Strategies for the development of an effective vaccine against Brucellosis

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Abstract: Brucellosis is a notorious zoonotic disease with global implications. Efforts to control the spread of the disease have been restricted to the agricultural livestock. Increasing incidences of accidental human infection have motivated researches to start working on alternative vaccines. At present, live attenuated vaccines are the only accepted type of vaccines used in developed countries for the prevention of brucellosis. Although serodiagnosis is occasionally unreliable, some countries have already claimed to have eradicated the disease, based on this testing. Live attenuated vaccines are not suitable for use in pregnant and immune-depressed animals. Moreover, these vaccines are not tolerated in humans. Therefore, many researches have been striving to discover alternative methods of vaccination. Most research has focused on the generation of subcellular, subunit, and DNA vaccines that are as efficient as the live attenuated vaccines. At present, none of the available vaccines has been able to replace the live attenuated vaccines. Therefore, additional research is necessary in order to discover a new brucellosis vaccine that is suitable for human use.

Key words: Brucella, zoonosis, live vaccine, animals

INTRODUCTION

Brucellosis is a notorious zoonotic disease that accounts for nearly 500,000 human infections every year around the world [16]. This disease is especially concerning because it induces abortions and causes sterility in the affected livestock, and causes major financial losses to the producers and consumers alike [16, 38]. Brucellosis is transmitted to humans through direct contact with infected animals and animal products [21]. It manifests itself as a systemic infection, causing undulant fever, endocarditis, and arthritis; diseases that require a lengthy and exorbitant antibiotic therapy in humans. Four of the species that are pathogenic to humans (in increasing order of severity) include Brucella canis, B. suis, B. abortus and B. melitensis, respectively [43].

The Brucella species comprises gram-negative, aerobic, and facultative intracellular bacteria (coccobacilli) that invade phagocytic cells including macrophages, placental trophoblasts, and epithelial cells [28, 34]. A key factor in the pathogenicity of the Brucella species is its surreptitious invasion of the professional and non-professional phagocytes; a process not easily detected by the primary host immune defenses. This invasion is made possible by the presence of pathogen-associated molecular patterns in the cell envelopes (particularly in the lipopolysaccharides and lipoproteins), which circumvent phagocytes and bactericidal effects, thereby establishing a niche conducive to pathogen survival and replication [29, 30]. This often results into chronic infection [31]. Vaccination is still the most effective approach in controlling Brucella infection, and the scientific community is striving relentlessly to develop new and effective vaccines for human and animal use [22]. However, effective vaccines to prevent brucellosis in humans are not yet available [29]. The effective control of infection in animals has limited the need for the development of a human brucellosis vaccine because of the relatively rare occurrences of infection in humans [20]. A better understanding of the diverse and extensive host-pathogen interaction is required in order to find new approaches for vaccine development [22]. It is evident that cell mediated responses are responsible for deparing intracellular infections, although humoral immunity is also similarly involved. The above-mentioned processes are always triggered by antigens.

B. abortus strain 19 was the first effective vaccine de-
veloped to combat Brucella infections. It is a live virulent strain that was attenuated after a year of being kept in the laboratory [48]. The major disadvantage of this vaccine is its inability to differentiate between infected and vaccinated animals, owing to the fact that it produces powerful antibodies against the O-side chain. B. melitensis Rev 1 is yet another live attenuated vaccine derived from B. melitensis. The administration of this vaccine during pregnancy, however, can result in spontaneous abortion, and this occurrence is usually dose-dependent. Modified live vaccines have been shown to stimulate cell-mediated immunity, thereby providing significant protection against the disease. However, the major drawbacks cannot be underestimated. The levels of antibodies mounted secondary to the vaccine interfere with the serologic diagnostic tests [48].

B. abortus strain RB51 was developed to address the serologic interference from the virulent strain 2308. RB51 is a rough mutant that does not produce antibodies against the O-polysaccharide chain of smooth lipopolysaccharides, thus making it possible to differentiate between infected and vaccinated animals [53]. Killed vaccines were also investigated, but the protection efficacies of live attenuated vaccines were found to be comparatively superior. This includes B. abortus strain 45/20 and B. melitensis H38, all of which produce persistent antibody titers [6].

