Rev. sci. tech. Off. int. Epiz., 2013, **32** (1), 207-217

Recent developments in livestock and wildlife brucellosis vaccination

S.C. Olsen

Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture (USDA), 1920 Dayton Ave, Ames, IA 50010, United States of America
E-mail: steven.olsen@ars.usda.gov

Summary

Live attenuated brucellosis vaccines have been available for protecting domestic livestock against *Brucella melitensis* and *B. abortus* for more than 60 years. Current vaccines are effective in preventing abortion and transmission of brucellosis, but poor at preventing infection or seroconversion. In addition, they can induce abortions in pregnant animals and are infectious to humans. It can be argued that current vaccines were developed empirically in that the immunological mechanism(s) of action were not determined. Current knowledge suggests that both the innate and adaptive immune responses contribute to immunity against intracellular pathogens and that binding of pathogen structures onto pattern recognition receptors (PRRs) is crucial to the development of adaptive immunity. The phagosome appears to be vital for the presentation of antigens to T-cell subtypes that provide protective immunity to intracellular pathogens. The observation that killed bacteria or subunit vaccines do not appear to fully stimulate PRRs, or mimic *Brucella* trafficking through phagosomes, may explain their inability to induce immunity that equals the protection provided by live attenuated vaccines. *Brucella* appears to have multiple mechanisms that subvert innate and adaptive immunity and prevent or minimise immunological responses. New technologies, such as DNA vaccines and nanoparticles, may be capable of delivering *Brucella* antigens in a way that induces protective immunity in domestic livestock or wildlife reservoirs of brucellosis. Because of the re-emergence of brucellosis worldwide, with an increasing incidence of human infection, there is a great need for improved brucellosis vaccines. The greatest need is for new or improved vaccines against *B. melitensis* and *B. suis.*

Keywords

Adaptive immunity – Brucellosis – DNA vaccine – Innate immunity – Nanoparticle – Vaccine.

Introduction

The persistence of brucellosis in domestic livestock reservoirs remains a continual source of significant numbers of human infections worldwide. Despite regulatory efforts, brucellosis remains endemic in many parts of the world and is re-emerging in many countries. Although the infections in wildlife reservoirs are a lesser threat for causing human infection, they can be a source for the reintroduction of infection into domestic livestock, which are more likely to transmit brucellosis to humans. Addressing the disease in natural hosts of *Brucella* is the most cost-effective mechanism to prevent human infection (43, 53).

The *Brucella* spp. within domestic livestock or wildlife capable of causing zoonotic infections and clinical illness in humans are, in order of pathogenicity: *B. melitensis*, *B. suis* and *B. abortus*. Within domestic livestock and wildlife, the genus *Brucella* typically causes reproductive losses, although bacteria can also become localised in joints, bones or other tissues, leading to osteoarthritic or necrosuppurative lesions. Although *Brucella* spp. are named according to their preferred hosts – *B. melitensis* (sheep and goats), *B. suis*

(swine), *B. abortus* (cattle) and *B. ovis* (sheep) – the more pathogenic species of *Brucella* have been isolated from other host species under field conditions. For example, *B. suis* has been isolated from cattle and reindeer (*Rangifer tarandus*); *B. melitensis* has been isolated from cattle, water buffalo (*Bubalus bubalis*), camels (*Camelus dromedarius*) and an Iberian wild goat (*Capra pyrenaica*); and *B. abortus* has been isolated from camels, water buffalo, bison (*Bison bison*), feral swine, elk (*Cervus elaphus*) and other species of cervid. Because of the risk of transmission to domestic livestock or humans, there is a significant need for vaccines for wildlife reservoirs of brucellosis, in addition to improved brucellosis vaccines for domestic livestock.

This review of recent developments in *Brucella* vaccines will focus on progress in protecting domestic livestock or wildlife against *B. melitensis*, *B. suis* and/or *B. abortus*, according to their zoonotic importance. Although the murine model has been the most common choice for experimental studies on *Brucella* vaccines, this review will be primarily limited to vaccine studies conducted in ruminants or swine, the natural hosts of *Brucella*. This choice is based on the fact that mice are not natural hosts for *Brucella* spp. that infect domestic livestock or wildlife. In addition, the responses of inbred strains of mice do not accurately reflect the immune responses of heterozygous livestock or wildlife populations, and it has previously been observed that data obtained from murine models of brucellosis have failed to accurately predict immunogenicity or efficacy of vaccines in domestic livestock or wildlife species of interest (17, 36). Because immunological responses and efficacy of brucellosis vaccines can differ between natural hosts, it is imperative that vaccine studies be conducted in the species of interest, using the relevant *Brucella* spp.

Current knowledge of the contributions of innate and acquired immunity against *Brucella* infection

Scientific data are increasingly demonstrating the strong connection between innate immunity and effective adaptive immune responses. Innate immunity is a non-specific response that recognises a limited number of microbial molecular structures (pathogen-associated molecular patterns or PAMPS) through pattern recognition receptors (PRRs), located at various sites on plasma membranes, endosomal vesicles and within the cytoplasm, enabling the recognition of microbes at these locations (32). These PRRs include Toll-like receptors (TLRs), nucleotide oligomerisation domain-like (NOD-like) receptors (NLRs), C-type lectin receptors (CLRs), and RIG-1-like receptors (RLRs). Microbial detection by PRRs on the cell membrane can lead to phagocytosis and the recruitment of proteins and organelles with antimicrobial activities to phagosomes (23). Through highly regulated membrane fusion events, newly formed phagosomes containing microbes undergo sequential interactions with early endosomes, late endosomes and lysosomes. But phagocytosis does more than degrade internalised microbes; PRRs are also present in the phagosome and can coordinate innate and adaptive immune processes. It has been postulated that TLRs are recruited to phagosomes, regardless of their cargo, to sample the compartment for the presence of microbial products (23, 49). Pattern recognition receptors are also present within the cytoplasm where they can sense DNA or RNA, with subsequent induction of type I interferons and other inflammatory cytokines.

