Review
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Development and trial of vaccines against *Brucella*

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The search for ideal brucellosis vaccines remains active today. Currently, no licensed human or canine anti-brucellosis vaccines are available. In bovines, the most successful vaccine (S19) is only used in calves, as adult vaccination results in orchitis in male, prolonged infection, and possible abortion complications in pregnant female cattle. Another widely deployed vaccine (RB51) has a low protective efficacy. An ideal vaccine should exhibit a safe profile as well as enhance protective efficacy. However, currently available vaccines exhibit one or more major drawbacks. Smooth live attenuated vaccines suffer shortcomings such as residual virulence and serodiagnostic interference. Inactivated vaccines, in general, confer relatively low levels of protection. Recent developments to improve brucellosis vaccines include generation of knockout mutants by targeting genes involved in metabolism, virulence, and the lipopolysaccharide synthesis pathway, as well as generation of DNA vaccines, mucosal vaccines, and live vectored vaccines, have all produced varying degrees of success. Herein, we briefly review the bacteriology, pathogenesis, immunological implications, candidate vaccines, vaccinations, and models related to *Brucella*.

Keywords: *Brucella*, animal models, brucellosis, vaccines

Introduction

Recently, brucellosis has garnered increased importance due to its zoonotic potential and possible use in bio-warfare. Though the disease-causal agent relationship was established in the 19th century [14], it never reached the center of attention in the medical field. In 2009, the World Health Organization's, third International Conference on Neglected Zoonotic Diseases assessed and acknowledged initiative works into the burden of key diseases such as bovine tuberculosis, brucellosis, toxoplasmosis, and zoonotic schistosomiasis [6]. Brucellosis is a sub-acute or chronic disease, and in cattle, sheep, goats, other ruminants, and pigs the initial phase following infection is often not apparent [29]. Recently recognized *Brucella* types associated with disease in marine animals may also have the capacity to cause disease in humans [29,72].

Control of zoonotic diseases in human populations has relied heavily on the control of animal disease. Over the last century, human brucellosis has been controlled by vaccination and culling within cattle, goat, and sheep herds [28]. Yet, despite past and current efforts to eradicate brucellosis many human cases are reported annually worldwide. There is a direct link between the disease and the economic status of a country [78]. It was the opinion of experts that resulted in the suggestion that vaccination efforts alone are insufficient to effectively control the disease. Still, the efficacy of vaccination-only strategies has not been seriously evaluated, and it depends in large part on the quality of the vaccine employed [28]. Since, brucellosis is closely tied to economic stature, improving livelihood would also alleviate disease burden and transmission in endemic areas.

Due to importance of controlling brucellosis in the animal population, *Brucella abortus* S19 and *Brucella melitensis* Rev. 1 vaccines have been widely used in certain developed countries, but both the vaccines induce abortions in pregnant animals and are virulent for humans; moreover, they elicit anti-*Brucella* antibodies that interfere with serodiagnosis. Further, Rev. 1 is streptomycin resistant, an important antibiotic used to treat the disease. However, the residual virulence issue can be solved by using further attenuated mutant strains, and the serodiagnosis problem can be solved by using lipopolysaccharide (LPS)-disrupted mutant strains, immunization via the conjunctival route, avoidance of adult vaccination, and by an individual serological follow up. Hence, a vaccine embodying all such prerequisite properties would be a boon to brucellosis control and a major breakthrough [70,85].

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Understanding *Brucella* for Strategic Development of Vaccine

Brucella organism

Interestingly, *Brucella* organisms are very closely related to plant microbes. It is remarkable how the proto-*Brucella* may jump between the two kingdoms, from plant to animal. *Brucellae* are classified under α-*Proteobacteria* phylogenetically, which are related to plant pathogens and symbionts such as *Rhizobium*, *Agrobacterium*, and an animal endosymbiont *Wolbachia*, to intracellular animal parasites such as *Bartonella* and *Rickettsia*, and to opportunistic and free-living bacteria like *Ochrobactrum* and *Caulobacter* [32,69]. Accurate identification of *Brucellae* and differentiating them from closely related opportunistic *Ochrobactrum* is important for accurate diagnosis [83]. It is not well understood whether pre-existing anti-*Ochrobactrum* immunity may hinder successful vaccination against *Brucella*. *Brucellae* are gram-negative, non-sporulating, capnophilic, facultative intracellular bacteria [28].

