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Review

Control of *Mycoplasma hyopneumoniae* infections in pigs

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Abstract

Mycoplasma hyopneumoniae, the primary pathogen of enzootic pneumonia, occurs worldwide and causes major economic losses to the pig industry. The organism adheres to and damages the ciliated epithelium of the respiratory tract. Affected pigs show chronic coughing, are more susceptible to other respiratory infections and have a reduced performance. Control of the disease can be accomplished in a number of ways. First, management practices and housing conditions in the herd should be optimized. These include all-in/all-out production, limiting factors that may destabilize herd immunity, maintaining optimal stocking densities, prevention of other respiratory diseases, and optimal housing and climatic conditions. Strategic medication with antimicrobials active against *M. hyopneumoniae* and, preferably, also against major secondary bacteria may be useful during periods when the pigs are at risk for respiratory disease. Finally, commercial bacterins are widely used to control *M. hyopneumoniae* infections. The main effects of vaccination include less clinical symptoms, lung lesions and medication use, and improved performance. However, bacterins provide only partial protection and do not prevent colonization of the organism. Different vaccination strategies (timing of vaccination, vaccination of sows, vaccination combined with antimicrobial medication) can be used, depending on the type of herd, the production system and management practices, the infection pattern and the preferences of the pig producer. Research on new vaccines is actively occurring, including aerosol and feed-based vaccines as well as subunit and DNA vaccines. Eradication of the infection at herd level based on age-segregation and medication is possible, but there is a permanent risk for re-infections.

Keywords: *Mycoplasma hyopneumoniae*; Control; Medication; Vaccination; Eradication

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1. Introduction

Mycoplasma hyopneumoniae is the primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs. Infections with *M. hyopneumoniae* are highly prevalent in almost all swine producing areas, and they cause significant economic losses due to increased medication use and decreased performance of the pigs. Moreover, *M. hyopneumoniae* is also considered to be one of the primary agents involved in the porcine respiratory disease complex (PRDC) (Thacker, 2006).

M. hyopneumoniae is extremely difficult to isolate because of its slow growth and interference with other swine mycoplasmas. The organism is primarily found on the mucosal surface of the trachea, bronchi, and bronchioles (Blanchard et al., 1992), and adherence of *M. hyopneumoniae* to the ciliated epithelium is a prerequisite for initiation of the infection. Zhang et al. (1995) identified the P97 protein to be a ciliary adhesin. Other adhesins may include a glycoprotein of 110 kDa (Chen et al., 1998), a P159 protein that is post-transitionally cleaved in proteins of 27, 51 and 110 kDa (Burnett et al., 2006) and a 146 kDa protein (Stakenborg et al., 2006). *M. hyopneumoniae* affects the mucosal clearance system by disrupting the cilia on the epithelial surface and, additionally, the organism modulates the immune system of the respiratory tract (Thacker, 2006). Therefore, *M. hyopneumoniae* predisposes animals to concurrent infections with other respiratory pathogens including bacteria, parasites and viruses. Limited information is available about the mechanisms which trigger the cilia damage. *M. hyopneumoniae* may increase the intracellular free calcium concentration in ciliated epithelial cells, which can induce loss of cilia (Park et al., 2002). A 54 kDa membrane protein of *M. hyopneumoniae* induced cytopathogenic effects in human lung fibroblast cell lines (Geary and Walczak, 1985). Epithelial cell damage may also be caused by the mildly toxic by-products of mycoplasma metabolism, such as hydrogen peroxide and superoxide radicals (Razin et al., 1998).

Chronic, non-productive coughing, the main clinical sign, appears 10–16 days after experimental infection, but this period can vary greatly under field conditions. Macroscopic lesions, consisting of purple to grey areas of pulmonary consolidation, are mainly found bilaterally in the apical, cardiac, intermediate and the anterior parts of the diaphragmatic lobes. Recovering

lesions consist of interlobular scar retractions, and in case of a pure *M. hyopneumoniae* infection macroscopic lesions are resolved 12–14 weeks after infection.

Clinical signs and lesions can lead to a tentative diagnosis, but laboratory testing is necessary for a conclusive diagnosis (Thacker, 2004). Although culturing of the organism is described as the gold standard, it is not used for routine diagnosis. The organism can be detected by immunofluorescence testing, but this test has limited sensitivity. Serology can be used to show presence of the organism at a herd level, but is not suited for diagnosis on individual animals (Sørensen et al., 1997). At present, (nested) polymerase chain reaction (PCR) testing is seen as the most sensitive tool to detect the infection (Calsamiglia et al., 1999).

Control of *M. hyopneumoniae* infections can be accomplished in a number of different ways namely by optimization of management practices and housing conditions, the use of antimicrobials, and vaccination. The present article reviews the current knowledge on these control measures, with emphasis on vaccination. Also strategies that can be used for elimination of the organism from pig herds are discussed.

2. Optimization of management practices and housing conditions

Improvement of the management practices is primordial in the control of *M. hyopneumoniae* infections and should be the first to be accomplished. Instituting management changes that reduce the possibilities of spreading *M. hyopneumoniae* or result in decreased lung damage by other pathogens may lead to considerable improvement in the control of enzootic pneumonia. However, also additional factors different from housing and management conditions, such as strain differences, may determine the infection pattern and clinical course of the disease (Vicca et al., 2002). A review on the influence of environmental and management factors on respiratory disease in pigs, including *M. hyopneumoniae* infections, has been published by Stärk (2000).

2.1. Production system

All-in, all-out (AIAO) production is probably the most important factor in the control of enzootic pneumonia since it can interrupt the cycle of pathogen transmissions from older to younger pigs (Clark et al., 1991). It allows the producer to tailor environmental conditions to a uniform population of pigs and to clean the facilities between groups of pigs. AIAO production also results in better performance and less lung lesions in slaughter pigs. Mixing or sorting pigs is a source of stress to the animals and it increases the probabilities of disease transmission. Therefore, an AIAO system in which the same pigs are moved as a group through the different production stages is to be preferred compared to one where pigs are regrouped during transfer from one unit to another.

Early weaning (<3 weeks) can reduce transmission of *M. hyopneumoniae* organisms from the sow to the offspring, but it is not allowed to be applied systematically in the EU. Parity segregation has been used in large production systems as a means to control several diseases in the breeding herd, including *M. hyopneumoniae* infections. Gilts and also their offspring are kept separated from the sows until they reach their second gestation. By that time, they are expected to have acquired the desired immune status and to pose no destabilization risk for the herd anymore (Hoy et al., 1986; Joo, 2003).