Toll-like receptor signalling pathways coordinate the process of phagocytosis, phagosome trafficking and autophagy; induce the expression of cytokines; activate dendritic cells and induce the surface expression of co-stimulatory and major histocompatibility complex (MHC) Class II molecules; and influence the processing and presentation of antigens. Dendritic cells process the peptides in phagosomes for presentation with MHC Class II molecules and TLR engagement induces translocation of the MHC-peptide complexes from the endosome to the plasma membrane, where they are presented to CD4+ T-cells. By the cross-presentation pathway, internalised antigen gains access to the endoplasmic recticulum by fusion of phagosomes with endoplasmic reticulum-derived vesicles, leading to the loading of antigen onto MHC Class I molecules and presentation on the cell surface to CD8+ T-cells (23). As protective immunity against intracellular pathogens is considered to be mediated through T-helper 1 (Th1) responses by CD4+ T-cells, and cytotoxic responses by CD8+ cells, TLRs of the innate immune system can influence the development of adaptive immunity after infection or vaccination. The ultimate goal of vaccination would be to induce protective immunity that, at the minimum, mimics protection induced by natural infection.

In accordance with its stealthy nature, *Brucella* has developed mechanisms to minimise stimulation of PRRs. The *Brucella* cell envelope has high hydrophobicity and its lipopolysaccharide (LPS) has a non-canonical structure that elicits a reduced and delayed inflammatory response when compared to other Gram-negative bacteria (33), and has lower stimulatory activity on TLR4 receptors (41). The O side-chain on the LPS can form complexes with the MHC Class II molecules that interfere with the ability of macrophages to present exogenous proteins. *Brucella* ornithine-containing lipids and lipoproteins in the outer membrane are poor activators of innate immunity.

Brucella bacteria are also devoid of many classical structures involved in virulence, such as pilli, fimbriae, capsules and plasmids that stimulate PRRs. In addition, *Brucella* prevents phagosome maturation and fusion with lysosomes – a stealth mechanism that may interfere with other innate and adaptive immune processes. As proteins have been identified in *Brucella* that demonstrate significant homology with TLR adaptor molecules, these peptides may be a mechanism to interfere with or subvert TLR signalling (33). Compared to other Gram-negative bacteria, *Brucella* induces a reduced innate immune response, and a lower rate of maturation and activation of dendritic cells. Lack of stimulation of innate immune responses by natural infection with *Brucella,* or after vaccination with attenuated strains, may impair the development of robust adaptive immune responses.

More recently, a role for Th17 and Th17-reg cells in combating infections at mucosal surfaces and protecting against other intracellular pathogens has been proposed, although the protective effect appears to be more dramatic for extracellular bacterial infections (48). Following recognition of bacterial moieties by PRRs, secretion of interleukin- (IL-) 12p40, IL-12p70 and IL-23 is induced, leading to IL-17 secretion by Th17 cells. IL-17 can promote IL-12 secretion by dendritic cells, stimulate inflammatory cytokines, and recruit protective immune cells to the site of infection. Th17 and IL-17 may be important in inducing memory Th1 cells that provide long-term protection, and may also mediate the balance between immune protection and inflammation/pathology, particularly at mucosal surfaces. Ruminants also have large circulating populations of γδ T-cells whose specific immunologic function is not yet known. Recent data suggest that γδ T-cells may be an early source of IL-17 in *Mycobacterium* infection, and they may also play a role in mucosal immunity. Treg cells, which play a crucial role in regulating immune responses, can be induced by low-dose engagement with the receptor on CD4+ cells and can suppress Th1, Th2 and Th17 responses (27). At the current time, little is known about the role of Th17 and Treg cells in *Brucella* infection or protective immunity after vaccination.

Protective immunity against *Brucella*

In regard to the development of protective immunity after brucellosis vaccination, it is generally agreed that vaccine-induced antibody responses do not correlate well with long-term protective immunity in natural hosts. For many years it has been hypothesised that protective immunity against intracellular pathogens is associated with a Th1-type response. In comparison, Th2 responses, which are associated with cytokines that promote humoral immunity, are not believed to provide long-term protection. CD4+ cells play a central role in coordinating and intensifying the adaptive immune response by separating themselves into functional subsets, such as the Th1 type, a subtype that is associated with production of interferon-γ (IFN-γ); IL-2; and tumour necrosis factor-α (TNF-α). CD4+ cells also provide growth factors and signals for the generation and maintenance of CD8+ T-cells. CD8+ cells are considered to be important for protective immunity because of their ability to lyse or kill infected cells, thereby releasing *Brucella* from intracellular hiding and exposing it to extracellular bactericidal mechanisms. Cytokines released by CD4+ and CD8+ cells may activate macrophages and dendritic cells, thereby increasing their bactericidal activity against *Brucella*. Antigen-presenting cells also present co-stimulatory molecules on their surface that are important for stimulation/activation of T-cells.

Many intracellular pathogens, and most likely live *Brucella* vaccines, evoke a mixed immune response containing characteristics of both Th1 and Th2 types of responses by CD4+ effector cells. Although Th1 responses, as indicated by the production of IFN-γ, have been reported in ruminants after *Brucella* vaccination, data suggest that the measurement of IFN-γ production by itself is not sufficient to predict protective immunity against the intracellular pathogens *Mycobacterium bovis* or *Leishmania major* (15, 19). It has been reported that IFN-γ expression in cattle is predictive of protection against *M. bovis* at the group level, rather than on an individual basis (5). Although many studies have used qualitative measurements of IFN-γ production as an indication that vaccination induces protective Th1 responses, recent data from other pathogens have suggested that a better indication of the quality of the immune response after immunisation may be gained by measuring the increases in antigen-specific polyfunctional T-cells that are capable of producing a triad of relevant cytokines (IFN-γ, TNF-α, and IL-2) (47). In other species, the memory T-cell pool is not monolithic, and different types of memory cells have different capabilities in regards to cytokine production, cytotoxic activity and protection (47).

Current *Brucella* vaccination strategies/options

Historically, brucellosis vaccines have been composed of live attenuated strains because, in general, heat-killed or subcellular fractions have failed to provide protection equivalent to that induced by live vaccines. It can be argued that all live attenuated strains were empirically developed, in that the mechanisms underlying immune protection against the pathogen by the designated host were not the impetus for the approach used in their identification.

Those vaccines (attenuated *B. abortus* or *B. melitensis* strains) that are currently available are effective in reducing production losses and reducing transmission, but are less effective at preventing animals from becoming infected or seroconverting after exposure to virulent field strains. Since most surveillance tests are based on detection of antibodies against the O side-chain of the *Brucella* LPS, vaccination with smooth attenuated strains can interfere with diagnostic procedures to identify animals infected with virulent field strains.