Brucella genetic composition

With the increased availability of the *Brucella* whole genome sequence, a wide variety of genetic modification studies related to microbiological and vaccinology have been undertaken. The type strains and other representative strains of the six species of *Brucella* form a very tight cluster, with G + C values between 57.9% and 59.2%, DNA homologies close to 100%, T_m values between 79.2°C and 80.5°C, and rRNA binding values between 0.166% and 0.198% [32]. *Brucella* species show a considerably high degree of homology, except for *B. ovis* which has been reported to show slight restriction endonuclease digestion pattern differences from other *Brucellae* [73]. The vaccine strain S19 genome is 3.2 Mbp and comprises two circular chromosomes: one 2122487 bp long and the other 1161449 bp long. The average G + C content of the two chromosomes of S19 strain is 57% [30]. It was shown that the S19 genome has remarkable similarity in size and structure to those of its virulent relatives, *B. abortus* strains 9−941 and 2308 [22,49]. The S19 genome sequence shows over 99.5% similarity to the genomes of strains 9−941 and 2308 [30].

Brucella host interaction and pathogenesis

Understanding host-pathogen interactions and pathogenesis helps in developing rational vaccine designs. Like other pathogenic intracellular bacteria, *Brucella* infection requires the four following steps: adherence, invasion, establishment, and dissemination within the host [58]. The most striking characteristics of *Brucella* infection is that it operates in "stealth mode", *i.e.*, it can evade immune detection [9,61]. This mode of infection eventually leads to chronicity and prolonged infection [9,31]. Earlier ultrastructural work characterizing the morphology of *B. abortus*-infected cells revealed that *Brucellae* multiply in an intracellular multimembranous compartment that has similarities to the rough endoplasmic reticulum [5]. Weak interactions between host and *Brucella* LPS probably has a substantial role in intracellular survival [17]. The chronicity of brucellosis is multifactorial, utilizing both the ability of *Brucella* to evade immune detection and to adapt to intracellular survival inside both phagocytic and nonphagocytic cells [31,80]. The inhibition of the phagosomal-lysosomal maturation pathway and the deviation of intracellular trafficking have important roles; in this scheme, the bacterium reaches its endoplasmic reticulum-derived replicative niche [20].

Brucella virulency with special reference to LPS

Unlike most common bacterial pathogens, *Brucella* species do not produce exotoxins, appendages such as flagella, pili, or fimbriae, antiphagocytic capsules or thick cell walls, resistant forms, and do not exhibit antigenic variation [39]. The success of invasion is due to the inability of the host's immune system to effectively detect or clear the bacteria, in this way the bacteria gain entry to its replicative niche and thus proliferate within professional and nonprofessional phagocytic host cells [18,80]. The successful evasion of host immunity is attributed to the LPS of the organism. *Brucella* possesses a peculiar nonclassical LPS that does not exhibit strong endotoxic activities similar to those of classical LPSs from enterobacteria such as *Escherichia coli* [56]. Further, LPS is recognized as a major virulence determinant as naturally occurring isolates lacking LPS showed reduced survival inside the host system.

