene expression profiles have been discovered at different experimental conditions, how to use these genes to design vaccine remains a challenge (Table 2).

Table 2
Trial vaccines for *Brucella*.

Vaccine name	Advantages	Disadvantages	Refs.
Engineered vaccines	Controlled attenuation; DIVA	The levels of protection need to be further confirmed in the natural hosts.	[58–65]
Vector-delivered vaccines	Higher levels of protection; DIVA	Multiple boosters; costly to produce	[66–73]
Subunit vaccines	High safe; no residual virulence; DIVA	Multiple boosters; low level of protection; costly to produce	[82–93]
Other potential vaccines	High safe; no residual virulence; DIVA	The levels of protection need to be further confirmed in the natural hosts	[94–101]

A comprehensive program, Vaxign, was designed by He et al to promote the *Brucella* vaccine development through reverse vaccinology approach [107,108]. This program comprised of a computational pipeline that utilized bioinformatics technology to find potential genes from the genomes for developing vaccines. The major predicted features included transmembrane domain, subcellular location of proteins, adhesion probability, sequence similarity to host proteome and MHC class I and II epitope binding. Among these features, subcellular localization was considered as one of the main criteria for target prediction. Based on the Vaxign program, 32 proteins were identified as vaccine targets from *Brucella* genomes [109].

Ontology-based analysis tools of *Brucella*, such as a Brucellosis Ontology (IDOBRU) and the vaccine ontology (VO) which can be used to support *Brucella* and brucellosis data exchange and data integration, also can provide support toward the development of a safe and effective *Brucella* vaccines [110,111].

7. Vaccines in clinical trials

Significant protective activity has been identified against *Brucella* using the following vaccines in natural hosts: Influenza viral vector *B. abortus* vaccine [112–114], *B. abortus* DNA vaccines encoding Bp 26 and trigger factor [115], Influenza viral vector *B. abortus* vaccine (Flu-BA) [116], Influenza A subtype H5N1 or H1N1 vector vaccine delivery of recombinant *Brucella* L7/L12 and Omp16 protein [117], *Salmonella* Typhimurium delivery-based combination *Brucella* antigen vaccine [118], *B. abortus* DNA vaccine encoding Ag85B, MPT64, MPT83, BCSP31, SOD, and L7/L12 [119], *B. abortus* DNA and RNA vaccines encoding *Brucella* Cu–Zn SOD gene [120], *B. ovis* particulate acellular vaccines containing outer membrane proteins and rough lipopolysaccharide (R-LPS) [97] (Table 3). However, since present government regulatory agencies, especially in endemic developing countries, are resistant to work with other vaccines than current commercial vaccines [121], license of these vaccine candidates are not obtained.

Table 3
Vaccines in clinical trials.

Vaccine name (protein, DNA)	Adjuvant	Challenge (*Brucella* species)	Refs.
Influenza viral vector *Brucella* abortus vaccine (Flu-BA) (L7/L12 or Omp16)	Montanide Gel01 adjuvant	*B. melitensisB. abortus*	[112,113]
B. abortus DNA vaccines encoding Bp 26 and trigger factor (TF)	CpG DNA vaccine adjuvant	*B. abortus*	[115]
Influenza viral vector *Brucella* abortus vaccine (Flu-BA) (Omp19 and SOD)	Montanide Gel01 adjuvant	*B. melitensis*	[116]
Influenza A subtype H5N1 or H1N1 vector vaccine delivery of recombinant *Brucella* L7/L12 and Omp16 protein	Montanide Gel01 or chitosan adjuvant	*B. abortus*	[117]
Salmonella Typhimurium delivery-based combination *Brucella* antigen vaccine (BLS, Omp19, PrpA, and SOD)	None	*B. abortus*	[118]
B. abortus DNA vaccine encoding Ag85B, MPT64, MPT83, BCSP31, SOD, and L7/L12	None	*B. abortus*	[119]
B. abortus DNA and RNA vaccines encoding *Brucella* Cu–Zn SOD gene	None	*B. abortus*	[120]
B. ovis particulate acellular vaccines containing outer membrane proteins and rough lipopolysaccharide (R-LPS)	Nanoparticles	*B. ovis*	[97]

8. Conclusions and prospective

Vaccination is a critical strategy for brucellosis control and eradication programs. However, due to some side effects shown by these smooth and rough vaccines, there are ongoing extensive efforts focused on the development of new and better vaccines. Considering that *Brucella* genome has been sequenced, plus the advances in recombinant DNA technology and bioinformatics, engineered smooth and rough vaccines have the potential to be the future of brucellosis control, but additional studies are still needed to do in terms of safety, efficacy and other desirable characteristics. Although the excellent results from some of subunit vaccines and other new types of vaccines were observed in mouse models, very few of these candidate vaccines have been evaluated in the target species. Moreover, they have the following obvious weaknesses: requirement of multiple boosters, adjuvants, and optimal combination of antigens and inducing poor cellular immune response. Therefore, the development of better vaccines has a long way to go.

Another barrier to develop novel vaccines is that present government regulatory agencies in endemic countries (such as China and other developing countries) are resistant to work with new vaccines than currently used vaccines. However, we believe that regulatory agencies will change their minds if the safety, efficacy and other desirable characteristics of these vaccines are shown to be enhanced significantly compared to attenuated vaccines in use. We anticipate that there will be several laboratories worldwide testing new brucellosis vaccines in the near future.

Declaration of Competing Interest

The authors declare no competing of interest.

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