Brucella abortus in domestic livestock and wildlife

At the present time, there are three primary vaccines being used worldwide to protect natural hosts against *B. abortus*. Vaccines using strains 19, RB51, and strain 82 have successfully been used to protect cattle against *B. abortus*, with mixed results in other domestic livestock and wildlife. Because strain 19 is a smooth strain which expresses the O side-chain on the surface of its LPS, vaccination of large ruminants with strain 19 is primarily limited to prepubescent heifers. As strain RB51 is rough and does not express the O antigen, it does not cause positive serological responses in brucellosis surveillance tests. Strain 82 is intermediate in that it does express some O antigen on its surface, but the humoral response has been reported to be less robust and shorter in duration when detected by diagnostic tests (21). As a result of the potential for inducing abortion, it is recommended that live vaccine strains be used with caution in pregnant cattle.

Over the years, numerous field studies with strain 19 have demonstrated the efficacy of this vaccine under field conditions. In a similar manner, more recent data have reported efficacy for the RB51 vaccine under field conditions (29). It should be noted that all currently available live vaccines are most effective when combined with an effective test-and-removal strategy, based on removing seropositive individuals.

In regard to *B. abortus* infections in other domestic livestock, limited data have suggested that single subcutaneous (SQ) vaccination with strain 19 ($6–12 \times 10^{10}$ colony-forming units [CFU]) or two SQ vaccinations with strain RB51 ($3–10 \times 10^{10}$ CFU) are efficacious in preventing infection in water buffalo (*B. bubalis*) after conjunctival challenge with *B. abortus* strain 544 (6). Others found that RB51 was cleared in water buffalo between 6 and 12 weeks post vaccination ($1–3 \times 10^{10}$ CFU) (13) but did not protect against field exposure (18) or intravenous challenge at 180 days of pregnancy with a local field strain of *B. abortus* (40). Although brucellosis infection of nomadic or domesticated camels (*Camelus bactrianus*) and yaks (*Bos grunniens*) is known to represent a zoonotic threat to humans, there is very little literature on the efficacy of any brucellosis vaccine in these species.

In terms of wildlife infection with *B. abortus*, in the United States, the primary focus is on bison and elk that live in Yellowstone National Park and the surrounding area. Strain 19 was reported to be highly abortigenic in adult bison and not efficacious as a calfhood vaccine in protecting bison against experimental challenge with *B. abortus* strain 2308 (9). Calfhood vaccination with strain RB51 (10^{10} CFU) has been found to protect bison against experimental challenge with strain 2308 during pregnancy (36, 38). However, the data suggest that the efficacy of strain RB51 in bison is less than that shown in comparable challenge studies in cattle. Limited data suggest that booster vaccinations of bison increase protection against abortion and infection after experimental challenge (35). Numerous studies in elk have found poor or no protection against experimental challenge after vaccination with strains RB51 or 19 (25, 26, 43). Immunological data suggest that elk primarily develop humoral responses after vaccination with RB51 or 19, and that cellular immune responses are poor or lacking (35).

Brucella melitensis in domestic livestock

At present, the only vaccine recommended for protecting sheep and goats against *B. melitensis* is strain Rev.1 (4). The Rev.1 vaccine has also been shown to protect sheep against *B. ovis*. Since it expresses the O side-chain on its LPS, vaccination with strain Rev.1 can induce serological titres on diagnostic tests. In an effort to prevent vaccination titres, SQ inoculations have been recommended only in young animals (< 4 months) and conjunctival vaccination with Rev.1 is recommended for older sheep and goats, particularly when not pregnant. Data on effective national campaigns to reduce the seroprevalence of *B. melitensis* have been reported in which Rev.1 vaccination of all juvenile animals (3–8 months of age or < 1 year of age) was combined with adult vaccination at approximately three-year intervals (51, 53).

Brucella suis in domestic livestock or feral swine

At the present time, there are no commercially available vaccines to protect domestic livestock or feral swine against *B. suis* infection. The development of efficacious vaccines against *B. suis* is impaired by the lack of a standardised repeatable challenge model which has been shown to correlate to tissue localisation and clinical disease caused by *B. suis* under field conditions.

Although some work has been conducted, publications on *B. suis* vaccine development are significantly fewer than citations related to *B. abortus* and *B. melitensis* vaccines.

Several decades ago, an oral *B. suis* strain 2 vaccine was described as being applied to livestock in the People's Republic of China to protect against all pathogenic *Brucella* spp.; the current literature would suggest it is not widely used and primarily limited to China (11). The strain RB51 vaccine has been evaluated in swine but was not found to effectively colonise tissues *in vivo*, induce detectable immune responses or protect against conjunctival challenge with a virulent strain of *B. suis* (46). As cattle can be infected with *B. suis*, a recent study evaluated protection in cattle after vaccination with strain RB51 and found that it did not protect against *B. suis* infection after conjunctival challenge (34).

Recently, immunological responses and protection induced by vaccinating swine with a natural rough strain of *B. suis* (strain 353-1) have been evaluated. When delivered subcutaneously (10^{10} CFU) or orally (10^{11} CFU) to swine, strain 353-1 did not cause clinical symptoms but did induce robust humoral and cell-mediated responses. After conjunctival challenge with a virulent *B. suis* strain at approximately 30 weeks after vaccination, swine vaccinated with strain 353-1 had reduced *B. suis* colonisation in the lymphoreticular tissues and major organs three weeks after challenge (W.S. Stoffregen, personal communication).

New *Brucella* vaccines or novel vaccination approaches

Numerous genes have been deleted in *Brucella* and the pathogenicity, immunogenicity, clearance and/or efficacy of recombinant *Brucella* strains evaluated. Unfortunately, at the present time, the majority of studies with recombinant *Brucella* strains have only been conducted in murine models of brucellosis. An early study evaluating recombinant *Brucella* strains in cattle found that vaccination with strain 19 derivatives, in which superoxide dismutase (SOD) or a 31 kilodalton *Brucella* protein of unknown activity were deleted, led to protection against experimental strain 2308 challenge equivalent to that provided by strain 19 vaccination (7). A more recent study, which also evaluated recombinants made from strain 19, found that vaccination with strain M1-luc, which has a deletion of the *Brucella* periplasmic protein BP26, induced similar protection against experimental challenge as vaccination with strain 19 (17). When an additional deletion was made in outer-membrane protein 19 into strain M1-luc, protection against experimental challenge was significantly reduced.