Brucella LPS is comprised of lipid A, core oligosaccharide, and O-polysaccharide (OPS). Lipid A contains 2, 3-diamino-2, 3-dideoxy-D-glucose (diaminoglucose) as backbone, amide, and ester-linked long chain saturated and hydroxylated fatty acids. The core oligosaccharide is composed of mannose, glucose, 2-amino-2, 6-dideoxy-D-glucose (quinovosamine), 2-amino-2-deoxy-D-glucose (glucosamine), 3-deoxy-D-manno-2-octulosonic acid, and unidentified sugars. The OPS is an unbranched homopolymer (n = 96−100) of 1, 2-linked 4, 6-dideoxy-4-formamido-α-D-mannopyranosyl [16,69]. In murine macrophages, it has been demonstrated that OPS is specifically involved in inhibition of early fusion between *Brucella suis* containing-phagosomes and lysosomes In contrast, phagosomes containing rough mutants, which fail to express the O antigen, rapidly fuse with lysosomes [81]. Due to its central role in virulency, much research has been undertaken into the disruption of LPS biosynthesis. The aim of that research is to generate a mutant vaccine strain that has a perfect balance between attenuation and protection, and also enables differentiating infected from vaccinated animals (DIVA) [54,102].

Apart from the LPS involvement in virulence, the *vir*B operon, encoding a type IV secretion system homologous to those encoded by *Agrobacterium tumefaciens vir*B, and the *Bordetella pertussis ptl* operon are present in the *Brucella*

genome [86]. The *A. tumefaciens vir*B operon encodes a pilus-like structure necessary for secretion of transfer DNA, and the *B. pertussis ptl* operon encodes an apparatus that allows the secretion of pertussis toxin, suggesting that *Brucella* may secrete regulatory DNA or protein for intracellular survival. Superoxide dismutase (SOD) forms part of the antioxidant defense system that protects bacteria from the toxic effects of reactive oxygen intermediates by converting superoxide radicals into hydrogen peroxide and oxygen [40], suggesting that SOD has a crucial role in *Brucella* intracellular survival. As anticipated, anti-*Brucella* SOD-based vaccines confer substantial amounts of protection [23,52,93].

Brucella interaction with host immunity

During the initial phase of infection, approximately 90% of bacteria are killed by phagocytic cells. Dendritic cells and macrophages have important roles in clearing the infection; however, failure to lyse the organism within phagosomes leads to formation of *Brucella*-containing vacuoles and, thus, replication in this niche [61]. *Brucella* can also subvert the autophagy process in order to evade efficient clearing [48,90]. Neutrophils may have an important role in subsiding the infection via phagocytosis, as *Brucella* does not replicate within neutrophils, but it does resist neutrophil-mediated bacterial killing [9].

Specific antibodies have important roles in reducing the initial phase of a *Brucella* infection; however, they have limited roles following intracellular localization. Hence, strong humoral immunity unaccompanied by cell-mediated immunity (CMI) cannot provide total protection against the *Brucella* organism. B lymphocytes may act in favor of the organism by providing a replication niche [43] or B cells may produce interleukin (IL)-10 cytokine thereby antagonizing the production of IL-12 and interferon-γ, which are important for orchestrating the CMI response. It has been demonstrated that mice deficient in B cells are highly resistant to *Brucella* infection [44]. Several researchers have demonstrated the importance of Th1 immune response in controlling *Brucella* infection, as well as the involvement of IL-12 and INF-γ [11,61]. Hence, it is prudent to design vaccines that can augment the Th1 response while reducing the Th2 response.

Approaches for Development of Anti-*Brucella* Vaccines

The general considerations associated with, and a summary of the different classes of, *Brucella* vaccines are listed in Table 1.

Classical and commercial vaccine

The most widely used vaccine for the prevention of brucellosis in cattle is the *B. abortus* S19 vaccine, which remains the reference vaccine to which other vaccines are compared. It is used as a live vaccine and is normally given to female calves aged between 3 and 6 months as a single subcutaneous dose of $5–8 \times 10^{10}$ viable organisms or as a reduced dose of from 3×10^8 to 3×10^9 organisms that can be administered subcutaneously to adult cattle. Alternatively, it can be administered to cattle of any age as either one or two doses of 5×10^9 viable organisms, given via the conjunctival route [50]. *B. abortus* S19 has the normal properties of the biovar 1 strain of *B. abortus*, but it does not require CO_2 for growth, does not grow in the presence of benzylpenicillin (3 μg/mL = 5 IU/mL), thionin blue (2 μg/mL), or i-erythritol (1 mg/mL) (all final concentrations), and presents with high L-glutamate use [50].