The more recent global advances in vaccine development underscore the continued challenges that biomedical researchers face in developing effective vaccines. An ideal vaccine should have the following characteristics to be considered effective: It should not induce disease in the vaccinated animals, it should prevent abortions, a single dose should be able to provide long-term protection, it should offer protection to animals belonging to both the genders, and it should be suitable for animals of all age groups. Biological stability should also be a prime consideration, in order to avoid the risk of virulence reversion. Moreover, the vaccine should not be pathogenic to humans. Therefore, the shedding of animal product derivative should be avoided. Mass production of the vaccine should also be considered for the vaccine to be considered ideal [43].

Besides the live attenuated vaccines, which offer a superior degree of protection, DNA and subunit vaccines have also been explored [43]. Subunit vaccines possess a distinct advantage, as they are noninfectious, nonviable, avirulent, and well defined [6]. However, they induce poor immunogenicity without adjuvants [46]. The generation of the memory Th1 cells (via T-cell antigens) offers great promise. Therefore, the first necessary step in this subunit approach is the determination of T-cell antigens. The DNA vaccine was mobilized using genes that encode for some specific antigenic proteins (e.g., the L7/L12 proteins) and it was found to offer some degree of protection. The DNA vaccine was clearly advantageous, because it induced both cell-mediated and humoral immunity. However, at present, it is still unable to supersede the efficacy of the licensed vaccines available [43]. A DNA vaccine expressing the Brucella groEL heat-shock protein displayed an ability to induce a Th1 type of immune response; however, it did not induce any protective immune response against an imposed challenge. The Cu-Zn superoxide dismutase (SOD) antigen fused with IL-2, was also expressed and tested as a possible DNA vaccine. It was found to positively elicit IFN-γ from the T cells of mice [23].

Recombinant proteins with or without an adjuvant have also been implicated in vaccine development [6]. The primary goal in vaccine production is to be able to identify antigenic proteins in the membrane of the Brucella species, which have particular epitopes to receptor B-cells. The vaccine should also be perceived by the T-cell receptors in the form of a complex comprising major histocompatibility complex molecules. This requirement poses the greatest challenge in the process of vaccine development. Another aspect that needs focus is the development of a delivery vehicle or adjuvant that can augment the low immunogenicity of subunit vaccines [22]. The literature abounds with a vast array of research on various adjuvant systems and their applications. The limitations are attributable to a lack of patent applications from scientists and investigators [23].

Outer membrane vesicles (OMVs), shed by gram-negative bacteria, have also been reported to function as potential vaccines. These vesicles derived from the outer membrane consist of outer membrane proteins, lipopolysaccharides, and to some extent, phospholipids. Several OMVs have been evaluated for to their immune-modulatory role, and equivalent levels of immunity have been observed, when OMVs are compared to the B. melitensis Rev. 1 live vaccine. The major advantage associated with OMVs is their capacity to act as adjuvants, owing to the many bacterial components, majority of which are phospholipids. The OMVs are also less expensive to produce and purify [6].

Future development of vaccines against the Brucella spe-
cies is expected to integrate optimal qualities that would result into a non-replicating, efficacious vaccine that would maximize immune stimulation and cost efficiency [35]. It is our general recommendation to navigate on the possibility of genetically engineering live attenuated vaccines that could replace the currently available vaccines [43].

Live attenuation and killed vaccines for brucellosis

The inactivated and live attenuated vaccines have been mostly studied historically during the process of vaccine development. To date, numerous vaccines belonging to the above category have been officially recognized by several countries. Live attenuated vaccines comprise live viruses or bacteria that have been weakened so as to not cause any disease in bodies with healthy immunity systems. However, the administration of these vaccines induces strong and long-lasting immune response in hosts [39]. Even though these vaccines are very effective, they cannot be tolerated by everyone. These vaccines cannot be administered to people with weakened immune systems or to patients undergoing chemotherapy. On the other hand, the inactivated or killed vaccines are safe for everyone; however, these vaccines trigger a completely different immune response than the live attenuated vaccines. Moreover, multiple doses of these vaccines are often required to maintain immunity, after being administered.