Brucella melitensis recombinant strains in which outer-membrane protein 25 (*omp25*), *asp24*, *cydA* and *virB2* genes have been deleted have been evaluated in goats (14). After SQ vaccination with 10^9 CFU, efficacy induced by the recombinant strains was compared to protection induced by Rev.1 vaccination (10^6 CFU) after conjunctival challenge with *B. melitensis* strain 16M during pregnancy. Although vaccination with the omp25 mutant gave similar protection against abortion as Rev.1, overall data from the two studies suggested that Rev.1 vaccination was more effective at preventing infection than the recombinant strains. In a similar manner, others have found that rough mutants of *B. melitensis* failed to provide equivalent protection to experimental challenge in sheep or goats, in comparison to vaccination with the Rev.1 strain (2, 16).

Others have tried increasing the immunogenicity and protection of vaccine strains by increasing the expression of genes believed to be associated with protection (50). Vaccinating bison with a recombinant RB51 overexpressing SOD and glycoyltransferase (*wboA*) genes resulted in reduced protection against experimental challenge when compared to vaccination with the parental RB51 strain (36). The observed reduction in protection was thought to be related to a more rapid *in vivo* clearance of the recombinant strain from lymphatic tissues. Vaccination of cattle with the recombinant RB51 strain overexpressing SOD and *wboA* genes also failed to demonstrate increased protection against experimental challenge, compared to the parental RB51 strain (P.H. Elzer, personal communication).

In an effort to increase the immunogenicity of *Brucella* vaccines, strain RB51 was incorporated into microspheres that also contained a *Fasciola hepatica* vitelline protein B and administered (10^{10} CFU) to red deer by SQ or oral routes (1). When compared to non-encapsulated RB51 vaccine, oral or SQ vaccinates had greater lymphocyte proliferative responses at 12 weeks after inoculation. Although the mechanism was not elucidated, co-administration of recombinant bovine IL-2 with strain 19 (10^6 CFU) vaccination of cattle increased protection against experimental challenge, as compared to vaccination with strain 19 alone (52).

Approximately 15 years ago, a novel vaccine approach was introduced in which immunogenic proteins were expressed *in vivo* by inoculation with plasmid DNA, expressing a gene of interest under the transcriptional control of a promoter. This approach, using what are known as DNA vaccines, offered great potential to develop targeted vaccines with high safety and efficacy that were also stable at room temperature. If properly optimised, proteins encoded within the plasmids would undergo post-translational modification and be processed and presented by antigen-presenting cells *in vivo* (12). At the current time, DNA vaccines expressing Cu-Zn SOD, glyceraldehyde-3-phosphate-dehydrogenase, or the combination of SOD, ribosomal L7/L12 and BCSP31 protein, have been evaluated in cattle and reported to induce immunological responses after multiple inoculations (20, 42, 44). In addition, bison that were inoculated three times with a DNA vaccine containing a periplasmic protein

(bp26) and chaperone protein (trigger factor) underwent increased lymphocyte proliferative responses and IFN-γ production, compared to non-vaccinated controls (8). At present, data on DNA vaccines in natural hosts are very limited, and published studies have evaluated the immunogenicity of multiple administrations of DNA vaccines in natural hosts without evaluating vaccine efficacy in a standardised challenge model. Therefore, current knowledge suggests that DNA vaccine candidates will require additional optimisation, refinement or improvement before they can be considered as viable vaccine candidates for use in domestic livestock or wildlife.

In a recent study, a non-pathogenic kinetoplastid parasite, *Trypanosoma theileri*, was engineered to express *Babesia* antigens for secretion or localisation on the interior or surface. This vector was proposed for use as a novel vehicle for enhancing immunity against multiple pathogens, as the vector is maintained within cattle for a long time and generates specific immune responses (31). Although proposed as a potential delivery vehicle for *B. abortus* proteins, the study characterised humoral, but not cellular, immune responses after vaccination. In a similar manner to DNA vaccines, a recombinant Semliki Forest virus was modified to express the *Brucella* Cu-Zn SOD gene *in vivo* (44). Cattle inoculated twice with the recombinant RNA virus had greater lymphocyte proliferative responses but not IFN-γ production, when compared to control cattle six weeks after the last immunisation. Efficacy against an experimental challenge was not evaluated.

More recently, synthetic particulate vaccine delivery systems are showing promise for sustained antigen release while enabling the transport of particles through extracellular and intracellular biological barriers (10). These vaccine delivery systems, known as nanoparticles because of their 1 nm to 100 nm size, can be targeted for endosomal disruption after internalisation, leading to cross-presentation of antigen to CD4+ and CD8+ cells. Nanoparticles may mimic the presentation of antigens during normal intracellular trafficking of *Brucella*. Nanoparticles are not only attractive because of their potential for releasing antigen within intracellular compartments, they also offer a means to deliver macromolecules, such as proteins, peptides or genes *in vivo*, using various routes of administration, and could even be targeted for delivery to specific tissues (28). As a result of their ability to protect encapsulated macromolecules from enzymatic and hydrolytic degradation, nanoparticles offer a potential approach for the development of oral vaccines. Orally delivered nanoparticles disseminate systemically, most probably after uptake by Peyer's patches within the gut. Their biological activity may occur because their small size enhances interaction with cell membranes and proteins, and mimics the natural nanostructures of pathogenic agents and protein complexes (28). Nanoparticles can also serve as an efficient delivery system for DNA vaccines, since they can be delivered orally, are readily taken up by macrophages (45), can escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment, and can increase the immunogenicity of DNA vaccines (28, 39). Nanoparticles can also incorporate adjuvants, as demonstrated in a recent publication in which a lipid-based nanoparticle expressing monophosphoryl lipid A and a candidate *Plasmodium vivax* antigen showed promise for eliciting protective immunity against malaria (30).