B. abortus strain 45/20 is a rough strain that is able to protect guinea pigs and cattle from *Brucella* infection; however, reversions to the wild smooth type has limited its use as a live

Table 1. Summary of *Brucella* vaccine and their properties

Vaccine	General properties and remarks
Live	1) S19, Rev. 1: high levels of protection; residual virulence; serointerference; reports of human infections; not suitable for human use; severe local reactions in actively infected individuals 2) RB51, Knock-out; differentiating infected from vaccinated animals (DIVA); comparatively safe; varying level of protection
Inactive cell lysate	Highly safe; no residual virulence; low level of protection; requires multiple boosters
Subunit	Highly safe; no residual virulence; low level of protection; requires multiple boosters; DIVA; a cross-protecting platform; suitable for human use
DNA	Highly safe; no residual virulence; low level of protection; requires multiple boosters; requires prime boosting; DIVA; a cross-protecting platform
Synthetic peptide	Highly safe; highly optimizable; no residual virulence; low level of protection; requires multiple boosters; DIVA; a cross-protecting platform; suitable for human use
Live vectored	Safe; customizable; no residual virulence; DIVA; a cross-protecting platform; varying level of protection; suitable for human use

vaccine [62,97].

B. abortus strain RB51, a rough attenuated organism, was originally derived from a rifampicin-resistant mutant of *B. abortus* strain 2308 and has replaced *B. abortus* S19 strain as a vaccine candidate in some developed countries. Strain RB51 is very stable and has no, or highly reduced, abortifacient characteristics [84]. Protective efficacy and immunity induced by strain RB51 is similar to or better than that induced by strain 19 [24,59]. However, although strain RB51 has an excellent record of stability, it is rifampicin resistant to important antibiotics used in the treatment of brucellosis; moreover, it is still infectious to humans and the exact nature of its mutations have not been described. Recently, it was reported that RB51 vaccinated cattle in the Greater Yellowstone Area in the USA were still susceptible to brucellosis [76,103].

Genetically modified *Brucella* mutant strains

To counteract the disadvantageous properties of the smooth *Brucella* vaccines, research attention had been focused on the development of rough phenotype vaccines with greater attenuation. Rough *Brucella* mutants lack the LPS immunodominant N-formylperosamine OPS and are substantially attenuated [70,85]. Some rough (R) vaccines or candidates are spontaneous mutants selected after repeated passage on antibiotic-containing media. Disruption of *per*, *pgm*, *wboA*, and *wbkA* (genes involved in the LPS biosynthesis pathway) results in rough mutants [67,101,108], showing that empirically, R vaccines can be improved. Other notable mutants that have metabolic genes disrupted are the *purL*, *purD*, and *purE* mutants that affect the purine biosynthesis pathway genes [1], the lipid A fatty acid transporting gene *bacA* mutant [38,57], the ferrochelatase *hemH* mutant [3], the type IV secretion *virB* mutant [34], and the phosphoglycerate kinase encoding gene *pgk* mutant [99]. Many mutants have shown promising results by exhibiting a protection level similar to or higher than RB51. In addition, the rough strains are DIVA enabled. Despite several encouraging results, trials have not been undertaken to evaluate the protective capabilities of these mutants in the target host. Thus, conclusive data and substantial definitive host-vaccine efficacy findings have not been validated. Also due to variations in evaluation protocols, accurate comparisons between the mutants have not been possible [45,110].

