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Review

Status of the development of a vaccine against *Mycoplasma bovis* ☆

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ABSTRACT

Mycoplasma bovis is an important pathogen of cattle and, despite numerous efforts an effective vaccine for control of the disease it causes remains elusive. Although we now know more about the biology of this pathogen, information is lacking about appropriate protective antigens, the type of immune response that confers protection and adjuvants selection. The use of conserved recombinant proteins, selected using in silico approaches, as components of a vaccine may be a better choice over bacterin-based vaccines due to the limited protection afforded by them and adverse reactions caused by them. More studies are needed on the characterization of host-pathogen interactions and to elucidate *M. bovis* products modulating these interactions. These products could be the basis for development of vaccines to control *M. bovis* infections in dairy farms and feedlots.

Contents

1. Introduction

Mycoplasma bovis is the causative agent of numerous diseases in cattle that have severe economic consequences for producers. The Chronic Pneumonia and Poly-arthritis Syndrome (CPPS) caused by *M. bovis* is associated with the Bovine Respiratory Disease (BRD) complex, an economically important disease in feedlot cattle [1]. In dairy cattle, *M. bovis* is probably the most common causative agent of mycoplasma mastitis although other mycoplasma species have been isolated from the milk of affected animals [2,3]. As a sequela of infection with *M. bovis*, arthritis and otitis media is sometimes observed in beef and dairy cattle. Affected animals present with clinical signs such as lameness, swelling of joints and ultimately weight loss as a consequence of impaired movement [1]. Keratoconjunctivitis, orchitis, infertility and decubital abscesses have been reported at lower frequency [1,4]. In a recent report, Gille et al. described post-surgical seromas as a new predilection site for *M. bovis* infections [5].

Due to their lack of a cell wall, the antibiotic arsenal available to treat *M. bovis* infections is limited, and numerous reports indicate that resistance to several antibiotics is on the rise (reviewed in [6]), compounding this problem, the cost of multiple antibiotic treatments adds considerable financial burden to the producer. This suggests that prevention and/or control of *M. bovis* infection by vaccination would be a valuable alternative. Research on *M. bovis* vaccines has been active for many years and this review is focused on the many vaccine candidate antigens identified so far; and the results of testing numerous experimental vaccines composed of bacterins, recombinant proteins, or live-attenuated strains.

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2. The challenges

There are numerous challenges that hinder the success of vaccines to prevent *M. bovis* infections. Among these challenges are colonization of young animals' upper respiratory tract, quality and modulation of the host immune response, the role of other respiratory pathogens, and the need for a challenge model that reproduces the disease seen in the field. The upper respiratory tract of young animals is colonized at a very early age by contact with shedding animals and also by ingestion of contaminated milk (reviewed in [4]). Adhesion to epithelial cells aids in the colonization but the adhesion capacity varies between isolates [7]. The variable-surface proteins (Vsps) and other surface proteins have been associated with attachment to host cells [7,8]. One complicating factor is that the capacity of Vsps for phase and antigenic variation [9]. The host-immune responses to *M. bovis* after natural or experimental infection provide important information that may help in designing a successful vaccine. In general, the immune response to *M. bovis* antigens is skewed to the Th-2 arm as they induce more IgG1 than IgG2 antibodies [10–12]. Activation of CD4+, CD8+ and γ/δ T-cells has been observed in response to heat-killed *M. bovis* [11], but not to live bacterial cells [13–17]. Although *M. bovis* is predominantly an extracellular pathogen, there is *in vivo* and *in vitro* evidence that suggests the potential for the bacterium to enter host cells [18–22]. Intracellular *M. bovis* can survive inside cells [13,20,21]; modulate cytokine expression [13,23–26] and apoptosis [13,20,27]; or directly play a role in pathogenesis [28]. Thus, because of the extra- and intracellular presence of *M. bovis* we believe that a vaccine that equally induces Th-1 and Th-2 responses would be more advantageous.

The current evidence strongly suggests that in cattle *M. bovis* is a secondary pathogen and that the contribution of other respiratory pathogens must be considered [29]. Immuno-suppressive viral pathogens such as bovine viral diarrhea virus (BVDV) and bovine herpes virus 1 (BHV-1) have long been associated with *M. bovis* respiratory disease in Canadian feedlots [30–34]. In 2014, Klima et al. reported the prevalence of BRD-associated pathogens in 68 cases in North American feedlots [35]. While *Mannheimia haemolytica* and BVDV-1 were the two pathogens most prevalent (91% and 69% respectively), the proportion of *M. bovis* detected by PCR was of 43%. The presence of BVDV-2 was only detected in Canadian animals however BHV-1 was not found. The authors suggest that this was due to limited sampling size and/or absence of viral DNA in the nasal mucus. The highest co-occurrence (16.2%) of pathogens was the combination of *Mannheimia* spp., BVDV-1 and *M. bovis*. In 11.2% of the cases, these three pathogens were also found with *Histophilus somni*. Finally, *Pasteurella multocida* was isolated from few samples. Thus, preventive measures against disease caused by *M. bovis* must take in consideration management of other respiratory pathogens by antimicrobial treatment and/or vaccination.

Testing of vaccine candidates greatly depends on the use of a challenge model that consistently reproduces the disease. Factors such as the age of the animals, the challenge dose, the challenge protocol and the role of other respiratory pathogens must be taken into account. A number of laboratories have reported success of their experimental vaccines after multiple challenges (up to three times) of young animals (ranging from 3 weeks to 5 months-old) with large doses of *M. bovis* (in the range of 10^9 to 10^{10} colony-forming units [cfu]) [36–38]. In these reports, the success of the challenges is associated with the onset of clinical signs such as dyspnea, nasal discharge, moderate fever, weight loss, the presence of characteristic macroscopic lesions, such as lung consolidation, adhesions, and caseonecrotic pneumonic lesions; microscopic lung lesions, such as suppurative bronchiolitis, lymphoid hyperplasia, intra-alveolar and intrabronchial exudates, and coagulative necrosis, and isolation of *M. bovis* from challenged animals. The clinical signs, gross and microscopic lung lesions, and isolation of *M. bovis* are consistent with the lesions seen in the feedlot animals. However the magnitude of these lesions, particularly the extent of lung involvement, and the number of caseonecrotic lesions and the degree of suppurative pneumonia [34,40,41] is less than seen in field cases. This could be due to the lesion age in feedlot animals or to the contribution of other respiratory pathogens.

In all these trials, the vaccines were solely tested against a *M. bovis* challenge but the role that other pathogens may have in the success or failure of the vaccines was not taken into account. Because of the association of *M. bovis* with other respiratory pathogens (see above), we wanted to establish a co-challenge model to test experimental vaccines. In 6 to 8 month-old Canadian feedlot cattle, a single intranasal challenge (5×10^8 cfu/ml) was sufficient to cause disease in animals previously exposed to BHV-1 [39]. We did not see disease in animals challenged with *M. bovis* only (intratracheal dose of 5×10^{10} cfu/ml) or in animals previously infected with BVDV-2 [39]. In this co-challenge model, the magnitude of the lesions more closely resembled the lesions seen in the feedlot animals [34,40,41].

3. *Mycoplasma bovis* vaccine candidates

3.1. Protein vaccine candidates

M. bovis cells display highly variable antigens on their surface. The most prominent of these are the variable surface proteins (Vsps). The Vsp family is composed of 13 lipoproteins that can generate a high degree of antigenic variation through genetic recombination [42,43]. Of the 13 Vsps identified, VspA, VspB and VspC are the most immunogenic [44] and thus they may be ideal targets for vaccines. However the high degree of antigenic variation in these lipoproteins may make the vaccines ineffective in the long run. Epitope mapping of the VspA, VspB, VspE and VspF proteins has identified several regions that are involved in adherence to embryonic bovine lung (EBL) cells [44]. The authors pointed out that because these epitopes were linear they may not be ideal targets for vaccines and as an alternative they suggested DNA vaccination with plasmids containing epitopes from variable and non-variable regions. To date, it is not clear whether such a DNA vaccination approach has been assessed. The surface expressed α-enolase protein of *M. bovis* has been characterized [45]. Its surface expression and binding to plasminogen combined with the fact that α-enolase of *Streptococcus iniae* has been shown to be protective in mice and zebra fish models [46,47], suggests that it has potential target for vaccine development in *M. bovis* but as yet, there have been no reports of the assessment of α -enolase in vaccine trials.

Numerous *M. bovis* proteins have been studied to evaluate their role in adherence. Sachse et al. described the capacity of a mAb against a 26 kDa *M. bovis* protein to inhibit adherence to EBL cells [48]. The mAb Mb4F6 was incubated with two strains of *M. bovis* that had differing adherence intensity. The mAb Mb4F6 more strongly inhibited adherence of the strain 454 than of the more adherent *M. bovis* strain 120. The strain 454 expressed less of the 26 kDa protein than the strain 120 suggesting that more mAb was able to bind to strain 454, resulting in more inhibition [48]. The identity of the 26 kDa protein remains unknown, but conceptually it could be used as a potential vaccine target.

Due to the high level of antigenic variation in *M. bovis*, the best vaccine targets are likely to be proteins that are conserved across strains. One example of such a protein is lipoprotein P48. Robino et al. reported that the P48 protein was detectable in all field isolates tested [49]. Compared to uninfected animals, antibody