Development of new vaccines or strategies against *Brucella*

Although they are a valuable tool for reducing the prevalence of brucellosis in natural hosts, the live vaccines that are currently available have a number of shortcomings. These problems include, but are not limited to:

– the potential to induce human infection

– the ability to induce abortions in pregnant animals

– a limited ability to prevent infection and seroconversion after exposure

– limited efficacy in other natural hosts of *Brucella*.

In this author's opinion, when protecting against *B. abortus*, *B. suis*, or *B. melitensis*, an ideal *Brucella* vaccine would:

– induce long-term protection against abortion and infection in a high proportion of animals after a single inoculation

– prevent colonisation and seroconversion after exposure to virulent field strains

– be clinically safe in young and adult animals (including males for *B. suis* vaccines)

– not be shed by vaccinates after inoculation

– possess stability that minimises the need for maintaining the cold-chain for the vaccine.

Properties that would also be beneficial in vaccines designed for delivery to wildlife would include the capability for targeted delivery to the targeted population in a manner that is safe and efficacious (preferably oral), and would be environmentally safe, even after accidental delivery to non-targeted wildlife species.

As precise correlates of protective immunity against brucellosis remain unknown at the present time in any natural host, the ideal vaccine will most likely require appropriate stimulation of PRRs associated with innate immunity, activation and presentation of the antigen by appropriate antigen-presenting cells, induction of a Th1

type of CD4+ T-cell response, cross-presentation of *Brucella* antigens leading to activation/recruitment of CD8+ cells, release of appropriate cytokines, and generation of effective memory cell populations, both systemically and at mucosal surfaces. Since current knowledge would suggest that the intracellular trafficking of live strains is more likely to induce these types of responses, this may explain the disappointing immunity associated with vaccines composed of killed bacteria or subunits of *Brucella*. As *Brucella* appears to have developed mechanisms to minimise the activation of innate immunity and PRRs, the identification and elimination of these stealth mechanisms may improve the immunogenicity of the current live vaccines. The possibility cannot be eliminated that new delivery vehicles may be capable of delivering *Brucella* proteins in a manner that mimics the intracellular trafficking of live *Brucella*. This might lead to the development of non-living vaccines that would eliminate many of the problems presently associated with live vaccines.

Although data from other species would suggest that vaccines which preferentially target or activate dendritic cells might be more immunogenic, the lack of knowledge on dendritic cell populations in natural hosts and on their interactions with *Brucella* spp. would suggest a need for more basic research to understand the role of these cells in protective immunity against brucellosis. In addition, the role of Th17 and Treg cells in relation to protective immunity must be considered, as the possibility cannot be eliminated that live *Brucella* vaccines may subvert this pathway in a way that reduces protective immunity and/or induces responses that mimic tolerance.

It should also be noted that knowledge of mucosal immunity against brucellosis in natural hosts is severely lacking. Parenteral vaccination most likely induces systemic immunity but may fail to induce adequate immunity at mucosal surfaces; the sites where *Brucella* invasion occurs. Data suggest that lymphocyte trafficking in mucosal tissues differs from trafficking in peripheral lymph nodes (3) and could also differ between natural hosts of *Brucella*. It could be hypothesised that one mechanism to improve the efficacy of conjunctival vaccination, particularly when used with Rev.1 vaccination, could be the stimulation of mucosal immunity. Perhaps a more effective approach to protect against infection after parenteral vaccination would be to develop a booster vaccination, which targets mucosal immunity. Such an approach would improve protection at the infection site while also enhancing systemic anamnestic immune responses. As there are significant numbers of γδ T-cells in intra-epithelial locations on the mucosal surfaces, and these cells represent a high percentage of circulating T-cells in the blood of ruminants, their contribution to immunity against brucellosis should be explored, particularly in light of their importance in the Th17 response.

Although protective mechanisms against *Brucella* may be similar across natural hosts, studies have demonstrated significant differences between species in their immunological responses and/or immune regulation after brucellosis vaccination. Examples include the differences between cervids and cattle, water buffalo and cattle, and field observations suggesting differences between the *Bos taurus* and *B. indicus* breeds of cattle. These observations point to the need to conduct vaccine studies in the species of interest, and to evaluate protection against the relevant *Brucella* spp. for that natural host. Current knowledge irrevocably demonstrates that laboratory animal models of brucellosis do not accurately predict *in vivo* clearance, immunogenicity or the efficacy of vaccines in domestic livestock or wildlife hosts of *Brucella*. Although there could be similarities in immune responses to vaccines across natural host species, appropriate safety and efficacy data for candidate vaccines must be acquired for each targeted host before the vaccine can be recommended for use. Studies in natural hosts will be more complicated and expensive and progress could be impaired by a lack of appropriate facilities or financial resources. However, the investment in new vaccines should be made, as human brucellosis is most effectively controlled by addressing the disease in its natural host. Moreover, several studies have definitively shown that the financial cost of brucellosis vaccination and control programmes is offset by a significant benefit-to-cost ratio in terms of the reduction in human health costs alone (22, 53).

Lastly, one could argue that, with the exception of the introduction of strain RB51 in 1996, no new brucellosis vaccines with acceptable safety and/or efficacy have been introduced for use in a natural host for more than 60 years. It should be noted that there is some controversy regarding this vaccine and some have argued that the strain RB51 vaccine does not provide acceptable efficacy in natural hosts. Parallel work to develop human vaccines against brucellosis has also failed to produce candidates with acceptable safety and efficacy. This lack of new vaccines could, in part, be related to the fact that the development of new brucellosis vaccines is complicated, challenging and difficult, and that the 'low-hanging fruit' was gathered more than 60 years ago. The current status of disease prevalence in humans and domestic livestock across the world strongly argues the need for a more efficacious and safe *B. melitensis* vaccine. It is hoped that recent developments in vaccine approaches and delivery, and new tools in basic research that characterise gene regulation and immunological responses, will provide innovative and productive approaches leading to the introduction of new brucellosis vaccines for use in domestic livestock or wildlife hosts in the future.