Inactivated vaccines

Cell fractions and lysate: Cell components from the killed organism were tested during the early development of a *Brucella* vaccine. Preparations that were evaluated included outer membrane protein [68], soluble and insoluble extracts of cell envelopes [36,107], whole killed cells [10], and periplasmic proteins and salt extractable proteins [94]. While infection-related issues were nil, several issues such as poor protection, local reactions, and serological problems hindered their popularity [64]. The local reactions were generally induced by adjuvants which were used to improve protective efficacy.

Subunit and DNA vaccine: Due to safety implications, several subunit vaccines have been evaluated. In addition, subunit vaccines have the advantage of being effective for pan-*Brucella* species because high homology protein can be selected as the candidate. Several subunit/DNA vaccine candidates have been evaluated with the central aim of evoking a Th1 response. These candidates include recombinant P39, bacterioferritin, L7/L12 [2,75], lumazine synthase [104], Bp26 and trigger factor together [109], InfC [21], L7/L12 [53], Omp16 and Omp19 [79], Omp25 [27], Omp28 [51], Omp31 [19], P39 [2], S-adenosyl-l-homocysteine hydrolase [111], DnaK and SurA [33], and SodC [71]. It has been demonstrated that subunit vaccines can be improved by including encapsulations such as escheriosomes and liposomes or via fortifying with IL-18 cytokines [60,88]. The major disadvantage of these vaccines is their relatively low protection and the requirement for several booster doses or prime boosting. Further studies are required to assess the efficacy of these candidate antigens in livestock [110].

Synthetic peptide vaccine: In 1994, Tabatabai and Pugh [93] synthesized three peptides derived from the primary structure of *B. abortus* Cu-Zn SOD and used those peptides in vaccines against brucellosis. Their studies showed that only peptide 3 (GGAPGEKDGKIVPAG) possessed protective biological activity, which was demonstrated by its ability to modulate both splenomegaly and the extent of *Brucella* infection in spleen [91]. It was concluded that peptide 3 probably contains a specific sequence preferentially recognized by the cellular immune system. Hence, this study showed that a highly selective epitope capable of activating T cells can be selected and synthesized accordingly. However, in general, this class of vaccine displays relatively low protection, which would hinder its effective deployment.

Vector-delivered *Brucella* vaccines: Due to its overall similarity in infection, an intracellular pathogen such as an attenuated *Salmonella* strain can be employed as a vector to deliver *Brucella* antigen at immunologically critical sites. It has been reported that oral immunization of mice with *Salmonella* expressing a 31 kDa *Brucella* protein resulted in the production of local as well as serum antibodies against the protein but resulted in poor CMI responses [89]. Recently, our group developed *Salmonella* Typhimurium delivery-based *Brucella* vaccines. Using a mouse model, the suitability of the vectored *Brucella* vaccine in different routes of immunization was studied [52]. To improve the protective efficacy, we also investigated the usefulness of these vectored vaccines when provided as a "cocktail". The cocktail of *Salmonella* delivering heterologous antigens of *Brucella* included SOD, BLS, PrpA, and Omp19 proteins. To further improve the vaccine's efficacy, purified LPS was included in the cocktail [55]. Single dose

immunization results were at par with the reference RB51 vaccination. Food-grade genetically modified *Lactococcus lactis* strains that express *B. abortus* GroEL heat-shock protein have been developed [66]; however, protective efficacies against wild-type challenges were not evaluated. It was reported that several recombinant vaccinia viruses were able to express a variety of *Brucella* antigens (HtrA, GroEL, GroES, Cu-ZnSOD, and YajC), and that study revealed that the recombinant vaccinia viruses induced specific immune responses to these antigens in mice, but the levels of protection were not significant [98].

Vaccine Trials for *Brucella*

A general overview and summary of the different types of trial hosts are presented in Table 2.