Presently, two major live attenuated vaccines, \textit{B. abortus} strain 19 (S19) and \textit{B. abortus} RB51, are recommended for controlling \textit{B. abortus} infection in cattle. \textit{B. melitensis} Rev. 1 is the only strain used for vaccinating goat and sheep against \textit{B. melitensis} infections [39, 48, 50]. Moreover, in countries where \textit{B. melitensis} infection in sheep and goat is widespread, cattle may also become infected with the same species. \textit{B. melitensis} Rev. 1 has also been considered for administration in cattle. However, the use of \textit{B. melitensis} Rev. 1 in cattle has been very limited [42]. The other live attenuated strain used for the vaccination of goat and sheep is \textit{B. suis} strain 2 (S2), a strain produced in China. Although the virulence of is \textit{B. suis} strain 2 (S2) is approximately the same as that of \textit{B. abortus} S19, and although its relatively stable, the efficacy of this strain against experimental \textit{B. melitensis} infections in pregnant ewes and against \textit{B. ovis} infections in rams, has been found to be lesser than that of the \textit{B. melitensis} Rev. 1 vaccine [10, 55, 56].

The most widely used vaccine for the prevention of brucellosis in cattle is \textit{B. abortus} S19. It is considered a reference vaccine, to which many other vaccines are compared. \textit{B. abortus} S19 is a smooth attenuated strain, which was isolated in the early twentieth century and was naturally attenuated when the virulent culture of \textit{B. abortus} was left at room temperature for one year [6, 52]. However, the molecular basis of the observed attenuation remains elusive. Although the strain S19 has been shown to contain a deletion in the erythritol catabolic genes, thereby rendering it sensitive to erythritol, a deletion of the same set of genes in virulent strains has not been shown to result into attenuation. Administration of \textit{B. abortus} S19 (a vaccine against virulent \textit{B. abortus} infections) with a single subcutaneous dose of $3 \times 10^{10}$ viable organisms to female calves aged 3–6 months, and with a dose of $3 \times 10^{4}$ viable organisms to cattle, has been shown to decrease the prevalence of this virus in herds, without any significant increase in the risk of abortion. However, S19 did exhibit several problems, which limited its use in cattle. Owing to the smooth nature of the strain S19 and because of the strong antibody response against the O-side chain, this strain is unable to discriminate between infected and vaccinated animals. Some of the animals administered with this vaccine undergo miscarriage, or release the vaccine strain into their milk [1, 51].

Since 1996, the live attenuated vaccine \textit{B. abortus} RB51 has become the globally accepted official vaccine for the prevention of brucellosis in cattle. This strain is stable, rifampin-resistant, and derived from a rough mutant of \textit{B. abortus} 2308. It has been found to contain an IS711 element disrupting the \textit{wboA} gene that encodes a glycosyl transferase responsible for O-side chain synthesis [47, 48, 54]. Similar to the S19 strain, vaccination of calves (aged 4–12 months) with $1 \sim 3.4 \times 10^{10}$ viable RB51 organisms, or with a reduced dose of $1 \sim 3 \times 10^{9}$ organisms, has been shown to prevent subsequent virulent \textit{B. abortus} infections [4, 25]. The rough strain RB51 is less virulent, and it does not induce a positive response in typical serological diagnostic tests. However, this strain does induce low levels of abortion (less than 0.2%), but is currently being used (instead of S19) in many countries [39, 47, 48]. However, RB51 still shows many drawbacks after being administered. Vaccination with full doses of RB51 can induce severe placentalitis and placental infections in most of the vaccinated cattle. It is also released into milk in a significant number of vaccinated animals [39]. Field experience also indicates that it can induce abortions in some cases when administered
to pregnant cattle. Additionally, although the sera from RB51 vaccinated cattle do not respond to standard diagnostic tests, they do contain antibodies that react to a dot-blot ELISA test containing the RB51 antigen. RB51 does not confer resistance to B. melitensis or to B. ovis, and other rough strains have also been evaluated as potential vaccines [24, 43, 45]. Similar to the S19 strain, RB51 can also infect humans and is therefore unsuitable as a vaccine for the prevention of human brucellosis.