Évolutions récentes de la vaccination des animaux d'élevage et sauvages contre la brucellose

S.C. Olsen

Résumé
Les vaccins contre la brucellose utilisant des souches vivantes atténuées sont utilisés depuis plus de soixante ans pour protéger les animaux d'élevage contre *Brucella melitensis* et *B. abortus*. Les vaccins disponibles actuellement sont efficaces pour prévenir les avortements et la transmission de la brucellose, mais leur efficacité est moindre pour empêcher l'infection ou l'apparition d'anticorps. En outre, ils induisent parfois des avortements chez les femelles gestantes et peuvent être une source d'infection chez l'homme. On peut rétorquer que ces vaccins ont été mis au point de manière empirique, dans le sens où les mécanismes immunologiques à l'œuvre n'étaient pas encore connus. Les connaissances actuelles tendent à montrer que les réponses immunes innées et adaptatives contribuent à l'immunité contre les agents pathogènes intracellulaires, et que la fixation des structures d'un agent pathogène avec les récepteurs de reconnaissance des motifs moléculaires (PRR) joue un rôle crucial dans l'initiation des réponses immunes adaptatives. Le phagosome semble indispensable pour la présentation des antigènes aux sous-types des lymphocytes T qui déclenchent une immunité protectrice contre les agents pathogènes intracellulaires. Le fait que les souches bactériennes tuées ou les vaccins sous-unitaires ne parviennent pas à activer complètement les PRR ni à simuler le transport des *Brucella* par les phagosomes pourrait expliquer leur incapacité à induire une immunité protectrice du niveau de celle conférée par les vaccins à souche vivante atténuée. *Brucella* semble disposer de nombreux mécanismes pour altérer l'immunité tant innée qu'adaptative et pour empêcher ou minimiser les réponses immunologiques. Grâce à certaines nouvelles technologies telles que les vaccins à ADN et les nanoparticules, il deviendra probablement possible de présenter les antigènes brucelliques de manière à induire une immunité protectrice chez les animaux d'élevage ou les réservoirs sauvages de la brucellose. La réémergence de la brucellose partout dans le monde et l'incidence croissante de l'infection humaine rendent indispensable une amélioration des vaccins utilisés contre cette maladie, en particulier (et en priorité) contre les infections à *B. melitensis* et à *B. suis*.

Mots-clés
Brucella – Brucellose – Immunité adaptative – Immunité innée – Nanoparticules – Vaccin – Vaccin à ADN.

Evolución reciente de la vacunación antibrucélica del ganado y la fauna salvaje

S.C. Olsen

Resumen
Hace más de 60 años que existen vacunas vivas atenuadas para proteger al ganado de la brucelosis por *Brucella melitensis* y *B. abortus*. Las vacunas actuales son eficaces para prevenir abortos y la transmisión de la brucelosis,

pero poco útiles para evitar la infección o la seroconversión. Además, pueden inducir abortos en las hembras grávidas, y son infecciosas para el ser humano. Cabe aducir que las actuales vacunas fueron obtenidas empíricamente, por cuanto no se habían determinado los mecanismos de acción inmunológica. De lo que ahora sabemos se infiere que la respuesta inmunitaria tanto innata como adquirida contribuye a la inmunidad contra los patógenos intracelulares, y que la unión de ciertas estructuras de los patógenos con receptores de reconocimiento de patrones (PPR, por sus siglas en inglés) es fundamental para la aparición de inmunidad adquirida. El fagosoma parece ser indispensable para presentar los antígenos a los subtipos de linfocito T que confieren inmunidad protectora contra los patógenos intracelulares. El hecho de que las bacterias muertas o las vacunas de subunidades no parezcan estimular por completo los PRR ni imitar el tránsito de *Brucella* por los fagosomas quizá explique que no induzcan un nivel de inmunidad equiparable a la protección que confieren las vacunas vivas atenuadas. *Brucella* parece disponer de múltiples mecanismos para alterar la inmunidad tanto innata como adquirida y desactivar o reducir al mínimo las respuestas inmunológicas. Tal vez algunas nuevas tecnologías, como las de vacunas de ADN o las nanopartículas, puedan presentar los antígenos brucélicos de tal modo que induzcan inmunidad protectora en el ganado doméstico o los reservorios salvajes de brucelosis. Ante el resurgimiento de la enfermedad en todo el mundo y la creciente incidencia de infecciones humanas que trae consigo, resulta muy necesario disponer de vacunas más eficaces contra la brucelosis, sobre todo de nuevas o mejores vacunas contra *B. melitensis* y *B. suis.*

Palabras clave
Brucella – Brucelosis – Inmunidad adquirida – Inmunidad innata – Nanopartículas – Vacuna – Vacuna de ADN.

References

1. Arenas-Gamboa A.M., Ficht T.A., Davis D.S., Elzer P.H., Wong-Gonzalez A. & Rice-Ficht A.C. (2009). – Enhanced immune response of red deer (*Cervus elaphus*) to live RB51 vaccine strain using composite microspheres. *J. Wildl. Dis.*, **45**, 165–173.

2. Barrio M.B., Grilló M.J., Muñoz P.M., Jacques I., González D., de Miguel M.J., Marín C.M., Barberán M., Letesson J.-J., Gorvel J.P., Moriyón I., Balsco J.M. & Zygmunt M.S. (2008). – Rough mutants defective in core and O-polysaccharide synthesis and export induce antibodies reacting in an indirect ELISA with smooth lipopolysaccharide and are less effective than Rev 1 vaccine against *Brucella melitensis* infection of sheep. *Vaccine*, **27**, 1741–1749.

3. Bimczok D. & Rothkotter H.J. (2005). – Lymphocyte migration studies. *Vet. Res.*, **37**, 325–338.

4. Blasco J.M. (2010). – Control and eradication strategies for *Brucella melitensis* infection in sheep and goats. *Prilozi*, **31**, 145–165.

5. Buddle B.M., Wedlock D.N., Denis M. & Skinner M.A. (2005). – Identification of immune response correlates for protection against bovine tuberculosis. *Vet. Immunol. Immunopathol.*, **108**, 45–51.

6. Caporale V., Bonfini B., Giannatale E.D., Di Provvido A., Forcella S., Giovanni A., Tittarelli M. & Scacchia M. (2010). – Efficacy of *Brucella abortus* vaccine strain RB51 compared to the reference vaccine *Brucella abortus* strain 19 in water buffalo. *Vet. ital.*, **46** (1), 13–19.

7. Cheville N.F., Stevens M.G., Jensen A.E., Tatum F.M. & Halling S.M. (1993). – Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *B. abortus*. *Am. J. vet. Res.*, **54**, 1591–1597.