Laboratory animals

Due to economical and ethical concerns associated with experimentation in hosts such as ruminants, humans, and other primates, various small laboratory animal species serve as important tools for investigating the pathophysiology and undertaking vaccine-related trials of brucellosis [46]. Many foremost experiments related to *Brucella* involve the use of chicken embryo as a model [15]. Rabbit has mostly served as an animal for producing antibodies against *Brucella* antigens as well as a model for *Brucella* toxicity and hypersensitivity, mainly due of the susceptibility of rabbit to bacterial endotoxins and toxins [4,35]. However, mouse (*Mus musculus*) has been the most convenient and commonly used brucellosis model [46,87]. For World Organisation for Animal Health (OIE) vaccine batch potency testing and residual virulence evaluation, mice are routinely used. CD1 mice at 5 to 6 weeks of age are used for screening of candidate vaccines. Due to ease of handling, availability, and presence of large amounts of information and literature, mice are often the laboratory animal of choice [46]. The efficacy of a *Brucella* candidate vaccine is usually measured as a reduction in splenic colonization as compared to that in non-vaccinated but challenged controls. However, due to matching phases and pathophysiology, a guinea pig model serves as a better model than mouse for human-related anti-*Brucella* vaccine studies [87]. Guinea pigs exhibit similar pulmonary, hepatic, spleen, and genital lesions and have similar hypersensitivity reactions to those observed in humans; moreover, they exhibit comparative stages of infection caused by *Brucella* in natural hosts, including abortion [12,13,41]. In addition, guinea pigs exhibit the highest susceptibility to *Brucella* infections among the tested laboratory animals [41]. Hence, guinea pig represents one of the best models for several immunological and vaccine studies [46,50,74].

Livestock

Large animal experimentation and trials involving anti-*Brucella* vaccine are comparatively uncommon due to limited availability of resources. However, it is critical to characterize the equivalent immune responses in livestock hosts. Such information will provide a better understanding of the safety and efficacy of the vaccine. Brucellosis in cattle is usually caused by biovars of *B. abortus*. In some countries, particularly in southern Europe and western Asia, where cattle are kept in

Table 2. Summary of hosts in vaccine development and trials

Host	Remarks
Mice	Most widely used model; useful for initial screening of a large number of vaccine candidates; availability of large datasets related to immunological and safety assays; screening model for vaccine batch; persistence in spleen is a useful virulence/attenuation index
Guinea pigs	Shows highest susceptibility to *Brucella* infections among laboratory animals; also shows infection similarities to those producing human pathology; produces pulmonary, hepatic, spleen and genital lesions and the hypersensitivity reactions similar to those observed in humans; exhibits the different phases of infection; one of the best models for use in immunological and vaccine studies
Companion animals	Limited use due to ethical concerns; requires final host trials; *Brucella canis* is the major causal agent and a rough strain *B. canis* vaccine is required; due to close proximity to humans, companion animals should be kept free of *Brucella*
Livestock	Limited use due to economic concerns; requires final host trials; vaccine capable of reducing zoonotic transmissions and abortions and being differentiating infected from vaccinated animals (DIVA) enabled are of prime importance
Human	Limited information related to *Brucella* vaccine trials; *B. melitensis* is the most common causal agent, however, other *Brucella* species may also be involved; *Brucella* human transmission is controlled indirectly via control of brucellosis in animal populations; Safe, human exclusive vaccines are required for effective deployment

close association with sheep or goats, infection can also be caused by *B. melitensis*. In small ruminants, brucellosis is mainly due to *B. melitensis* [42]. Such brucellosis is mainly controlled by vaccination with the vaccine strain Rev. 1. This strain confers excellent immunity; however, it has two disadvantages: i) prolonged sensitization of animals resulting in interference with subsequent allergic tests; ii) formation of anti-Rev. 1 antibodies, which disappear at different rates among individual animals [37]. Occasionally, *B. suis* may produce a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion or spread to other animals [50]. Considering the disease's transmission cycle and zoonotic implications, livestock animals may be more suitably vaccinated with multivalent pan-*Brucella* species cross-protecting vaccines.

