



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Available online at www.sciencedirect.com



Comparative Immunology, Microbiology
& Infectious Diseases 31 (2008) 227–238

C O M P A R A T I V E
I M M U N O L O G Y
M I C R O B I O L O G Y &
I N F E C T I O U S
D I S E A S E S

www.elsevier.com/locate/cimid

Contagious bovine pleuropneumonia: A rationale for the development of a mucosal sub-unit vaccine

Laurence Dedieu-Engelmann*

*CIRAD, Département BIOS, UPR15, TA A15/G, Campus International de Baillarguet,
34398 Montpellier Cedex 5, France*

Accepted 12 July 2007

Abstract

Contagious bovine pleuropneumonia (CBPP) remains a major cattle disease in Africa with serious socio-economic consequences. Its eradication requires the development of improved vaccines. Knowledge on this disease and its causing agent, *Mycoplasma mycoides* subsp. *mycoides* biotype Small Colony (*MmmSC*), has been progressing significantly in the last years, opening new areas for vaccine design. Advances were achieved in the understanding of the protective immune responses to *MmmSC* infection and immunopathological mechanisms allowing the pathogen to escape the host immune response. Based on sequencing and genomic studies, some virulence factors and metabolic pathways were unraveled leading to the identification of potential *MmmSC* vaccine candidates. Based on these findings, this review presents a scientific strategy to design multi-component sub-unit vaccines for mucosal delivery as the most promising approach for efficient long-term protective vaccines to prevent CBPP. © 2007 Elsevier Ltd. All rights reserved.

Keywords: *Mycoplasma mycoides* subsp. *mycoides* SC (*MmmSC*); Mycoplasmas; Immune responses; Vaccine antigens; Mucosal vaccines; Sub-unit vaccines; Oral vaccines

*Tel.: +33 4 67 59 38 16; fax: +33 4 67 59 37 98.

E-mail address: laurence.dedieu@cirad.fr

Résumé

La péripneumonie contagieuse bovine reste une maladie majeure des bovins en Afrique avec de sérieuses conséquences socio-économiques. Son éradication nécessite le développement de vaccins améliorés. Ces dernières années, les connaissances de cette maladie comme de l'agent pathogène responsable, *Mycoplasma mycoides* subsp. *mycoides* biotype Small Colony (*MmmSC*), ont largement évolué, ouvrant la voie à de nouvelles approches vaccinales. Ces avancées ont été obtenues dans la compréhension des réponses immunitaires protectrices face à *MmmSC* ainsi que des mécanismes immunopathologiques permettant à *MmmSC* d'échapper à la réponse de l'hôte. De plus, grâce au séquençage et à des études génomiques, certains facteurs de virulence et cycles métaboliques de *MmmSC* ont été caractérisés permettant l'identification de candidats vaccinaux potentiels. A partir de ces données, cette revue présente une stratégie scientifique pour le développement de vaccins sous-unitaires multi-composants à délivrance mucoale, comme étant l'approche la plus prometteuse pour des vaccins protecteurs à long terme contre la PPCB.

© 2007 Elsevier Ltd. All rights reserved.

Mots clés: Péripneumonie contagieuse bovine (PPCB); *Mycoplasma mycoides* subsp. *mycoides* SC (*MmmSC*); Mycoplasmes; Réponse immunitaire; Antigènes vaccinaux; Vaccins muqueux; Vaccins sous-unitaires; Vaccins oraux

1. Introduction and rationale

Contagious bovine pleuropneumonia (CBPP) remains one of the major cattle diseases in Africa. It is caused by *Mycoplasma mycoides* subsp. *mycoides* biotype Small Colony (*MmmSC*). CBPP is responsible for heavy economic losses due to mortality, loss of weight, reduced working ability or fertility. A recent study evaluated the total economic cost of CBPP in Africa (direct and indirect production losses and disease control costs) at 44.8 million euros [1]. Accurate data, requiring efficient epidemiological surveillance networks, are however lacking [2]. CBPP is included in the Office International des Epizooties (OIE) list of pathologies requiring official declaration (<http://www.oie.int>). Infected countries are thus excluded from international trade.

CBPP was eradicated during the twentieth century from most of the world, except limited areas in Europe and Asia, based on a program combining slaughtering, control of cattle movement and vaccination. However, in Africa, the sanitary measures used on other continents to successfully eradicate CBPP are impracticable since cattle raising rely on nomadism and transhumance. The disease was reported in 17 countries in 2001 and in 20 countries in 2004 (<http://www.oie.int/hs2/>). In 1994, in Botswana, stamping out the whole cattle population in the affected zones was the ultimate choice of the veterinary services, because vaccination with the available T1 vaccine failed to stop the spread of the disease. This strategy proved very efficient and enabled Botswana to regain its freedom status, but it remains too expensive to be applied in other African countries [2].