8. Clapp B., Walters N., Thornburg T., Hoyt T., Yangh X. & Pascual D.W. (2011). – DNA vaccination of bison to brucellar antigens elicits elevated antibody and IFN-γ responses. *J. Wildl. Dis.*, **47**, 501–510.

9. Davis D.S., Templeton J.W., Ficht T.A., Huber J.D., Angus R.D. & Adams L.G. (1991). – *Brucella abortus* in bison. II. Evaluation of strain 19 vaccination of pregnant cows. *J. Wildl. Dis.*, **27**, 258–264.

10. Demento S.L., Cui W., Criscione J.M., Stern E., Tulipan J., Kaech S.M. & Fahmy T.M. (2012). – Role of sustained antigen release from nanoparticle vaccines in shaping the T cell memory phenotype. *Biomaterials*, **33**, 4957–4964.

11. Deqiu S., Donglou X. & Jiming Y. (2002). – Epidemiology and control of brucellosis in China. *Vet. Microbiol.*, **90**, 168–182.

12. Dhama K., Mahendran M., Gupta P.K. & Rai A. (2008). – DNA vaccines and their applications in veterinary practice: current perspectives. *Vet. Res. Commun.*, **32**, 341–356.

13. Diptee M.D., Adesiyun A.A., Asgarali Z., Campbell M. & Fosgate G.T. (2005). – Evaluation of cell-mediated immune responses and bacterial clearance in 6-10 months old water buffalo (*Bubalus bubalis*) experimentally vaccinated with four dosages of commercial *Brucella abortus* strain RB51 vaccine. *Vet. Immunol. Immunopathol.*, **106** (3–4), 209–220.

14. Edmonds M.D., Cloeckaert A., Hagius S.D., Samartino L.E., Fulton W.T., Walker J.V., Enright F.M., Booth N.J. & Elzer P.H. (2002). – Pathogenicity and protective activity in pregnant goats of a *Brucella melitensis* Deltaomp25 deletion mutant. *Res. vet. Sci.*, **72** (3), 235–239.

15. Elias D., Alruffo H. & Britton S. (2005). – Induced *in vitro* interferon gamma production is not a reliable correlate of protection against *Mycobacterium tuberculosis*. *Trans. roy. Soc. trop. Med. Hyg.*, **99**, 363–368.

16. Elzer P.H., Enright F.M., McQuiston J.R., Boyle S.M. & Schurig G.G. (1998). – Evaluation of a rough mutant of *Brucella melitensis* in pregnant goats. *Res. vet. Sci.*, **64**, 259–260.

17. Fiorentino M.A., Campos E., Cravero S., Arese A., Paolicchi F., Campero C. & Rossetti O. (2008). – Protection levels in vaccinated heifers with experimental vaccines *Brucella abortus* M1-luc and INTA 2. *Vet. Microbiol.*, **132**, 302–311.

18. Fosgate G.T., Adesiyun A.A., Hird D.W., Johnson W.O., Hietala S.K., Schurig G.G., Ryan J. & Diptee M.D. (2003). – Evaluation of brucellosis RB51 vaccine for domestic water buffalo (*Bubalus bubalis*) in Trinidad. *Prev. vet. Med.*, **58**, 211–225.

19. Gicheru M.M., Olobo J.O., Anjili C.O., Orago A.S., Modabber F. & Scott P. (2001). – Vervet monkeys vaccinated with killed *Leishmania major* parasites and interleukin-12 develop a type 1 immune response but are not protected against challenge infection. *Infect. Immun.*, **69**, 245–251.

20. Hu X.D., Yu D.H., Chen S.T., Li S.X. & Cai H. (2009). – A combined DNA vaccine provides protective immunity against *Mycobacterium bovis* and *Brucella abortus* in cattle. *DNA Cell Biol.*, **28**, 191–199.

21. Ivanov A.V., Salmakov K.M., Olsen S.C. & Plumb G.E. (2011). – A live vaccine from *Brucella abortus* strain 82 for control of cattle brucellosis in the Russian Federation. *Anim. Hlth Res. Rev.*, **12**, 113–121.

22. Jelastopulu E., Bikas C., Petropoulos C. & Leotsinidis M. (2008). – Incidence of human brucellosis in a rural area in Western Greece after the implementation of a vaccination programme against animal brucellosis. *BMC Public Hlth*, **8**, 241.

23. Kagan J.C. & Iwasaki A. (2012). – Phagosome as the organelle linking innate and adaptive immunity. *Traffic*, **13** (8), 1053–1061.

24. Kahl-McDonagh M.M., Elzer P.H., Hagius S.D., Walker J.V., Perry Q.L., Seabury C.M., den Hartigh A.B., Tsolis R.M., Adams L.G., Davis D.S. & Ficht T.A. (2006). – Evaluation of novel *Brucella melitensis* unmarked deletion mutants for safety and efficacy in the goat model of brucellosis. *Vaccine*, **24**, 5169–5177.

25. Kreeger T.J., Cook W.E., Edwards W.H., Elzer P.H. & Olsen S.C. (2002). – *Brucella abortus* strain RB51 vaccination in elk II. Failure of high dosage to prevent abortion. *J. Wildl. Dis.*, **38**, 27–31.

26. Kreeger T.J., Miller M.W., Wild M., Elzer P.H. & Olsen S.C. (2000). – Safety and efficacy *of Brucella abortus* strain RB51 vaccine in captive pregnant elk. *J. Wildl. Dis.*, **36**, 477–483.

27. Liu J., Liu S. & Cao X. (2012). – Highlights of the advances in basic immunology in 2011. *Cell Molec. Immunol.*, **9**, 197–207.

28. Mahapatro A. & Singh D.K. (2011). – Biodegradable nanoparticles are excellent vehicle for site directed *in-vivo* delivery of drugs and vaccines. *J. Nanotechnology*, **9**, 55.

29. Martins H., Garin-Bastuji B., Lima F., Flor L., Pina Fonseca A. & Boinas F. (2009). – Eradication of bovine brucellosis in the Azores, Portugal: outcome of a 5-year programme (2002–2007) based on test-and-slaughter and RB51 vaccination. *Prev. vet. Med.*, **90** (1–2), 80–89.