2.2. Purchase of animals

Closed pig herds or production systems have a more stable herd immunity compared to herds where (breeding) pigs are purchased. The risk for destabilization of the herd immunity especially increases in case purchasing of animals is performed at a regular basis, when replacement rates are high, or in case the animals originate from different sources. However, the pressure to improve genetics in high performing pig herds forces many pig producers to purchase their breeding stock. In these cases, it is important to evaluate the health status of the origin herd. Gilts should originate from a herd with a similar or higher health status, and a quarantine or adaptation period of at least 30 days should be respected (Amass and Baysinger, 2006).

2.3. Animal stocking density

Decreasing animal density during the different production stages has been shown to reduce the level

of respiratory disease (Pointon et al., 1985). Crowding may lead to increased transmission of pathogens, and to stress reactions, making the pigs more susceptible to infectious diseases. Too low stocking densities are financially not justified. Therefore, it is important to find a reasonable compromise between stocking densities that are appropriate for the health of the pigs and those that maximize the returns on the building's cost. In general, the stocking density for finishing pigs on fully slatted floors should equal or exceed 0.7 m^2 per pig (Madec, 1984).

2.4. Herd size

Herd size is generally considered as a risk factor for respiratory disease in pigs. However, study results are not always consistent. Flesja and Solberg (1981) found an increased prevalence of *M. hyopneumoniae* lesions in larger pig herds whereas in other studies, no significant associations could be found between herd size and seroprevalence of *M. hyopneumoniae* (Maes et al., 1999b, 2000) or prevalence of *Mycoplasma*-like lung lesions at slaughter (Maes et al., 2001). Biologically plausible reasons for a positive association between herd size and *M. hyopneumoniae* infections include a greater risk of introduction of the pathogen from outside the herd, greater risk of transmission of *M. hyopneumoniae* within and among herds when the herd is large, and effects of management and environmental factors that are related to herd size. However, compared with owners of small herds, owners of large herds might more frequently and more rapidly adopt management and housing practices that mitigate this potential increased risk (Gardner et al., 2002).

2.5. Prevention of other diseases

Appropriate parasite control measures are necessary to avoid any lung damage caused by migrating *Ascaris suum* larvae. Lung damage may also arise from infections with other respiratory pathogens including bacteria and viruses. Porcine reproductive and respiratory syndrome virus (PRRSV), influenza viruses, porcine circovirus type 2 (PCV-2) and porcine respiratory corona virus (PRCV), in combination with *M. hyopneumoniae*, are considered to play an important role in the development of the porcine respiratory disease complex (PRDC). The impact of these infections can be reduced by means of medication, vaccination or by appropriate management practices.

2.6. Biosecurity measures

Strict biosecurity measures such as hygiene, insect and rodent control, and restricted movement of personnel and equipment between animals of different age groups should be applied as much as possible in pig herds. The importance of these measures for the control *M. hyopneumoniae* infections however is not clear. Indirect transmission of *M. hyopneumoniae* through fomites has been suggested (Goodwin, 1985), but is only of limited importance compared to direct pig-to-pig transmission which is by far the most important transmission route. Batista et al. (2004) found that, under standard hygiene protocols, *M. hyopneumoniae* was not transmitted to naive pigs by personnel who were in a weekly contact with infected pigs.

2.7. Improvement of housing conditions

Housing and/or environmental changes that optimize the climate of the pigs' environment are important in the control of *M. hyopneumoniae* infections. Special attention should be paid to the temperature set points, fan staging, air inlet and curtain settings, sensor placement, heater capacity, drafts and building maintenance (Gonyou et al., 2006). However, making environmental changes for improving the climate in inappropriate or old barns frequently entail extensive remodeling, and therefore, they may be difficult and expensive to institute and maintain.

3. Antimicrobial medication

To control and treat respiratory disease including *M. hyopneumoniae* infections in pigs, tetracyclines and macrolides are most frequently used. Also, other potentially active antimicrobials against *M. hyopneumoniae* include lincosamides, pleuromutilins, fluoroquinolones, florfenicol, aminoglycosides and aminocyclitols. Fluoroquinolones and aminoglycosides have mycoplasmacidal effects. Since the organism lacks a cell wall, it is insensitive to

β-lactamic antibiotics such as penicillins and cephalosporins.

Although acquired antimicrobial resistance of *M. hyopneumoniae* has been reported to tetracyclines (Inamoto et al., 1994), and recently also to macrolides, lincosamides and fluoroquinoles (Vicca et al., 2004), it does not seem to constitute a major problem for treatment of *M. hyopneumoniae* infections to date. Antimicrobials or combinations of antimicrobials that are also active against secondary bacteria that often complicate *M. hyopneumoniae* infections are indicated.

Several studies have been conducted to assess the efficacy of various antimicrobials used for the prevention and the treatment of *M. hyopneumoniae* infections. An overview of peer reviewed studies performed under experimental as well as under field conditions is given by Vicca (2005). It can be concluded that for most antimicrobials tested, performance parameters were improved and lung lesions as well as clinical signs were decreased in treated animals.

The control of the disease by medication can be approached by strategic administration of antimicrobials. The product is added for about 1–3 weeks, commencing approximately 1 week prior to the expected time of disease onset. Such medication regimen can limit the severe consequences of the disease and the infection load (Thacker et al., 2004), but it does not prevent pigs from becoming infected with *M. hyopneumoniae*. Treatment and control of enzootic pneumonia outbreaks may be disappointing because the symptoms may reappear after cessation of the therapy. Pulse medication in which medication is provided intermittently during critical production stages of the pigs, can also be used (Le Grand and Kobisch, 1996). Pulse medication during extended periods of time as well as continuous medication during one or more production stages should be discouraged because of both the increased risk of antimicrobial resistance development and the possible risk for antimicrobial residues in the pig carcasses at slaughter.

In endemically infected farms, strategic medication of the reproductive herd is sometimes practiced as an attempt to decrease the bacterial shedding from sows to the newly introduced gilts. Antimicrobial medication of recently weaned pigs has been shown to reduce the number of *M. hyopneumoniae* organisms in the respiratory tract (Vicca et al., 2005; Thacker et al., 2006), but further research is necessary to quantify the shedding of *M. hyopneumoniae* organisms in sows receiving antimicrobial medication.