Currently, the live attenuated B. melitensis Rev. 1 (Rev1) is the only available vaccine for protection against B. melitensis infections in small ruminants. In 1957, a smooth attenuated strain of B. melitensis was obtained by growing a streptomycin-dependent population in a streptomycin deficient medium [6, 9]. In countries where B. melitensis frequently infects cattle, Rev. 1 has been hypothesized to be more effective than S19 in protecting them from B. melitensis infections. However, there is a dearth of information related to this issue [27]. Vaccination with Rev1 induces significant protection in sheep and goats, and it has been found to be much more protective in goats and sheep than in animals vaccinated with S19 [4]. However, these vaccines are not administered to cattle older than 12 months, as the organisms in the viable strain can elicit a strong immune safety response in these animals, and can cause abortions, when used in pregnant cattle and other animals [4, 5]. Additionally, it is resistant to 2.5 μg/mL of streptomycin and susceptible to 5 IU penicillin G, which allows its differentiation from field strains. Moreover, vaccination with Rev1 can result into persisting agglutinins that can interfere with various serological diagnostic tests. Rev1 is also pathogenic to humans via aerosol exposure or self-inoculation, as it causes generalized brucellosis in the affected individuals [5, 12, 19, 24].

B. abortus strain 45/20 was obtained in 1938 by following 20 passages in guinea pigs [39, 48]. This rough strain is used only as a heat-killed vaccine to avoid its reversion to a virulent strain. It is also much safer in pregnant animals and does not interfere with serological diagnosis. However, the genetic defects in this strain are unknown, and the administration of this vaccine in cattle requires an adjuvant [13, 26, 39].

The currently available live attenuated vaccines have limited use, as they tend to maintain their virulence even after undergoing attenuation. Although killed vaccines have been thought to be safe for everyone, they elicit remarkably different immune responses than the live attenuated vaccines. Besides insufficient protection, killed vaccines such as 45/20 can also induce persistent antibody titers that can interfere with the commonly used serological tests [7, 14, 18].

Subcellular vaccine

Subcellular, acellular, and subunit vaccines harbor dead organisms and contain only fragments of the pathogen against which they work. Cell-mediated immunity is the dominant immune response required for protection against brucellosis [57]. Thus, the concept of a subunit vaccine for combating brucellosis is based on the generation of memory Th1 cells following immunization with the T-cell antigen [11, 32, 33]. Subcellular vaccines are cheap and quite easy to produce, administer, and maintain. Being nonliving, they also do not cause any disease. Subcellular vaccines do not exhibit the drawbacks displayed by live attenuated vaccines, because they do not interfere with immune-diagnosis [15]. The disadvantages of subcellular vaccines include poor immunogenicity and the need for booster shots, due to insufficient antigen dose or because of the lack of antigen persistence in vivo [2, 10, 57].

The development and optimization of an effective subunit vaccine against brucellosis is indeed an intense and interesting area for research [15]. Subcellular vaccines used for brucellosis harbor high concentrations of immunoreactive recombinant proteins. Subcellular vaccines containing the outer membrane components from the hot saline extracts of B. ovis were noted to be as effective as the live B. melitensis Rev 1 vaccine, in protecting against B. ovis [8]. There have been numerous studies on outer membrane fragments and on single or double protein vaccines against brucellosis. In general, the obtained results show that subcellular vaccines do not have a clear advantage over other vaccine strains. One approach being assessed for designing new, safe, and efficient varieties of vaccines is based on the use of a universal subcellular vaccine against brucellosis [41]. The level of protection can be improved using multiple subunit vaccines, with a more powerful antigen, a better adjuvant, or both [3]. After weighing the advantages and disadvantages, it has been found that subcellular vaccines could be accepted as an alternative vaccination strategy in the case of animals that cannot be vaccinated by the live vaccine strain as well as in humans at a high risk of
acquiring the disease.

DNA vaccines

Brucellosis in domestic livestock still remains a continual source of human infection worldwide, and is endemic in many parts of the world. It is currently re-emerging in many countries. Therefore, there is a need to address the disease in natural hosts, as this strategy would be more cost-effective in preventing human infections. The development of improved vaccines is urgently necessary for preventing infections in humans [44]. Currently available live attenuated \textit{Brucella} vaccines, although effective in preventing abortion and transmission of brucellosis, cannot effectively prevent the infection or seroconversion. They also tend to induce abortions in pregnant animals and cause infections in humans [44]. On the other hand, the use of dead bacteria or subunit vaccines elicits a lesser degree of protective immunity as compared to live attenuated vaccines, most likely because of the inability to fully stimulate the pathogen's pattern recognition receptors or to mimic the pathogen's trafficking through phagosomes [44].

Immunization with plasmid DNA encoding the protective antigen is a novel and promising strategy that is safe, effective, and capable of inducing protective immunity [36]. This technology was developed in the 1990s for gene therapy applications and since then it has initiated the exploration of DNA vaccines for the induction of protective immunity against microbial pathogens [48]. DNA vaccination is a powerful method of immunization that targets the \textit{in vivo} genes of intracellular pathogens such as \textit{Brucella}, thereby inducing both humoral and cellular immune responses. The gene on the plasmid vector has the ability to replicate in prokaryotes without expressing the protein, but also has the ability to replicate and express the protective antigen in the immunized eukaryotes [40, 48]. This method offers several benefits, such as a zero risk of infection, a long-lived immune response, an enhanced stability at room temperature, ease of preparation, as well as low cost and easy validity. It also protects the host from many intracellular pathogenic infections [36]. At present, the DNA vaccines that have been observed to induce immunological responses in cattle after several inoculations, include those expressing Cu-Zn SOD, glyceraldehydes-3-phosphate-dehydrogenase, or a combination of SOD, ribosomal L7/L12, and the BCSP31 protein [44]. Furthermore, a DNA vaccine containing bp26 (a periplasmic protein) and a cha
erone protein, after being inoculated thrice into a bison, also showed increased lymphocyte proliferative responses and an increased production of IFN-\(\gamma\), as compared to the non-vaccinated controls [44]. As the data on DNA vaccines in natural hosts are very limited, there has been no evaluation of its standard vaccine efficacy. Therefore, newly developed DNA vaccines require further optimization, refinement, and improvement. Moreover, a thorough investigation on enhanced delivery mechanisms seems necessary before these vaccines are administered to domestic livestock or wild animals [37, 44, 49].

Recent synthetic particulate vaccine delivery systems, known as nanoparticles, have shown several significant advantages as an alternative methodology for vaccine development. The advantages include a sustained antigen release, and the transport of particles into the extracellular and intracellular biological barriers. Therapeutic nanoparticles can also be targeted for endosomal disruption after internalization, and they enable the cross presentation of antigens to elicit both CD4+ and CD8+ T cell responses, inducing a comprehensive antigen-specific immune response [17]. Furthermore, nanoparticles can be used to protect macromolecules such as proteins or genes \textit{in vivo} from enzymatic and hydrolytic degradation, and to deliver the encapsulated macromolecules to specific tissues through various routes of administration [34, 44]. Therefore, this system offers a great potential for the efficient delivery of DNA vaccines, as they can be orally administered, systematically disseminated, and readily taken up by the macrophages. Vaccine-harboring nanoparticles can also escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment, thereby increasing the overall immunogenicity of the embedded vaccines [34, 44].

CONCLUSION

In this review, we have highlighted the various types of vaccines used to combat brucellosis. These include the live attenuated vaccines, the subcellular and DNA vaccines, as well as the vaccines comprising dead organisms. This review may help us in developing an effective strategy for the eradication of brucellosis in domestic animals including cattle, goats, sheep, and dogs. Further research needs to be conducted in order to develop an improved variety of the brucellosis vaccine, intended for use in humans.
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