To achieve eradication of the disease from Africa, the development of improved vaccines remains the only realistic approach. The T1-based vaccines currently in use are of low efficacy and require annual, costly vaccination campaigns [3]. These vaccines, made from freeze-dried broth cultures of live attenuated *MmmSC* strains (T1/44 and T1SR), present severe limitations such as the short duration of immune protection, although longer for the T1/44 strain compared to the T1/SR, and persistent side effects, mainly for the T1/44 strain [3]. Several studies have suggested a possible improvement of their efficacy through the combined use of antibiotics, changes in vaccine formulation, or the use of a prime-boost strategy [2,4]. However, this might be too expensive or insufficient to overcome the intrinsic limitations of the vaccine. Based on immunological knowledge, another approach could rely on new routes of vaccine delivery. Indeed, it is well known for mucosal diseases, such as CBPP, that mucosal delivery would significantly improve the efficacy of the host immune response [5]. So far, however, this approach has not received the attention it deserves. There is, therefore, a need for improved vaccines that could overcome the T1 vaccine limitations and trigger long-term protection and which, coupled to effective surveillance systems and control of animal movements, would represent the most relevant tool to achieve eradication of CBPP from Africa.

The proof of feasibility of triggering a long-term protective immune response against CBPP by vaccination stems from the observation that cattle recovering from an *MmmSC* infection are then protected against new *MmmSC* infections [3,6,7]. Recovering cattle, therefore, not only succeed in controlling the *MmmSC* infection by mounting an appropriate immune response, but also retain in their lymph nodes an *MmmSC*-specific immune memory able to trigger an anamnestic protective response [8]. This objective is also supported by the experiments made by Willems in 1852 showing that subcutaneous inoculation of CBPP-infective lymph protected cattle against an *MmmSC* challenge [9]. These findings demonstrate that a long-term protective immune response against *MmmSC* can be naturally developed and most probably also vaccine induced in cattle.

Rational development of optimized vaccines requires a good understanding of the immunological bases of protection against infection, as well as an understanding of the immunopathological mechanisms underlying the disease and the identification of the components of the pathogen involved in both these aspects. Recent studies have led to improved knowledge of CBPP, which leads to defining more relevant vaccine strategies against the disease. This review gives a rapid overview of most recent results and discusses rational strategies for the development of optimized sub-unit vaccines. Because the safety of vaccines is paramount and because live vectored vaccines, although powerful, raise some concerns regarding safety, stability, potential reversion to virulence or effectiveness in the presence of pre-existing immunity, the trend in vaccinology now focuses on the design of safer and better-defined sub-unit vaccines.

2. Rational strategy to develop sub-unit vaccines to prevent CBPP

A vaccinal strategy is determined by (1) the protective immune parameters to trigger; (2) the immunopathological mechanisms to control; and (3) the antigens from the pathogen to include in the vaccine.

2.1. Protective immune parameters

The objectives of a vaccine are to elicit, or at least prime the relevant immune responses allowing the vaccinated host to quickly mount an efficacious protective response when in contact with a new infection. Identifying the *MmmSC*-specific immune protective mechanisms is thus a major prerequisite to the development of efficient vaccines. To this aim, recent studies were implemented to characterize the immune response elicited in cattle after natural *MmmSC* infection. Comparative analyses of the *MmmSC*-specific humoral and cellular immune responses were performed between animals recovering or dying from the *MmmSC* infection. Cattle recovering from an *MmmSC* infection are known to develop long-term immunity [6,7]. Characterization of the *MmmSC*-specific immune mechanism generated, therefore, during the primary immune response and persisting in the regional lymph nodes of these recovered animals will thus unravel the basis of immune protection against CBPP and the *MmmSC*-specific memory response, responsible for the protective anamnestic response. Understanding the mechanisms operative in the development of these memory T-cells is a central goal in the development of effective vaccination strategies.

The results of these studies revealed that cattle recovering from an *MmmSC* infection, compared to animals with acute infection, were characterized by (1) a stronger and persisting local IgA response [10] and (2) a higher *MmmSC*-specific CD4+ T-cell response with IFN γ production detected in blood and persisting in the respiratory lymph nodes several months after infection [8,11]. Data on the *MmmSC*-specific local cell-mediated immune response, although very valuable to unravel the complete protective mechanisms against CBPP, are not yet available due to the difficulty to assess this aspect in cattle. Nevertheless, a key finding is the persistence of the *MmmSC*-specific IFN γ -secreting CD4+ T-cells in the draining lymph nodes of recovered cattle several months after infection. Indeed, studies of several viral or bacterial diseases indicate that while a pool of effector T-cells is expanded during active infection, only memory cells remains detectable after infection resolution [12]. Both arms of the immune system are, therefore, playing a role in protection against CBPP, the local humoral response relying on secretory IgAs and the cellular response based on CD4+ T-cells of the Th1-like type. Consequently, both represent the main target to be elicited or primed by a vaccine with the induction and expansion of a subset of *MmmSC*-specific CD4+ memory T-cells as the required goal to develop long-term protection against CBPP.

CBPP being a respiratory disease whose pathogen generally remains restricted to the lungs, a key role for the local humoral response was predictable. Immunoglobulin A is, indeed, the main element of the humoral response to provide protection against microbial antigens entering mucosal surfaces and represents an important first line of defense against invasion of deeper tissues [5]. The IgA response might play a direct role by inhibiting *MmmSC* growth and colonization and/or blocking some yet unknown virulence factors. A CD4+ T-cell response was also expected since *MmmSC* is an extracellular pathogen [13]. Extracellular pathogens are generally taken up by antigen presenting cells (APC) and processed in association

with the class II molecules of the major histocompatibility complex (MHC) for presentation to the CD4⁺ T-lymphocytes. CD4⁺ T-cells should contribute to protection against CBPP mainly through their cytokine network including IFN γ that will enhance phagocytosis and cell killing ability and promote the expression of MHC class II molecules, thus increasing the host cell capacity for antigen processing and epitope presentation to T-lymphocytes [14]. A protective role for the IFN γ -induced IgG2 antibody, only able to promote killing by neutrophils in cattle, is also expected since neutrophil recruitment has been observed in CBPP lung lesions [15–17]. Besides, CD4⁺ T-cells play also a key role in facilitating B-cell proliferation and class-switching to give rise to antigen-specific IgAs in mucosal effector sites [5]. Whether Th1 or Th2 cells are beneficial for optimal IgA production is not known as either Th1 or Th2 cells or their combination can support antigen-specific IgA responses [5].

Based on these findings, CBPP vaccines should be able to trigger both a local antibody response and a persistent CD4⁺, Th1-like T-cell-mediated response to selected *MmmSC* components.

2.2. Immunopathological mechanisms

Understanding the immunopathological aspects of CBPP is also an important step for the rational design of an optimized vaccine. Indeed, stimulating a vaccine-induced protective immune response implies that the host still maintains its ability to mount an efficient anamnestic response when in contact with an *MmmSC* infection. However, *MmmSC* appears to possess an efficient mechanism to escape the host immune response, as suggested by its ability to survive in encapsulated lung lesions but also in lymph nodes of infected cattle [18,19].

To characterize this immunomodulatory property, *MmmSC* interactions with bovine immune cells were analyzed *in vitro*. We thus demonstrated that (1) viable *MmmSC* was able to trigger a time-dependent apoptosis of bovine blood leukocytes whereas heat-killed *MmmSC* had no such effect [20]; (2) soluble *MmmSC*-secreted components appeared to play a role in this process [20]; and (3) viable *MmmSC* strongly depressed the mitogenic activity of ConA on bovine T-cells [21]. This *MmmSC*-mediated immunosuppressive effect might thus allow the pathogen to escape the host response *in vivo* by diminishing its ability to mount an immune response to antigenic challenge. Although further studies are necessary to fully unravel this mechanism, these results confirm the importance of taking into account the immunopathological aspects of the disease in the rational development of a vaccine.

Besides this immunosuppressive effect on T-cell responsiveness, *MmmSC* also appears able to escape the innate and humoral host responses. *MmmSC* is characterized, indeed, by the presence of a polysaccharide (PS) external pseudo-capsule. Genome sequencing has identified the metabolic pathway leading to the synthesis of the capsular PS [22]. The pseudo-capsule was shown to have a role in virulence and to protect *MmmSC* from the host antibody response and cell phagocytosis [23,24].

These data confirm that *MmmSC* did evolve efficient ways to escape the bovine immune response. These should be controlled to achieve full vaccine efficacy, otherwise the impairment of the host immune system (innate, humoral and cellular) by exposure to *MmmSC* infection will lead to inefficient vaccine-induced anamnestic responses. To this aim, elucidation of the *MmmSC* component(s) which mediate these pathological effects will help identifying critical vaccine candidate antigens able to control these mechanisms and thus enhance vaccine efficacy.

2.3. Potential *MmmSC* candidate antigens for a multi-component sub-unit vaccine

Screening of *MmmSC* components and genetic libraries with sera and bronchoalveolar lavages taken from naturally *MmmSC*-infected cattle, has already revealed a panel of IgA- and IgG2-specific proteins ([25] Du Plessis, personal communication). Several of these components are membrane lipoproteins (Lpps), which might have a central role in interaction between *MmmSC* and the host cells and in the inflammatory reaction [25,26]. Besides the capacity of Lpps to elicit an humoral response, their strong potential to trigger also a cell-mediated response and their major role in the early host response was reported [27,28]. Some proteins are known to display both B-cell and T-cell epitopes and several vaccines such as those against influenza, malaria or *Yersinia pestis* are based on this type of antigens [29–31]. A similar approach to select *MmmSC* vaccine candidates is currently being undertaken by *in vitro* assessment of the ability of a panel of antibody-inducing *MmmSC* antigens to elicit an *MmmSC*-specific CD4⁺ Th1-like T-cell response (Dedieu, unpublished data). To this aim, the capacity of four *MmmSC* Lpps (LppA, LppB, LppC and LppQ) to recall *in vitro* a cellular immune response from lymphocytes taken from three *MmmSC*-infected cattle was evaluated. The results showed that LppA was the only *MmmSC* protein recognized by the *MmmSC*-primed lymphocytes (Dedieu, submitted). This finding demonstrates that *MmmSC*-infected cattle, besides their known humoral response against *MmmSC* LppA, do also develop an LppA-specific T-cell response. The *MmmSC* LppA, a highly conserved lipoprotein, might be retained as a potential vaccine candidate against CBPP due to its ability to trigger both arms of the host immune response. At this time, however, the protective efficacy of an LppA-triggered immune response has to be further evaluated.

Besides the screening of *MmmSC* components for their immunogenicity, another approach could target selected *MmmSC* virulence factors. However, in contrast to other pathogenic bacteria, where virulence relies mainly on adhesins or toxins, no such typical virulence factors have been identified in the genome of the *MmmSC* reference strain PG1, recently published, nor on the genomes of the 10 other *mycoplasmas* species that have been completely sequenced [22,26]. This might be due to their small genome, leading *mycoplasmas* to drastic savings in genetic resources, reduced to essential functions of life [32]. *Mycoplasmas* have thus adopted a parasitic mode of life, acquiring macromolecular precursors and high-energy compounds from the host cells.

Genome sequencing and comparative genomic studies have, nevertheless, led to identify some *MmmSC* pathogenic mechanisms, such as the glycerol transport system leading to the production of cytotoxic H_2O_2 , as well as metabolic pathways such as the phosphotransferase system for sugar transport or the several ATP-binding cassette (ABC) transporters for transfer of components such as phosphate or oligopeptides [22,32]. Both represent interesting targets for a vaccine-induced antibody response aiming at controlling *MmmSC* growth and virulence.

Blocking the glycerol transport membrane proteins GtsABC or the membrane-located glycerophosphate oxidase GlpO by specific antibodies resulted, indeed, in inhibition of glycerol uptake or glycerol metabolism and in significant reduction of H_2O_2 production and cytolytic activity [32,33]. Such antibody response might thus control *MmmSC* growth, as glycerol metabolism is presumably required for triglyceride synthesis. Control of the pathogenicity should also be achieved, since H_2O_2 is considered as a virulence factor [32–34]. Similarly, an antibody response blocking membrane components of metabolic pathways such as the ABC transporters will likely limit *MmmSC* growth and thus host infection. Indeed, due to its drastic parasitic mode, *MmmSC* is dependent on these ABC transporters to scavenge precursors from the host cells. Therefore, a screening of these potential *MmmSC* components for their ability to elicit an antibody response, and mainly local IgAs, with a functional role in limiting *MmmSC* growth and virulence, would lead to promising vaccine candidates.

In parallel, a vaccine-induced *MmmSC*-specific $INF\gamma$ -secreting $CD4^+$ T-cell response is also needed. Therefore, *MmmSC* components able to prime such a response also have to be included. These vaccine candidates should be selected by T-cell screening, as described above, either from the selected antibody-inducing *MmmSC* antigens or from other purified *MmmSC* proteins.