30. Moon J.J., Suh H., Polhemus M.E., Ockenhouse C.F., Yadava A. & Irvine D.J. (2012). – Antigen-displaying lipid-enveloped PLGA nanoparticles as delivery agents for a *Plasmodium vivax* malaria vaccine. *PLoS ONE*, **7**, e31472.

31. Mott G.A., Wilson R., Fernando A., Robinson A., MacGregor P., Kennedy D., Schaap D., Matthews J.B. & Matthews K.R. (2011). – Targeting cattle-borne zoonoses and cattle pathogens using a novel trypanosomatid-based delivery system. *PLoS Pathog.*, **7**, 1–9.

32. Oh J.E. & Lee H.K. (2012). – Modulation of pathogen recognition by autophagy. *Front. Immunol.*, **3**, 1–8.

33. Oliveira S.C., Giamartolomei G.H. & Cassataro J. (2011). – Confronting the barriers to develop novel vaccines against brucellosis. *Expert Rev. Vaccines*, **10**, 1291–1305.

34. Olsen S.C. & Hennager S.G. (2010). – Immune responses and protection against experimental *Brucella suis* biovar 1 challenge in nonvaccinated or *B. abortus* strain RB51-vaccinated cattle. *Clin. vaccine Immunol.*, **17**, 1891–1895.

35. Olsen S.C. & Johnson C.S. (2012). – Efficacy of dart or booster vaccination with strain RB51 in protecting bison against experimental *Brucella abortus* challenge. *Clin. vaccine Immunol.*, **19**, 886–890.

36. Olsen S.C., Boyle S.M., Schurig G.G. & Sriranganathan N.N. (2009). – Immune responses and protection against experimental challenge after vaccination of bison with *Brucella abortus* strain RB51 or RB51 overexpressing superoxide dismutase and glycosyltransferase genes. *Clin. vaccine Immunol.*, **16**, 535–540.

37. Olsen S.C., Fach S.J., Palmer M.V., Sacco R.E., Stoffregen W.S. & Waters W.R. (2006). – Immune responses of elk to initial and booster vaccinations with *Brucella abortus* strain RB51 or 19. *Clin. vaccine Immunol.*, **13**, 1098–1103.

38. Olsen S.C., Jensen A.E., Stoffregen W.C. & Palmer M.V. (2003). – Efficacy of calfhood vaccination with *Brucella abortus* strain RB51 in protecting bison against brucellosis. *Res. vet. Sci.*, **74**, 17–22.

39. Paryam J., Zhou W.Z., Prabha S., Sahoo S.K. & Labhasentvar V. (2002). – Rapid endolysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. *FASEB J.*, **16**, 1217–1226.

40. Ramnanan A. (2010). – Response of water buffalo (*Bubalus bubalis*) to experimental challenge with *Brucella abortus* biovar 1 following vaccination with two dosage of *Brucella abortus* strain RB51. M. Phil. thesis submitted to the University of the West Indies, St Augustine Campus, Trinidad and Tobago.

41. Rittig M.G., Kaufmann A., Robins A., Shaw B., Sprenger H., Gensa D., Foulongne V., Rouot B. & Dornand J. (2003). – Smooth and rough lipopolysaccharide phenotypes of *Brucella* induce different intracellular trafficking and cytokine/chemokine release in human monocytes. *J. Leukoc. Biol.*, **74**, 1045–1055.

42. Rivers R., Andrews E., González-Smith A., Donoso G. & Oñate A. (2006). – *Brucella abortus*: immunity, vaccines and prevention strategies based on nucleic acids. *Arch. Med. vet.*, **38**, 7–18.

43. Roth F., Zinsstag J., Orkhon D., Chimedj-Ochir G., Hutton G., Cosivi O., Carrin G. & Otte J. (2003). – Human health benefits from livestock vaccination for brucellosis: case study. *Bull. WHO*, **81**, 867–876.

44. Sáez D., Guzmán I., Andrews E., Cabera A. & Oñate A. (2008). – Evaluation of *Brucella abortus* DNA and RNA vaccines expressing Cu-Zn superoxide dismutase (SOD) gene in cattle. *Vet. Microbiol.*, **129**, 396–403.

45. Scheerlinck J.P. & Greenwood D.L.V. (2008). – Virus-sized vaccine delivery systems. *Drug Discov. Today*, **13**, 882–887.

46. Stoffregen W.C., Johnson C.A. & Olsen S.C. (2013). – Immunogenicity and safety of a natural rough mutant of *B. suis* as a vaccine for swine. *Rev. vet. Sci.* (in press).

47. Thakur A., Pedersen L.E. & Jungersen G. (2012). – Immune markers and correlates of protection for vaccine-induced immune responses. *Vaccine*, **30**, 4907–4920.

48. Torrado E. & Cooper A.M. (2011). – IL-17 and Th17 cells in tuberculosis. *Cytokine Growth Fact. Rev.*, **21**, 455–462.

49. Underhill D.M., Ozinsky A., Hajar A.M., Stevens A., Wilson C.B., Bassetti M. & Aderem A. (1999). – The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature*, **401**, 811–816.

50. Vemulapalli R., He Y., Cravero S., Sriranganathan N., Boyle S.M. & Schurig G.G. (2000). – Overexpression of protective antigen as a novel approach to enhance vaccine efficacy of *Brucella abortus* strain RB51. *Infect. Immun.*, **68** (6), 3286–3289.

51. Ward D., Jackson R., Karomatullo H., Khakimov T., Kurbonov K., Amirbekov M., Stack J., El-Idrissi A. & Heuer C. (2012). – Brucellosis control in Tajikistan using Rev1 vaccine: change in seroprevalence in small ruminants from 2004 to 2009. *Vet. Rec.*, **170**, 100.

52. Wyckoff J.H., Howland J.L., Scott C.M., Smith R.A. & Confer A.W. (2005). – Recombinant bovine interleukin 2 enhances immunity and protection induced by *Brucella abortus* vaccines in cattle. *Vet. Microbiol.*, **111**, 77–87.

53. Zinsstag J., Roth F., Orkhon D., Chimed-Ochir G., Nansalmaa M., Kolar J. & Vounatsou P. (2005). – A model of animal-human brucellosis transmission in Mongolia. *Prev. vet. Med.*, **69**, 77–95.