4. Vaccination

4.1. Commercial vaccines

Commercial vaccines, consisting of inactivated, adjuvanted whole-cell preparations, are widely applied worldwide. In many countries, vaccination for controlling *M. hyopneumoniae* infections is applied in more than 70% of the pig herds. The major advantages of vaccination include improvement of daily weight gain (2–8%), feed conversion ratio (2–5%) and sometimes mortality rate. Additionally, shorter time to reach slaughter weight, reduced clinical signs, lung lesions and lower treatment costs are observed (Maes et al., 1998, 1999a).

Although protection against clinical pneumonia is often incomplete and vaccines do not prevent colonization (Thacker et al., 1998), some studies indicate that the currently used vaccines may reduce the number of organisms in the respiratory tract (Meyns et al., 2006) and may decrease the infection level in a herd (Sibila et al., 2007a). This apparent contradictory situation is comprehensible by the fact that maximum beneficial effects of vaccination are reached several months after the initiation of vaccination (Haesebrouck et al., 2004). Meyns et al. (2006) showed using an experimental transmission model that, although vaccination against *M. hyopneumoniae* with a commercial vaccine significantly reduced the clinical symptoms and lung lesions in pigs, only a limited and non-significant reduction in the transmission of *M. hyopneumoniae* was achieved. They concluded that vaccination alone with the current vaccines will likely not be sufficient to eliminate *M. hyopneumoniae* from infected pig herds.

4.2. Mechanisms of protection after vaccination

The exact mechanisms of protection are not yet fully understood. Intramuscular injection of a commercial bacterin (Thacker et al., 2000a) has been

shown to induce the secretion of specific antibodies in the serum and the respiratory tract, as well as the generation of IFN-γ secreting cells in blood. Based on the findings of this study, the authors suggested that both local mucosal antibodies and systemic cell-mediated immunity (CMI) responses are important for protection. However, the correlation of local *M. hyopneumoniae*-specific antibody production and protection against pneumonia remains unclear. Djordjevic et al. (1997) and Thacker et al. (1998) found that antibody concentrations in respiratory tract washings did not correlate with protection against pneumonia.

CMI against *M. hyopneumoniae* in response to vaccination or challenge has been measured by determining the proliferative responses of lymphocytes following *in vitro* stimulation with *M. hyopneumoniae*-specific antigens (Djordjevic et al., 1997; Thacker et al., 1998). Results of these studies indicate a considerable variation among individual pigs, although proliferative responses in peripheral blood lymphocytes were more pronounced in vaccinated compared to non-vaccinated pigs. Conversely, suppression of T cell responses by thymectomy and treatment with anti-thymocyte serum resulted in decreased severity of microscopic pneumonic lesions after challenge, although the multiplication of *M. hyopneumoniae* organisms was enhanced compared to immune-competent pigs (Tajima et al., 1984). These findings suggest that the CMI may both help and hinder the development of mycoplasmal pneumonia. IFN-γ, a cytokine produced by helper cells, subtype 1 (T_H1) lymphocytes, and natural killer cells, has been shown to be important for macrophage activation. The generation of IFN-γ secreting cells in blood following vaccination might suggest that IFN-γ may also play a role in protection against *M. hyopneumoniae* through the activation of macrophages (Thacker et al., 2000a). Increases in concentrations of pro-inflammatory cytokines such as TNF-α, are thought to be associated with lesion development (Meyns et al., 2007) and to be one possible mechanism for the potentiation of PRRSV-induced pneumonia by *M. hyopneumoniae* (Thacker et al., 1999). Vaccination against *M. hyopneumoniae* reduced the potentiation of PRRSV-induced pneumonia by *M. hyopneumoniae* (Thacker et al., 2000c). This may be because the vaccine prevents an increase in lung TNF-α concentrations in response to challenge with *M. hyopneumoniae*, thus impeding potentiation of PRRSV-induced pneumonia.

Commercial vaccines also induce serum *M. hyopneumoniae* specific antibodies. The percentage of animals seroconverting after *M. hyopneumoniae* vaccination ranges from 30 to 100% (Sibila et al., 2004a), and serological responses also differ among vaccines (Thacker et al., 1998). Antibody titers following vaccination may, in the absence of natural infections that boost the immune system, decrease below detection limits 1–3 months after vaccination (Maes et al., 1999a). It is generally accepted that serum antibodies are not suited to evaluate protective immunity, since no direct correlation could be demonstrated between serum antibody concentration and protection against *M. hyopneumoniae* challenge (Djordjevic et al., 1997; Thacker et al., 1998).

4.3. Vaccination strategies

In *M. hyopneumoniae*-free herds or in herds with very low infection levels, vaccination may not be recommended since under these conditions, the benefits of vaccination may not be sufficient to outweigh the costs of vaccination. In herds with higher infection levels without obvious clinical signs, or in herds with clinical disease, vaccination is economically justified (Maes et al., 2003).

Different vaccination strategies have been adopted, depending on the type of herd, the production system and management practices, the infection pattern and the preferences of the pig producer. Moreover, under field conditions, optimal vaccination strategies must balance the advantage of delayed vaccination with the need to induce immunity before exposure to pathogens. Since infections with *M. hyopneumoniae* may already occur during the first weeks of life (Vicca et al., 2002; Sibila et al., 2007b), vaccination of piglets is most commonly used. Its efficacy has been demonstrated by means of numerous studies under experimental as well as field conditions (Jensen et al., 2002). Vaccination of suckling piglets (early vaccination; <4 weeks of age) is more common in single-site herds, whereas vaccination of nursery/early fattening pigs (late vaccination; between 4 and 10 weeks) is more often practiced in three-site systems where late infections are more common.

Traditionally, double vaccination was the most frequent practice. During the last years, one-shot vaccines have been shown to confer similar benefits than two-shot vaccines and are more often used now (Baccaro et al., 2006). One-shot vaccination is especially popular because it requires less labor and it can be implemented more easily in routine management practices on the farm. With one-shot vaccines however, the skill of the pig producer or employee to vaccinate properly is more critical for vaccine compliance since only one injection is given.