The final selection of *MmmSC* vaccine candidates will include an *in vitro* safety control evaluating pathological effects such as the inflammatory potential which is a major characteristic of this pathogen.

This strategy should hopefully lead to the selection of appropriate antigens that, included in a safe, multi-components sub-unit vaccine, will be able to trigger an immune response that will reduce bacterial load and the clinical outcome of infection, while also controlling the immunopathological mechanisms involved in disease, thus allowing the establishment of efficient innate and vaccine-induced immune responses able to clear the infection with *MmmSC* while maintaining an *MmmSC*-specific immune memory.

3. Relevant vaccine delivery system and route of delivery

Non-replicating antigens, such as purified proteins, are often of low immunogenicity. The major challenge to an effective vaccine, once the protective antigens are identified, lies, therefore, in their formulation and delivery. First, the choice of a relevant delivery system and the addition of immunostimulating molecules will allow enhancing, modulating and orientating the host immune response. Then, of major importance is the selection of an effective system capable of inducing a significant

immune response at a mucosal site. Indeed, in CBPP, *MmmSC* enters and initiates infection at the mucosal respiratory surface, which constitutes the best target for effective vaccination. As systemic immunization is largely ineffective at providing immunity at mucosal surfaces [35,36], the mucosal surface should be the choice site for immunization. In fact, mucosal immunization can induce both mucosal and systemic immunity [35].

Mucosal immunization benefits from the existence of a “common mucosal immune system” where immunization at some mucosal inductive sites can activate mucosal B- and T-cells to migrate from these sites and home to mucosal effector sites [5,35]. Accordingly, although intranasal delivery appears the most relevant, the oral route can also be very promising and may offer an inexpensive (no need for needles, professional health care infrastructure required for injectable preparations, or for a cold chain) and convenient way of vaccination (edible vaccine). The effectiveness of this approach is demonstrated by the panel of vaccines currently licensed for mucosal delivery in domestic animals, e.g. vaccines against BHV, coronavirus or rotavirus in cattle [35]. Although these are live vectored vaccines, a large panel of *potent adjuvants* (bacterial toxins or cell wall components, CpG oligonucleotides, antimicrobial peptides) and *delivery systems* (biodegradable microspheres, liposomes, novasomes, VLP, ISCOMs, etc.) are now available for mucosal vaccine delivery [35]. Some of them are able to induce both a mucosal antibody and cellular immune response. Studies in domestic animals, nevertheless, are limited and the potential of mucosal immunization with purified antigens remains unexploited in veterinary medicine [35].

Some experiments in cattle have shown the potential of the cholera toxin and *E. coli* heat-labile (LT) enterotoxin as mucosal immunoadjuvants and the efficacy of oral or intranasal immunization with microparticles [35,37–39]. ISCOMs also have great potential in the delivery of antigens to the mucosa [35]. However, in the two experiments conducted in cattle with ISCOM vaccines against CBPP, the vaccines were delivered parenterally and failed to protect the animals against an *MmmSC* challenge [40,41]. Another possible reason for this failure is that these vaccines were based on either whole inactivated *MmmSC* or the cytotoxic LppQ protein. In contrast, oral delivery in pigs of another pathogenic mycoplasma, *Mycoplasma hyopneumoniae*, included in microspheres led to effective protection [42]. Therefore, these new antigen delivery systems might offer promising approaches for mucosal vaccine delivery in large animals.

Advances in the understanding of the protective immune responses to *MmmSC* infection are providing promising opportunities for the rational development of improved vaccines. Protection was shown to require *MmmSC*-specific pulmonary IgAs and activation of the local macrophages through stimulation of *MmmSC*-specific CD4+ IFN γ -secreting T-cells. Both immunological arms could be effectively targeted by a mucosal vaccine. Indeed, induction of protective secretory IgAs was shown to be efficiently achieved after mucosal antigenic stimulation, such as the targeting of the mucosa-associated lymphoid tissues and local lymph nodes [5]. To enhance the priming of *MmmSC*-specific IFN γ secreting CD4+ T-cells, a Th1-inducing adjuvant will have to be used with a delivery system allowing the

vaccine antigens to be presented by the APC MHC class II pathway. This objective could be achieved using *microparticles* (biodegradable microspheres, chitosan, liposomes, ISCOM, etc.) which can increase host cell phagocytosis, antigen uptake and MHC class II presentation to CD4⁺ T-cells. Besides, the ability of these particulate systems to also elicit local IgAs has largely been demonstrated [35,36]. The potency of these delivery vehicles can be further enhanced by incorporating adjuvants or coating with immunostimulating molecules such as lectins or Toll-like receptor ligands, which would reduce the quantity of antigen needed and thus the cost of the vaccine. Various strategies are already under evaluation to enhance particulate vaccine uptake and efficacy [35].

Selection of the most appropriate system for a CBPP vaccine will depend on the nature of the selected *MmmSC* antigens and on the immunological properties of the available delivery systems. However, in our context of tropical veterinary diseases, where the final objective is vaccine technology transfer to our African partners for local production, other criteria have to be taken into account, such as thermostability, cost effectiveness, manufacturing process and energy requirement, etc. While these are very stringent criteria, one can hope that due to the rapid progress in this field, it will be achievable in the near future. The final choice will then rely on preliminary evaluation by *in vitro* assays for efficacy testing in the presence of the *MmmSC* vaccinal candidates. Unfortunately, no small animal models are relevant for CBPP studies and cattle experiments are very costly as they require high security facilities when dealing with tropical diseases. Therefore, although not fully relevant in terms of mucosal immunity, *in vitro* cellular assays will help determine the capacity of the delivery systems to be efficiently captured by bovine APCs and their efficacy to trigger DC maturation, epitope presentation and *MmmSC*-specific CD4⁺ Th1-like T-cell response from autologous T-cells taken from recovering cattle. Cattle experiments will then permit to evaluate the vaccine-induced mucosal immunity and the protective efficacy against CBPP infection.

4. Conclusion

The objectives of an efficacious CBPP vaccine would be (1) to limit *MmmSC* colonization, thus reducing host infection and the strong inflammatory process typical of the disease; (2) to control the *MmmSC*-induced immunosuppression so as to maintain the responsiveness of the host immune system; and (3) to stimulate an *MmmSC*-specific immune memory response, responsible, in case of a new antigen encounter, of an anamnestic protective response. To this aim, it was shown that the immune parameters that correlate with protection are the mucosal production of *MmmSC*-specific IgAs and a persistent *MmmSC*-specific INF γ -secreting CD4⁺ T-cell response. Accordingly, the *MmmSC* vaccine candidates will have to be selected on these criteria.

The advances in our understanding of CBPP and the better characterization of the causing agent, *MmmSC*, together with the improved knowledge in mucosal immunology and vaccine delivery systems, offer very promising approaches for the development of efficient long-term protective mucosal vaccines to prevent CBPP.

Mucosal sub-unit vaccines against *MmmSC* infection will hopefully limit the shedding of *MmmSC*, thereby reducing the pathogen load in the environment and consequently reducing the rate of herd infection and transmission of CBPP through the herd. It is hoped, therefore, that the availability of optimized vaccines, coupled with an effective surveillance system and stricter control of animal movements will allow one to control and even eventually to eradicate CBPP from the African continent.

References

- [1] Tambi NE, Maina WO, Ndi C. An estimation of the economic impact of contagious bovine pleuropneumonia in Africa. *Rev Sci Tech* 2006;25(3):999–1011.
- [2] Thiaucourt F, Aboubakar Y, Wesonga H, Manso-Silvan L, Blanchard A. Contagious bovine pleuropneumonia vaccines and control strategies: recent data. In: Schudel A, Lombard M, editors. *Control of infectious animal diseases by vaccination*, vol. 119. Dev. Biol. Basel: Karger; 2004. p. 99–111.
- [3] Thiaucourt F, Dedieu L, Maillard JC, et al. Contagious bovine pleuropneumonia vaccines, historic highlights, present situation and hopes. In: Brown F, Roth B, editors. *Vaccines for OIE List A and emerging diseases*, vol. 114. Dev. Biol. Basel: Karger; 2003. p. 111–24.
- [4] March J. Improved formulations for existing CBPP vaccines—recommendations for changes. *Vaccine* 2004;22:4358–64.
- [5] Van Ginkel FW, Nguyen HH, McGhee JR. Vaccines for mucosal immunity to combat emerging infectious diseases. *Emerg Infect Dis* 2000;6(2):123–32.
- [6] Masiga WN, Domenech J, Windsor RS. Manifestation and epidemiology of contagious bovine pleuropneumonia in Africa. *Rev Sci Tech Off Int Epiz* 1996;15:1283–308.
- [7] Provost A, Perreau P, Breard A, et al. Contagious bovine pleuropneumonia. *Rev Sci Tech Off Int Epiz* 1987;6:625–79.
- [8] Dedieu L, Balcer-Rodrigues V, Cisse O, et al. Characterisation of the lymph node immune response following *Mycoplasma mycoides* subsp. *mycoides* SC-infection in cattle. *Vet Res* 2006;37:579–91.
- [9] Willems L. Mémoire sur la pleuro-pneumonie épizootique du gros bétail. *Rec. Méd. Vét. Pratique* 1852;9:401–34.
- [10] Niang M, Diallo M, Cisse O, et al. Pulmonary and serum antibody responses elicited in zebu cattle experimentally infected with *Mycoplasma mycoides* subsp. *mycoides* SC by contact exposure. *Vet Res* 2006;37(5):733–44.
- [11] Dedieu L, Rodrigues V, Yaya A, et al. Gamma interferon-producing CD4 T-cells correlate with resistance to *Mycoplasma mycoides* subsp. *mycoides* S.C. infection in cattle. *Vet Immunol Immunopathol* 2005;107:217–33.
- [12] Goletti D, Butera O, Bizzoni F, et al. Region of difference 1 antigen-specific CD4+ memory T-cells correlate with a favourable outcome of tuberculosis. *J Infect Dis* 2006;194:984–92.
- [13] Wise KS, Foecking MF, Röske K, et al. Distinctive repertoire of contingency genes conferring mutation-based phase variation and combinatorial expression of surface lipoproteins in *Mycoplasma capricolum* subsp. *capricolum* of the *Mycoplasma mycoides* phylogenetic cluster. *J Bacteriol* 2006;188(13):4926–41.
- [14] Gehring AJ, Rojas RE, Canaday DH, et al. The *Mycobacterium tuberculosis* 19-kilodalton lipoprotein inhibits gamma interferon-regulated HLA-DR and Fc gamma R1 on human macrophages through Toll-like receptor 2. *Infect Immun* 2003;71(8):4487–97.
- [15] Brown WC, Shkap V, Zhu D, et al. CD4 T-lymphocyte and immunoglobulin G2 responses in calves immunized with *Anaplasma marginale* outer membranes and protected against homologous challenge. *Infect Immun* 1998;66:5406–13.
- [16] Naessens J. Immunoglobulins. In: Pastoret PP, Griebel P, Bazin H, Govaerts A, editors. *Handbook of vertebrate immunology*. Academic Press: New York; 1998. p. 456–9.

- [17] Rodriguez F, Kennedy S, Bryson TD, et al. An immunohistochemical method of detecting *Mycoplasma* species antigens by use of monoclonal antibodies on paraffin sections of pneumonic bovine and caprine lungs. Zentralbl Veterinarmed 1996;B.43:429–38.
- [18] Bashiruddin JB, De Santis P, Persson A, et al. Detection of *Mycoplasma mycoides* subsp. *mycoides* SC in bovine lung and lymph node tissues by culture, sandwich ELISA and polymerase chain reaction systems. Res Vet Sci 2005;78:199–205.
- [19] Scanziani E, Paltrinieri S, Boldini M, et al. Histological and immunohistochemical findings in thoracic lymph nodes of cattle with contagious bovine pleuropneumonia. J Comp Path 1997;117:127–36.
- [20] Dedieu L, Chapey E, Balcer-Rodrigues V. *Mycoplasma mycoides* subsp. *mycoides* SC-secreted components induce apoptotic cell death in bovine leukocytes. Scan J Immunol 2005;62(6): 528–38.
- [21] Dedieu L, Balcer-Rodrigues V. Viable *Mycoplasma mycoides* subsp. *mycoides* SC-mediated depression of the bovine cell responsiveness to the mitogen Concanavalin A. Scan J Immunol 2006;64:376–81.
- [22] Westberg J, Persson A, Holmberg A, et al. The genome sequence of *Mycoplasma mycoides* subsp. *mycoides* SC type strain PG1, the causative agent of contagious bovine pleuropneumonia (CBPP). Genome Res 2004;14:221–7.
- [23] Marshall AJ, Miles RJ, Richards L. The phagocytosis of mycoplasmas. J Med Microbiol 1995;43:239–50.
- [24] Waite ER, March JB. Capsular polysaccharide conjugate vaccines against contagious bovine pleuropneumonia: immune response and protection in mice. J Comp Path 2002;126:171–82.
- [25] Abdo EM, Nicolet J, Miserez R, et al. Humoral and bronchial immune responses in cattle experimentally infected with *Mycoplasma mycoides* subsp. *mycoides* small colony type. Vet Microbiol 1998;59:109–22.
- [26] Pilo P, Frey J, Vilei EM. Molecular mechanisms of pathogenicity of *Mycoplasma mycoides* subsp. *mycoides* SC. Vet J 2006, in press.
- [27] Chambaud IH, Wroblewski H, Blanchard A. Interactions between mycoplasma lipoproteins and the host immune system. Trends Microbiol 1999;7:493–9.
- [28] Lührmann A, Deiters U, Skokowa J, et al. In vivo effects of a synthetic 2-kilodalton macrophage activating lipopeptide of *Mycoplasma fermentans* after pulmonary application. Infect Immun 2002;70(7):3785–92.
- [29] Ben-Yedidia T, Arnon R. Towards an epitope-based human vaccine for influenza. Hum Vaccines 2005;1(3):95–101.
- [30] Calvo-Calle JM, Oliveira GA, Watta CO, et al. A linear peptide containing minimal T- and B-cell epitopes of *Plasmodium falciparum* circumsporozoite protein elicits protection against transgenic sporozoite challenge. Infect Immun 2006;74(12):6929–39.
- [31] Tripathi V, Chitrakha KT, Bakshi AR, et al. Inducing systemic and mucosal immune responses to B-T construct of F1 antigen of *Yersinia pestis* in microsphere delivery. Vaccine 2006;16:3279–89.
- [32] Vilei EM, Frey J. Genetic and biochemical characterization of glycerol uptake in *Mycoplasma mycoides* subsp. *mycoides* SC: its impact on H₂O₂ production and virulence. Clin Diag Lab Immunol 2001;8:85–92.
- [33] Pilo P, Vilei EM, Peterhans E, et al. A metabolic enzyme as a primary virulence factor of *Mycoplasma mycoides* subsp. *mycoides* Small Colony. J Bacteriol 2005;187(19):6824–31.
- [34] Rice P, Houshaymi BM, Abu-Groun EAM, et al. Rapid screening of H₂O₂ production by *Mycoplasma mycoides* and differentiation of European subsp. *mycoides* SC (small colony) isolates. Vet Microbiol 2001;78:343–51.
- [35] Gerdtts V, Mutwiri GK, Tikoo SK, Babiuk LA. Mucosal delivery of vaccines in domestic animals. Vet Res 2006;37:487–510.
- [36] Sedgmen BJ, Meeusen ENT, Lofthouse SA. Alternative routes of mucosal immunization in large animals. Immunol Cell Biol 2004;82:10–6.
- [37] Bowersock TL, HogenEsch H, Toregrosa S, et al. Induction of pulmonary immunity in cattle by oral administration of ovalbumin in alginate microspheres. Immunol Lett 1998;60:37–43.

- [38] Rebelatto MC, Guimond P, Bowersock TL, HogenEsch H. Induction of systemic and mucosal immune response in cattle by intranasal administration of pig serum albumin in alginate microparticles. *Vet Immunol Immunopathol* 2001;83(1/2):93–105.
- [39] Yokomizo Y, Watanabe F, Imada Y, et al. Mucosal immunoadjuvant activity of the low toxic recombinant *Escherichia coli* heat-labile enterotoxin produced by *Bacillus brevis* for the bacterial subunit or component vaccine in pigs and cattle. *Vet Immunol Immunopathol* 2002;87:291–300.
- [40] Hubschle OJ, Tjipura-Zaire G, Abusugra I, et al. Experimental field trial with an immunostimulating complex (ISCOM) vaccine against contagious bovine pleuropneumonia. *J Vet Med B Infect Dis Vet Public Health* 2003;50(6):298–303.
- [41] Nicholas RAJ, Tjipura-Zaire G, Mbulu RS, et al. An inactivated whole cell vaccine and LppQ subunit vaccine appear to exacerbate the effects of CBPP in adult cattle. In: Proceedings of the 3rd meeting of the FAO-OIE-OAU/IBAR-IAEA consultative group on CBPP. Towards sustainable CBPP control programmes for Africa, Rome, 12–14 November 2003. Rome, Italy: FAO; 2004. p. 91–7.
- [42] Lin JH, Weng CN, Liao CW, et al. Protective effects of oral microencapsulated *Mycoplasma hyopneumoniae* vaccine prepared by co-spray drying method. *J Vet Med Sci* 2003;65(1):69–74.