Companion animals

Brucella canis is important pathogen resulting in brucellosis in dogs, and, to date, no effective anti-*B. canis* vaccine is available. Very limited research has been initiated into this type of anti-*Brucella* vaccine [25,26,82]. The site of injection and the type of adjuvant to be used needs to be determined before initiating vaccine trials [63]. Vaccines containing recombinant antigens may be less reactogenic and also less immunogenic, thus necessitating the inclusion of an adjuvant [26].

Other animals

Brucellosis has been reported in the one-humped camel (*Camelus dromedarius*), in the two-humped camel (*Camelus bactrianus*), and in South American camelids including llama (*Lama glama*), alpaca (*Lama pacos*), guanaco (*Lama guinicoe*), and vicuna (*Vicugne vicugne*). Those infections have been related to contact with large and small ruminants infected with *B. abortus* or *B. melitensis* [50,106]. In addition, brucellosis has been observed in the domestic buffalo (*Bubalus bubalus*), American and European bison (*Bison bison*, *Bison bonasus*), yak (*Bos grunniens*), elk/wapiti (*Cervus elaphus*), the African buffalo (*Syncerus caffer*), and various African antelope species. The clinical manifestations of brucellosis in these animals are similar to those in cattle [50]. However, it is uncertain whether current vaccine candidates and immunization strategies would be suitable application in feral populations of those species. Rough strain RB51 has been tested for use in wild animals, such as bison in North America, and it conferred considerable protection in test subjects; moreover, the level of protection was improved with a booster vaccine regime [76,77].

Human

To date, no vaccine licensed for human anti-*Brucella* is available. In addition, there is very little information and relevant data regarding human clinical vaccine trials. Although the threat due to bioterrorism has recently toned down [47], developing a safe and effective vaccine is of prime importance due to the chronic debilitating nature of brucellosis. The most extensive trial and study was held in the former Soviet Union in which over 3 million people were vaccinated with the S19 vaccine strain [105]. Most human brucellosis cases have been linked with infected sheep and goats [28]. There was a nearly 60% reduction in the number of human cases over the period 1952 to 1958. Despite conferring strong protection due to the prolonged persistency in vaccinated individuals [105], the deployment of S19 in humans has been limited.

Several candidate anti-*Brucella* vaccines have been proposed for the use against human brucellosis. Since *B. melitensis* is the most common species found in humans, development of a vaccine based on *B. melitensis* is prudent. Several candidate vaccines are reported to be capable of protecting against a virulent *B. melitensis* challenge in mice model [7,8,95]. However, validation in a primate model may be necessary prior to undertaking large scale trials. Success stories involving the control of human brucellosis via its control in an animal population have been documented in different parts of the world [65,78]. Nevertheless, with the increasing reports and re-emergence of brucellosis around the globe [92,96,100], research into developing safe, effective, cross-protecting, human exclusive vaccines must be continued.

Conclusions

The most effective strategy to control human brucellosis in the absence of an appropriate human vaccine is to control brucellosis in animal populations; thereby reducing zoonotic transmission and the number of carrier hosts. For human anti-*Brucella* vaccine, safety properties should be considered of prime importance, and, ideally, the vaccine should be cross protecting. Vaccine formulation comprising a cocktail of protective antigens may be used in conjunction with an appropriate adjuvant to augment the vaccine's protective capabilities. Newer immuno-modulators such as viability-associated pathogen-associated molecular patterns may also be included in such formulations in order to mimic live bacterial infections. A major challenge in the development of an ideal vaccine lies in evoking robust CMI in the host. In general, vaccine candidates that evoke a strong CMI response confer a better level of protection. Hence, targeting the CMI branch of host immunity via induction of IL-12 and INF-γ should prove to be useful.

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Conflict of Interest

The authors declare no conflicts of interest.

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