Vaccination of suckling piglets has the advantage that immunity can be induced before pigs become infected, and that less pathogens are present that can interfere with immune response. Possible disadvantages of vaccinating piglets before weaning include the presence of maternal antibodies (see further) and an increased risk for more severe porcine circovirus type 2 (PCV2) infections after weaning. Some studies have shown that administration of *M. hyopneumoniae* bacterins shortly before experimental/natural PCV2 infection increased the severity of PCV2 induced lesions/postweaning multisystemic wasting syndrome (Opriessnig et al., 2003). However, the potentiation of PMWS by certain vaccines is still a controversial issue since other studies (Haruna et al., 2006) concluded that routine vaccination against swine diseases, including *M. hyopneumoniae* infections, may not significantly contribute to the occurrence of PMWS under field conditions. It is likely that timing of vaccination in relation to PCV2 infection, among many other factors, plays a role in the influence vaccination has on the clinical outcome of this viral infection (Opriessnig et al., 2006).

Vaccination of nursery pigs has no or less interference with possible maternally derived antibodies. However, nursery pigs may already be infected with *M. hyopneumoniae*. In addition, the age of infection or the age-window in which the piglets become infected may vary between successive groups within a herd (Sibila et al., 2004b). Finally, many infections such as PRRSV or PCV2 mainly take place after weaning and may affect the general health status of the pigs, and consequently also interfere with proper immune responses after vaccination.

In contrast with the numerous studies that investigated the efficacy of vaccination of piglets, only a few studies have assessed the effects of sow vaccination. Vaccination of sows at the end of gestation aims to both reduce the shedding of *M. hyopneumoniae* from the sow to the offspring and to protect the piglets against infection via maternally derived immunity. It has been shown that vaccinating sows 5 and 3 weeks before farrowing was associated with a lower number of positive piglets at weaning using nested PCR on nasal swabs, both in farrow-to-finish operations and multi-site production systems (Sibila et al., 2006). However, maternally derived antibodies only provide partial protection against lesion development following challenge infection, and under experimental conditions, they provide limited to no effect on colonization of *M. hyopneumoniae* (Thacker et al., 2000b). The role of antigen-specific maternally derived immune cells in protection against *M. hyopneumoniae* is not known. Bandrick et al. (2006) showed *in vivo* response by delayed-type hypersensitivity and *in vitro* proliferation of maternally derived cells when newborn piglets were stimulated with *M. hyopneumoniae* antigen. Since piglets from vaccinated sows can still be infected, additional measures to control *M. hyopneumoniae* during the nursery and finishing phases may be warranted. Further research is needed to investigate the effects of sow vaccination on infection levels in pigs in different production systems.

Vaccination of gilts is recommended in endemically infected herds to avoid destabilization of breeding stock immunity (Bargen, 2004). This is particularly the case when gilts are purchased from herds that are free from *M. hyopneumoniae* or from herds with a low infection level of *M. hyopneumoniae*.

4.4. Factors influencing the efficacy of vaccination

Although vaccination confers beneficial effects in most infected herds, the effects are variable between herds. The variable results may be due to different factors such as improper vaccine storage conditions and injection technique, antigenic differences between field strains and vaccine strains, presence of disease at the time of vaccination, and interference of vaccine induced immune responses by maternally derived (colostral) antibodies.

The existence of high proteomic heterogeneity between *M. hyopneumoniae* isolates from different

herds has been clearly demonstrated (Calus et al., 2007). It remains to be investigated however whether this high protein variability may account partially for reduced efficacy of vaccination as observed in some herds.

Under experimental conditions, PRRSV infection or administration of a modified live virus (MLV) PRRS vaccine (US strain) at the time of *M. hyopneumoniae* vaccination appeared to significantly reduce the efficacy of the *M. hyopneumoniae* vaccine (Thacker et al., 2000c). On the contrary, administration of a MLV PRRS vaccine to pigs 1 week prior to vaccination with *M. hyopneumoniae* vaccine did not interfere with vaccine efficacy or immune responses to *M. hyopneumoniae* infection (Boettcher et al., 2002). It is likely that also other infections or factors *e.g.* mycotoxins affecting the health and/or immune system of the pig at the moment of vaccination may interfere with vaccine efficacy.

The influence of maternally derived antibodies on vaccine responses in piglets has not been definitively established. In general, pigs with high maternally derived antibody titers may elicit similar (Martelli et al., 2006) or lower (Maes et al., 1999a; Hodgins et al., 2004) serological responses after vaccination. One study (Jayappa et al., 2001) showed that high titers of maternally derived antibodies, induced by infection and vaccination of the sows, had negative effects on the efficacy of piglet vaccination. Hodgins et al. (2004) also showed that, apart from the colostral antibody titers, the age of the pigs at vaccination (2, 3 or 4 weeks of age) did not influence the serological response following vaccination. Martelli et al. (2006) indicated that the immune system of the pigs is primed in the absence of a clear serological response after vaccination, and that passively acquired antibodies have little or no effect on either a vaccine induced priming or subsequent anamnestic response. It can be expected that the interaction of vaccine and maternally derived antibodies varies with the exact formulation of the vaccine and also with the titers of maternal antibodies present in the pig.

4.5. Experimental vaccines

Investigation of new vaccines is actively occurring, including the use of aerosol and feed-based vaccines as well as subunit and DNA vaccines (Fagan et al., 2001; Lin et al., 2003; Murphy et al., 1993). Intradermal vaccination with a commercial bacterin has been shown to be efficacious (Jones et al., 2004). If *M. hyopneumoniae* vaccines could be delivered to the animals via aerosols or via the feed, this would provide an easy means for mass vaccination since it would substantially reduce labor costs and it would also be better for the welfare of the pigs as well as for stimulating a mucosal immune response at the respiratory tract. However, aerosol vaccination given three times with 2 weeks interval provided insufficient protection, in contrast with the intramuscular application of the same commercial vaccine which was efficacious (Murphy et al., 1993). On the other hand, Lin et al. (2003) showed that an oral micro-spheres experimental vaccine based on the PRIT-5 *M. hyopneumoniae* strain and prepared by a co-spray drying method significantly reduced pneumonia lesions following challenge infection with *M. hyopneumoniae* in pigs.

King et al. (1996) found only minimal and non-significant protection in a pig challenge infection model using a recombinant subunit vaccine based on the P97 adhesin of *M. hyopneumoniae*. Intranasal immunization of pigs with the attenuated *Erysipelothrix rhusiopathiae* YS-19 strain expressing a recombinant protein of *M. hyopneumoniae* P97 adhesin significantly reduced the severity of pneumonic lung lesions following challenge infection (Shimoji et al., 2003). However, apparently significant humoral and cell-mediated immune responses were not observed in the immunized pigs. Okamba et al. (2007) showed that a replication-defective adenovirus expressing the C-terminal portion of *M. hyopneumoniae*-P97 adhesin applied intranasally and intramuscularly in BALB/c mice, induced significant immune responses. Also several experimental DNA vaccines have been developed and tested for immune responses in mice or pigs. Significant immune responses with DNA vaccines were elicited in mice, based on the expression of a heat shock protein gene P42 (Chen et al., 2003) or a ribonucleotide reductase R2 subunit gene fragment of *M. hyopneumoniae* (Chen et al., 2006). The studies suggest that these vaccines may represent new strategies for controlling *M. hyopneumoniae* infections in pigs, but they need to be validated in pigs under experimental and practical circumstances.

Further studies are necessary for improving vaccines and vaccination strategies. From an immunological point of view, challenges include induction of immunity at the mucosal level. For rational design of vaccines, a comprehensive understanding of the pathobiology of *M. hyopneumoniae* infections and the molecular basis of pathogenicity of this microorganism is required. Bacterial genes and antigens involved in survival of the bacterium in the host or that render the bacterium harmful to the host need to be identified. This may be facilitated by the fact that the genome of three different *M. hyopneumoniae* isolates has recently been sequenced (Minion et al., 2004; Vasconcelos et al., 2005).

5. Preventive medication versus vaccination

The use and efficacy of either vaccination or preventive (strategic) medication are frequently discussed among swine veterinarians and the question always arises whether medication and/or vaccination should be used. Antimicrobials can be used in a flexible way, they are often effective against several (respiratory) pathogens and their administration is less labor-intensive since in-feed or in-water medication is mostly used. Vaccination, on the other hand, does not select for antimicrobial resistance in pathogenic bacteria and in bacteria belonging to the microbiota of the animal. It also avoids risks for antimicrobial residues in the pig carcasses at slaughter. While an immediate effect can be expected for antimicrobial treatment, the effect of vaccination of young piglets will only be evident at herd level if it is practiced for at least several months. Although vaccines are directed towards control of *M. hyopneumoniae* infections, also other secondary bacterial infections (*Pasteurella multocida*, *Actinobacillus pleuropneumoniae*) or lung lesions caused by these pathogens less frequently occur after vaccination (Maes et al., 1998, 1999a,b; Meyns et al., 2006).

Neither vaccination nor preventive medication can prevent infection and adherence of *M. hyopneumoniae* to the ciliated cells of the respiratory tract (Le Grand and Kobisch, 1996). Finally, in case of high infection levels and/or in herds with poor management and housing conditions, the use of antimicrobials may remain necessary or may confer additional clinical and performance benefits in vaccinated herds (Mateusen et al., 2002).

6. Eradication

Eradication of *M. hyopneumoniae* infections would result in significant savings each year and in improvement of the health and welfare of the pigs. A depopulation and restocking programme was proposed and conducted in 1960 by the Pig Health Control Organisation. This programme was expensive and re-infections occurred in 20% of the herds (Whittlestone, 1990).

Currently, eradication of *M. hyopneumoniae* is mainly applied in Denmark, Finland and Switzerland. In Switzerland, a total and partial sanitation programme was set up (Zimmerman et al., 1989), which is nowadays known as "the Swiss system" of eradication of infectious diseases. The total sanitation programme involved complete emptying of the animal facilities by selling or culling all animals. The partial sanitation programme included a piglet and gilt free (<10 months) interval and the temporary feeding of a medicated diet during 10–14 days. The feed contained either tiamulin (6 mg/kg body weight [BW]) or a combination of chlortetracycline (20 mg/kg BW), tylosin (4 mg/kg BW) and sulfadimidin (30 mg/kg BW). Once finished this medication period, the farm continues with its normal animal flow. The partial sanitation programme is well suited for small farms. In the US and Canada, large swine operations have developed a modified version of the Swiss method. The program includes the features of the partial depopulation scheme along with multiple vaccinations of the breeding herd and an offsite breeding program. The method is usually combined with herd closure when PRRSV elimination is also targeted.

Another method used mainly in the US includes medicated early weaning (MEW) wherein intensive medication of the sow during late gestation and immediately following parturition as well as the newborn piglets is used to derive pigs free of *M. hyopneumoniae*. Piglets are weaned at 5 days of age, placed in an isolated nursery on a separate site and medicated for the first 10 days after weaning (Harris and Alexander, 1999). Modifications of the MEW programme have been applied, based on postponing

weaning until 21–25 days of age, keeping sows and nursery pigs at the same site, vaccination strategies and/or combined with medication of the sows and medications of the piglets before and after weaning (Clark et al., 1994). It was concluded that isolating the pigs was as effective as medication and vaccination protocols in controlling the transmission of the pathogens investigated, including *M. hyopneumoniae*, *Bordetella bronchiseptica* and *P. multocida*. *M. hyopneumoniae* was not detected when any MEW procedure was used.

Although several attempts have been made to eradicate *M. hyopneumoniae* from a herd, re-infection frequently occurs (2.6–10% per year). The high re-infection rates may be due to airborne transmission from infected neighbourhood herds, or to purchase of infected animals that were tested negative using ELISA techniques on serum or colostrum samples. Although ELISA has proved to be a very useful technique, pigs can be colonized with low numbers of *M. hyopneumoniae* organisms in the lung without eliciting a serological detectable response (Thacker, 2006). Other risk factors for the re-infection include being a finishing farm, large mixed breeding–finishing farm, and parking site for pig transport vehicles close to the farm.

7. Conclusions

Control of enzootic pneumonia can be accomplished by optimizing management practices and housing conditions, strategic medication and vaccination. These measures can decrease the infection level in a herd and the number of organisms in the lungs, and improve health conditions of the animals but they do not guarantee the absence of *M. hyopneumoniae*. Elimination of the micro-organism at herd level based on age-segregation and medication is possible, but there is a permanent risk for re-infections. Further efforts are needed for development of more effective vaccines and vaccination strategies.

References

Amass, S., Baysinger, A., 2006. Swine disease transmission and prevention. In: Straw, B.E., Zimmerman, J.J., D'Allaire, S., Taylor, D.J. (Eds.), Diseases of Swine. 9th ed. Blacwell Publishing Ltd., Oxford, UK, pp. 1075–1098.

Baccaro, M., Hirose, F., Umehara, O., Gonçalves, L., Doto, D., Paixão, R., Shinya, L., Moreno, A., 2006. Comparative efficacy of two single-dose bacterins in the control of *Mycoplasma hyopneumoniae* in swine raised under commercial conditions in Brazil. Vet. J. 172, 526–531.

Bandrick, M., Pieters, M., Pijoan, C., Molitor, T., 2006. Cellular immune response in piglets following sow vaccination wit *Mycoplasma hyopneumoniae*. In: Proceedings of the Allen D. Leman Swine Conference, vol. 33, Supplement. College of Veterinary Medicine, University of Minnesota, Minneapolis MN, USA, p. 11.

Bargen, L., 2004. A system response to an outbreak of enzootic pneumonia in grow/finish pigs. Can. Vet. J. 45, 856–859.

Batista, L., Pijoan, C., Ruiz, A., Utrera, V., Dee, S., 2004. Assessment of transmission of *Mycoplasma hyopneumoniae* by personnel. J. Swine Health Prod. 12, 75–77.

Blanchard, B., Vena, M., Cavalier, A., Lannic, J., Gouranton, J., Kobisch, M., Le-Lannic, J., 1992. Electron microscopic observation of the respiratory tract of SPF piglets inoculated with *Mycoplasma hyopneumoniae*. Vet. Microbiol. 30, 329–341.

Boettcher, T., Thacker, B., Halbur, P., Waters, W., Nutsch, R., Thacker, E., 2002. Vaccine efficacy and immune response to *Mycoplasma hyopneumoniae* challenge in pigs vaccinated against porcine reproductive and respiratory syndrome virus and *M. hyopneumoniae*. J. Swine Health Prod. 10, 259–264.

Burnett, T., Dinkla, K., Rohde, M., Chatwal, G., Uphoff, C., Srivastava, M., Cordwell, S., Geary, S., Liao, X., Minion, C., Walker, M., Djordjevic, S., 2006. P159 is a proteolytic processed surface adhesin of *Mycoplasma hyopneumoniae*: defined domains of P159 bind heparin and promote adherence to eukaryote cells. Mol. Microbiol. 60, 669–686.

Calsamiglia, M., Pijoan, C., Trigo, A., 1999. Application of a nested polymerase chain reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. J. Vet. Diagn. Invest. 11, 246–251.

Calus, D., Baele, M., Meyns, T., de Kruif, A., Butaye, P., Decostere, A., Haesebrouck, F., Maes, D., 2007. Protein variability among *Mycoplasma hyopneumoniae* isolates. Vet. Microbiol. 120, 284–291.

Chen, A., Fry, S., Forbes-Faulkner, J., Daggard, G., Mukkur, T., 2006. Comparative immunogenicity of *Mycoplasma hyopneumoniae* NrdF encoded in different expression systems delivered orally via attenuated *S.* Typhimurium aroA in mice. Vet. Microbiol. 114, 252–259.

Chen, J.R., Lin, L.H., Weng, C.N., Lai, S.S., 1998. Identification of a novel adhesin-like glycoprotein from *Mycoplasma hyopneumoniae*. Vet. Microbiol. 62, 97–110.

Chen, Y.L., Wang, S.N., Yang, W., Chen, Y.J., Lin, H.H., Shiuan, D., 2003. Expression and immunogenicity of *Mycoplasma hyopneumoniae* heat shock protein antigen P42 by DNA vaccination. Infect. Immun. 71, 1155–1160.

Clark, L., Freeman, M., Scheidt, A., Knox, K., 1991. Investigating the transmission of *Mycoplasma hyopneumoniae* in a swine herd with enzootic pneumonia. Vet. Med. 86, 543–550.

Clark, L., Hill, M., Kniffen, T., VanAlstine, W., Stevenson, G., Meyer, K., Wu, C., Scheidt, A., Knox, K., Albregts, S., 1994. An

evaluation of the components of medicated early weaning. Swine Health Prod. 2, 5–11.

Djordjevic, S., Eamens, G., Romalis, L., Nicholls, P., Taylor, V., Chin, J., 1997. Serum and mucosal antibody responses and protection in pigs vaccinated against *Mycoplasma hyopneumoniae* with vaccines containing a denatured membrane antigen pool and adjuvant. Aust. Vet. J. 75, 504–511.

Fagan, P., Walker, M., Chin, J., Eamens, G., Djordjevic, S., 2001. Oral immunization of swine with attenuated *Salmonella* Typhimurium aroA SL3261 expressing a recombinant antigen of *Mycoplasma hyopneumoniae* (NrdF) primes the immune system for a NrDF specific secretory IgA response in the lungs. Microb. Pathogenesis 30, 101–110.

Flesja, K., Solberg, I., 1981. Pathological lesions in swine at slaughter. Acta Vet. Scand. 22, 272–282.

Gardner, A., Willeberg, P., Mousing, J., 2002. Empirical and theoretical evidence for herd size as a risk factor for swine diseases. Anim. Health Res. Rev. 3, 43–55.

Geary, S., Walczak, E., 1985. Isolation of a cytopathic factor from *Mycoplasma hyopneumoniae*. Infect. Immun. 48, 576–578.

Gonyou, H., Lemay, S., Zhang, Y., 2006. Effects of the environment on productivity and disease. In: Straw, B.E., Zimmerman, J.J., D'Allaire, S., Taylor, D.J. (Eds.), Diseases of Swine. 9th ed. Blacwell Publishing Ltd., Oxford, UK, pp. 1027–1038.

Goodwin, R., 1985. Apparent reinfection of enzootic-pneumonia-free pig herds: search for possible causes. Vet. Rec. 116, 690–694.

Haesebrouck, F., Pasmans, F., Chiers, K., Maes, D., Ducatelle, R., Decostere, A., 2004. Efficacy of vaccines against bacterial diseases in swine: what can we expect? Vet. Microbiol. 100, 255–268.

Harris, H., Alexander, T., 1999. Methods of disease control. In: Straw, B., D'Allaire, S., Mengeling, W., Taylor, D. (Eds.), Diseases of Swine. 8th ed. Iowa State University Press, Ames, pp. 1077–1110.

Haruna, J., Hanna, P., Hurnik, D., Ikede, B., Miller, L., Yason, C., 2006. The role of immunostimulation in the development of postweaning multisystemic wasting syndrome in pigs under field conditions. Can. J. Vet. Res. 70, 269–276.

Hodgins, D., Shewen, P., Dewey, C., 2004. Influence of age and maternal antibodies on antibody responses of neonatal piglets vaccinated against *Mycoplasma hyopneumoniae*. J. Swine Health Prod. 12, 10–16.

Hoy, S., Mehlhorn, G., Hörügel, K., Dorn, W., Eulenberger, K., Johannsen, U., 1986. Der Einfluss ausgewählter endogener Faktoren auf das Auftreten entzündlicher Lungenveränderungen bei Schweinen. Mh. Vet. Med. 41, 397–400.

Inamoto, T., Takahashi, H., Yamamoto, K., Nakai, Y., Ogimoto, K., 1994. Antibiotic susceptibility of *Mycoplasma hyopneumoniae* isolated from swine. J. Vet. Med. Sci. 56, 393–394.

Jayappa, H., Davis, B., Rapp-Gabrielson, V., Wasmoen, T., Thacker, E., 2001. Evaluation of the efficacy of *Mycoplasma hyopneumoniae* bacterin follwoing immunization of young pigs in the presence of varying levels of maternal antibodies. In: Proceedings of the 32nd Annual Meeting, Am. Assoc. Swine Vet., Nashville, Tennessee, pp. 237–241.

Jensen, D., Ersboll, A., Nielsen, J., 2002. A meta-analysis comparing the effect of vaccines against *Mycoplasma hyopneumoniae* on daily weight gain in pigs. Prev. Vet. Med. 54, 265–278.

Jones, G., Rapp-Gabrielson, V., Wilke, R., Thacker, E., Thacker, B., Gergen, L., Sweeney, D., Wasmoen, T., 2004. Intradermal vaccination for *Mycoplasma hyopneumoniae*. J. Swine Health Prod. 13, 19–27.

Joo, H.S., 2003. Segregated parity production: a potential method to improve production and health. Int. Pigletter 23, 5–6.

King, K., Faulds, D., Rosey, E., Yancey, R., 1996. Characterization of the gene encoding Mhp1 from *Mycoplasma hyopneumoniae* and examination of Mhp1's vaccine potential. Vaccine 15, 25–35.

Le Grand, A., Kobisch, M., 1996. Comparaison de l'utilisation d'un vaccin et d'un traitement antibiotique séquentiel dans un élevage infecté par *Mycoplasma hyopneumoniae*. Vet. Med. 27, 241–253.

Lin, J., Weng, C., Liao, C., Yeh, K., Pan, M., 2003. Protective effects of oral microencapsulated *Mycoplasma hyopneumoniae* vaccine prepared by co-spray drying method. J. Vet. Med. Sci. 65, 69–74.

Madec, F., 1984. The risk factors of respiratory diseases on fatteners in intensive breeding–finishing units. In: Proceedings of the 8th IPVS Congress, Ghent, Belgium, p. 349.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Lein, A., Vrijens, B., de Kruif, A., 1998. Effect of vaccination against *Mycoplasma hyopneumoniae* in pig herds with a continuous production system. J. Vet. Med. B 45, 495–505.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Vrijens, B., Verbeke, W., Viaene, J., de Kruif, A., 1999a. Effect of vaccination against *Mycoplasma hyopneumoniae* in pig herds with an all-in/all-out production system. Vaccine 17, 1024–1034.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Vrijens, B., de Kruif, A., 1999b. Risk indicators for the seroprevalences of *Mycoplasma hyopneumoniae*, porcine infuenza viruses and Aujeszky's disease virus in slaughter pigs from fattening pig herds. J. Vet. Med. B 46, 341–352.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Vrijens, B., de Kruif, A., 2000. Risk indicators for the seroprevalences of *Mycoplasma hyopneumoniae*, porcine influenza viruses and Aujeszky's disease virus in slaughter pigs from farrow-to-finish pig herds in Belgium. Vet. Res. 31, 313–327.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Vrijens, B., Ducatelle, R., de Kruif, A., 2001. Non-infectious herd factors associated with macroscopic and microscopic lung lesions in slaughter pigs from farrow-to-finish pig herds. Vet. Rec. 148, 41–46.

Maes, D., Verbeke, W., Vicca, J., Verdonck, M., de Kruif, A., 2003. Benefit to cost of vaccination against *Mycoplasma hyopneumoniae* in pig herds under Belgian market conditions from 1996 to 2000. Livest. Prod. Sci. 83, 85–93.

Martelli, P., Terreni, M., Guazzetti, S., Cavirani, S., 2006. Antibody response to *Mycoplasma hyopneumoniae* infection in vaccinated pigs with or without maternal antibodies induced by sow vaccination. J. Vet. Med. B 53, 229–233.

Mateusen, B., Maes, D., Van Goubergen, M., Verdonck, M., de Kruif, A., 2002. Effectiveness of treatment with lincomycin

hydrochloride and/or vaccination against *Mycoplasma hyopneumoniae* for controlling chronic respiratory disease in a herd of pigs. Vet. Rec. 151, 135–140.

Meyns, T., Dewulf, J., de Kruif, A., Calus, D., Haesebrouck, F., Maes, D., 2006. Comparison of transmission of *Mycoplasma hyopneumoniae* in vaccinated and non-vaccinated populations. Vaccine 24, 7081–7086.

Meyns, T., Maes, D., Calus, D., Ribbens, S., Dewulf, J., Chiers, K., de Kruif, A., Cox, E., Decostere, A., Haesebrouck, F., 2007. Interactions of highly and low virulent *Mycoplasma hyopneumoniae* isolates with the respiratory tract of pigs. Vet. Microbiol. 120, 87–95.

Minion, F.C., Lefkowitz, E.J., Madsen, M.L., Cleary, B.J., Swartzell, S.M., Mahairas, G.G., 2004. The genome sequence of *Mycoplasma hyopneumoniae* strain 232, the agent of swine mycoplasmosis. J. Bacteriol. 186, 7123–7133.

Murphy, D., Van Alstine, W., Clark, K., Albregts, S., Knox, K., 1993. Aerosol vaccination of pigs against *Mycoplasma hyopneumoniae* infection. Am. J. Vet. Res. 54, 1874–1880.

Okamba, F., Moreau, E., Bouh, C., Gagnon, C., Massie, B., Arella, M., 2007. Immune responses induced by replication-defective andenovirus expressing the C-terminal portion of *Mycoplasma hyopneumoniae*-P97 adhesin. Clin. Vaccine Immunol. 14, 767–774.

Opriessnig, T., Halbur, P., Yu, S., Thacker, E., Fenaux, M., Meng, X., 2006. Effects of the timing of *Mycoplasma hyopneumoniae* bacterin on the development of lesions associated with porcine circovirus type 2. Vet. Rec. 158, 149–154.

Opriessnig, T., Yu, S., Gallup, J., Evans, R., Fenaux, M., Pallares, F., Thacker, E., Brockus, C., Ackermann, M., Thomas, P., Meng, X., Halbur, P., 2003. Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. Vet. Pathol. 41, 624–640.

Park, S.C., Yibchok-Anun, S., Cheng, H., Young, T.F., Tacker, E.L., Minion, F.C., Ross, R.F., Hsu, W.H., 2002. *Mycoplasma hyopneumoniae* increased intracellular calcium release in porcine ciliated tracheal cells. Infect. Immun. 70, 2502–2506.

Pointon, A., Heap, P., McCloud, P., 1985. Enzootic pneumonia of pigs in South Australia—factors relating to incidence of disease. Aust. Vet. J. 62, 98–100.

Razin, S., Yogev, D., Naot, Y., 1998. Molecular biology and pathogenicity of Mycoplasmas. Microbiol. Mol. Biol. Rev. 62, 1094–1156.

Sørensen, V., Ahrens, P., Barfod, K., Feenstra, A., Feld, N., Friis, N., Bille-Hansen, V., Jensen, N., Pedersen, M., 1997. *Mycoplasma hyopneumoniae* infection in pigs: duration of the disease and evaluation of four diagnostic assays. Vet. Microbiol. 54, 23–34.

Shimoji, Y., Oishi, E., Muneta, Y., Nosaka, H., Mori, Y., 2003. Vaccine efficacy of the attenuated *Erysipelothrix rhusiopathiae* YS-19 expressing a recombinant protein of *Mycoplasma hyopneumoniae* P97 adhesin against mycoplasmal pneumonia of swine. Vaccine 21, 532–537.

Sibila, M., Bernal, R., Torrent, D., March, R., Llopart, D., Riera, P., Calsamiglia, M., 2006. Effect of *Mycoplasma hyopneumoniae* sow vaccination on colonization, seroconversion and presence of enzootic pneumonia compatible lung lesions. In: Proceedings of the 19th IPVS congress, Copenhagen, Denmark, p. 103.

Sibila, M., Calsamiglia, M., Nofrarías, M., López-Soria, S., Espinal, A., Segalés, J., Riera, P., Llopart, D., Artigas, C., 2004b. Longitudinal study of *Mycoplasma hyopneumonicae* infection in naturally infected pigs. In: Proceedings of the 18th IPVS congress, Hamburg, Germany, p. 169.

Sibila, M., Calsamiglia, M., Vidal, D., Badiella, Ll., Aldaz, Á., Jensen, J.D., 2004a. Dynamics of *Mycoplasma hyopneumonicae* infection in twelve farms with different production systems. Can. J. Vet. Res. 68, 12–18.

Sibila, M., Nofrarıas, M., Lopez-Soria, S., Segales, J., Riera, P., Llopart, D., Calsamiglia, M., 2007b. Exploratory field study on *Mycoplasma hyopneumoniae* infection in suckling pigs. Vet. Microbiol. 121, 352–356.

Sibila, M., Nofrarías, M., López-Soria, S., Segalés, J., Valero, O., Espinalm, A., Calsamiglia, M., 2007a. Chronological study of *Mycoplasma hyopneumoniae* infection, seroconversion and associated lung lesions in vaccinated and non-vaccinated pigs. Vet. Microbiol. 122, 97–107.

Stakenborg, T., Vicca, J., Maes, D., Peeters, J., de Kruif, A., Haesebrouck, F., Butaye, P., 2006. Comparison of molecular techniques for the typing of *Mycoplasma hyopneumoniae* isolates. J. Microbiol. Methods 66, 263–275.

Stärk, K., 2000. Epidemiological investigation of the influence of environmental risk factors on respiratory diseases in swine—a literature review. Vet. J. 159, 37–56.

Tajima, M., Yagihashi, T., Nunoya, T., Takeuchi, A., Ohashi, F., 1984. *Mycoplasma hyopneumoniae* infection in pigs immunosuppressed by thymectomy and treatment with antithymocyte serum. Am. J. Vet. Res. 45, 1928–1932.

Thacker, B., Thacker, E., Halbur, P., Minion, C., Young, T., Erickson, B., Thanawonguwech, T., 2000b. The influence of maternally-derived antibodies on *Mycoplasma hyopneumoniae* infection. In: Proceedings of the 16th IPVS Congress, Melbourne, Australia, p. 454.

Thacker, E., 2004. Diagnosis of *Mycoplasma hyopneumoniae*. Anim. Health Res. Rev. 5, 317–320.

Thacker, E., 2006. Mycoplasmal diseases. In: Straw, B.E., Zimmerman, J.J., D'Allaire, S., Taylor, D.J. (Eds.), Diseases of Swine. 9th ed. Blacwell Publishing Ltd., Oxford, UK, pp. 701–717.

Thacker, E., Halbur, P., Ross, R., Thanawongnuwech, R., Thacker, B., 1999. *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. J. Clin. Microbiol. 37, 620–627.

Thacker, E., Nilubol, D., Halbur, P., 2006. Efficacy of Aureomycin® chlortetracycline (CTC) granulated premix in decreasing the potentiation of PRRSV pneumonia by *Mycoplasma hyopneumoniae*. In: Proceedings of the 37th Annual Meeting, Am. Assoc. Swine Vet., Kansas City, Missouri, pp. 153–155.

Thacker, E., Thacker, B., Boettcher, T., Jayappa, H., 1998. Comparison of antibody production, lymphocyte stimulation, and protection induced by four commercial *Mycoplasma hyopneumoniae* bacterins. J. Swine Health Prod. 6, 107–112.

Thacker, E., Thacker, B., Kuhn, M., Hawkins, P., Waters, W., 2000a. Evaluation of local and systemic immune responses induced by intramuscular injection of a *Mycoplasma hyopneumoniae* bacterin to pigs. Am. J. Vet. