Brucella melitensis Vaccines

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Brucella melitensis is recognized as one of the predominant zoonotic pathogens globally. Live-attenuated vaccine Rev 1 is currently the most effective vaccine for controlling *B. melitensis* in small ruminants. While *Brucella* inactivated, nanoparticle, and subunit vaccines are less effective and require multiple doses, live-attenuated vaccines are less expensive and more efficacious. Several drawbacks are associated with the administration of current attenuated *B. melitensis* vaccines, including interference with serological diagnostic tests, inducing abortion in pregnant animals, shedding in milk, and zoonotic infections in humans.

Keywords: brucellosis ; B. melitensis ; vaccines ; Rev 1 ; small ruminants

1. Introduction

Because of the high number of human cases identified each year, *Brucella melitensis* is one of the most important zoonotic pathogens worldwide [1]. Three biovars of *B. melitensis*, 1, 2, and 3, are known but are not known to differ in virulence [2]. Goats and sheep are the most common natural hosts of *B. melitensis*. The most common route of transmission in natural hosts is most likely through oral or respiratory mucosa. The predilection of *B. melitensis* for colonization of trophoblasts in the placenta and fetal tissues leads to fetal stress or death and the induction of premature parturition (abortion) [1][2]. Although abortion is the primary clinical sign, it can be minimal in chronically infected herds. In addition to the shedding of high numbers of bacteria after abortion or birth of infected lambs or kids, some animals shed high levels of bacteria in the milk. Bacterial shed after parturition poses a risk for lateral transmission to other animals [2], whereas shedding in milk is a risk for vertical transmission. It is worth noting that contaminated milk can contribute to the human transmission of this infection. Failure to prevent exposure of uninfected animals to those shedding *Brucella* makes control of the disease difficult. Co-housing of ruminant species is a risk factor for brucellosis transmission [3]. Camels are highly susceptible to *B. melitensis* infection and play an important role in its epidemiology in some countries [4]. In other countries, endemic infection has been identified in Alpine ibex (*Capra ibex*) and chamois (*Rupicapra rupicapra*) and has occasionally led to brucellosis spillover into humans and domestic livestock [5].

Since the beginning of the 20th century, researchers have been searching for vaccines to prevent brucellosis in animals and humans [6]. Both live and inactive vaccines have been developed, but live-attenuated vaccines have been found to be better at inducing protective immunity against this intracellular pathogen [7]. Although a variety of killed vaccines have been developed, their success and acceptance have been limited, and none have induced the level of protection of live-attenuated vaccines. Historically, the first *Brucella* vaccine using killed *Brucella* was made in 1906 by Eyre who used it to vaccinate 51 soldiers [7]. Two killed vaccines that had more widespread use were *B. melitensis* H38 and *B. abortus* strain 45/20 with strain 45/20 utilized in cattle and sheep and H38 in cattle [8]. Due to their limitations in protective immunity as compared to the attenuated strains and their induction of persistent antibody titers, both are no longer utilized under field conditions.

The live vaccine *B. melitensis* Rev 1 (Rev 1) is currently utilized for the control of brucellosis in small ruminants. This strain was developed by Herzberg and Elberg in the mid-1950s and retains common characteristics of *Brucella* but is resistant to streptomycin and susceptible to penicillin G [9]. Subcutaneous or conjunctival immunization with Rev 1 confers protective immunity in small ruminants. However, Rev 1 vaccination stimulates an antibody response that reacts in serological tests, which cannot be differentiated from the humoral responses of infected animals. As *B. melitensis* can infect cattle, limited data have been reported that suggest that vaccination with Rev 1 has some efficacy in controlling infection with this *Brucella* species in cattle [10].

Due to the incubation period and potential latent infections, it is difficult to eradicate brucellosis by using serologic methods to detect and remove infected animals. The inclusion of vaccination programs to improve herd resistance is generally required to control the spread of the disease. To date, Rev 1 is the best available vaccine for the prevention of *B. melitensis* infection in small ruminants [9] and is typically administered to young animals (3–5 months old) at 0.5–2 ×

10^9 colony-forming units (CFU) subcutaneously. Although a bacteremia occurs after vaccination, it has been reported that the vaccine strain is cleared in approximately 14 weeks in goats. Although a reduced dose of the Rev 1 vaccine (10^3–10^6 CFU) has been suggested for subcutaneous administration, it has been found to offer limited protection against disease and does not prevent abortions [11][12]. When administered to adult sheep or goats during pregnancy at 10^9 CFU, abortions and shedding of Rev 1 in milk are common [11][12]. This presents a challenge in endemic areas where mass vaccination is necessary for control and pregnant sheep and goats may need to be vaccinated despite the risk of adverse effects [13]. In addition to the disadvantages listed above, the strain is also virulent to humans, and zoonotic infections have occurred during vaccination from exposure to abortions or consumption of infected milk [14][15].

Administered subcutaneously, Rev 1 vaccine stimulates protective immunity against *B. melitensis* but can elicit long-lasting antibody responses that interfere with serological testing. In contrast, conjunctival administration of the vaccine confers immunity similar to the standard subcutaneous approaches but reduces the magnitude and persistence of serological responses. For this reason, conjunctival vaccination has been utilized in endemic areas, as it has less impact on serologic screening and facilitates control programs. When eradication is the ultimate goal of a control program, conjunctival vaccination of Rev 1 in adult animals is ideal for enhancing herd immunity and preventing *B. melitensis* infections. It has been hypothesized that Rev 1 vaccination may induce a high level of protective immunity for up to 4.5 years, which essentially is lifelong immunity for small ruminants in most production systems. This hypothesis that Rev 1 vaccination induces lifelong immunity has contributed to the belief that the vaccination of young stock is sufficient for adequate control of *B. melitensis* infection in small ruminants. However, this vaccination strategy is tenuous, and even developed countries have failed to control brucellosis in small ruminants with this approach. The failure of vaccination of young stock only could be due to (i) failure to obtain high vaccination coverage in a herd due to animal movement or introduction of unvaccinated animals; (ii) use of poor quality vaccines or products not maintained with appropriate cold chain conditions; and (iii) a possible decrease over time of protective immunity induced by vaccination. Therefore, vaccination of whole flocks is the only viable alternative to control *B. melitensis* infection in small ruminants under extensive control conditions characteristic of many developing countries [16].

To develop promising vaccines against brucellosis, it is critical to produce T helper 1 (Th1)-derived cytokines (interleukin-12, tumor necrosis factor α, interferon gamma γ) associated with cellular immunity and activation of macrophages, dendritic cells, and CD4+ and CD8+ T cells. T helper 2 (Th2) immune responses associated with humoral responses do not appear to have a major role in the clearance of infection [17]. A study has demonstrated that cytokines, such as IL-4 (Th2 cytokines) and IFN-γ (Th1 cytokines), stimulate different IgG subtypes (IgG1 and IgG2 antibodies, respectively) that may be indicative of differences in the immune response [17].

2. *Brucella melitensis* Vaccines in Small Ruminants

Of the 18 included publications, nine contained information only relevant to recombinant vaccines, one to a live *B. melitensis* vaccine, five to nanoparticle vaccines, two to subunit vaccines, and one to a DNA vaccine. The recombinant *B. melitensis* 16M hfq (16MΔhfq) strain induced humoral and cellular responses in mice after intraperitoneal vaccination (ip) with increased expression of IgG1, IgG2a, IFN-γ, and IL-4. The recombinant strain did not differ from Rev 1 in the induction of cytokine responses, was cleared at approximately eight weeks post-vaccination (PV), and demonstrated protective immunity that was slightly less than Rev 1 vaccination after experimental challenge with *B. melitensis* 16M at eight weeks PV. [18]. Another study utilized a *B. melitensis* TcfSR promoter (16MΔTcfSR) recombinant strain in which one of the two-component regulatory systems that allow host cells to detect environmental changes and adapt to *Brucella* infection was mutated. The recombinant induced a high level of protective immunity when challenged approximately 1 week after vaccine strain clearance. Vaccination did not induce humoral responses that interfered with serodiagnostic tests [19]. The M5-90ΔwboA recombinant is a reduced virulence, attenuated vaccine that induces reduced inflammatory responses as compared to the parental strain. The reduced virulence and safety of this recombinant were based on the observation that splenomegaly did not occur in a murine host. When compared to the parental strain, comparable protection to the parental strain was observed in mice after experimental challenge approximately 1 week after vaccine strain clearance. Humoral responses in vaccinated sheep and mice allowed for vaccinates to be distinguished from infected animals [20]. Others have demonstrated that a DNA vaccine based on pcDNA3.1, encoding the ORF of *B. melitensis* Omp25 and Opm31 genes, may be a viable vaccine candidate due to induction of humoral (IgG) and cellular (Th1 cytokines IFN-ɣ and Th2 cytokines IL-10) in mice. The DNA vaccine construct elicited cellular and humoral responses to *B. melitensis* antigens after four inoculations at 1-week intervals [21]. Vaccination of mice with nanoparticle *B. melitensis* and *B. abortus* vaccines conferred less protection than Rev 1 vaccination when experimentally challenged 1 month after the last of three oral vaccinations. Protection was correlated with a mixed Th1-Th17 response [22]. In a different study, the authors demonstrated that a different nanoparticle vaccine (Omp31-loaded N-trimethyl chitosan

nanoparticles) induced Th1–Th17 immune responses in mice after three dosages delivered orally or two dosages administered ip. Lower antibody titers were observed in mice orally immunized as compared to ip [23]. When experimentally challenged with *B. melitensis* strain 16M at 1 month after vaccination, oral vaccinates had greater protection than intraperitoneal vaccinates but less protection than Rev 1 vaccinates. A third nanoparticle vaccine (based on poly lactic-co-glycolic acid nanoparticles 50:50 and containing oligopolysaccharide antigens) induced humoral responses in mice that increased after each inoculation. Experimental challenge with *B. melitensis* 2 weeks after the third inoculation demonstrated reduced splenic infection when compared to the control mice [24].

Subunit vaccines offer the advantages of better safety profiles, induction of humoral responses [25], and faster production with reduced costs [26]. Candidate subunit vaccines identified in this search included OMVs vaccines in either Poly (I:C) or 327 CpG + Montanide ISA adjuvant formulations. Humoral responses increased after two vaccine dosages with adjuvanted vaccines demonstrating the greatest responses. Spleenocytes demonstrated greater cytokine responses (IFN-γ and IL-2) in vitro after stimulation with OMV antigens [27]. Another study that used a recombinant protein-based subunit vaccine (Omp10, Omp28, L7/L12 combinations) alone or with Taishan Pinus massoniana pollen polysaccharide adjuvants (TPPPS) demonstrated increased humoral responses after inoculation, but the responses were greater in mice inoculated with a live *B. melitensis* M5 vaccine. Serum IL-2, IL-4, and IFN-γ were increased in the vaccinated mice. After experimental challenge at 4 weeks after vaccination, the mice inoculated with subunit vaccines containing all antigens and adjuvant had reductions in splenic infection that were similar to but less than the reductions observed in the mice inoculated with the live vaccine [28].

Unfortunately, most *B. melitensis* candidate vaccines have only been evaluated in murine models in which disease pathogenesis can be markedly different from what occurs in ruminant hosts. Murine models are inbred as compared to outbred domestic livestock hosts. There are significant differences in ruminant immunologic responses from those of mice, including the observation that ruminants have a high percentage of circulating T cells expressing γδ markers. Lastly, infection in mice is generally quantified by the evaluation of hepatic and splenic colonization, whereas in ruminant hosts, infection is predominantly within lymphatic tissues. These differences emphasize the need for the evaluation of brucellosis vaccine candidates in the species of interest to address research needs. In addition, some studies in murine models administer experimental challenges prior to the immune system returning to a senescent state, and therefore, colonization data might be influenced by nonspecific immune activation.

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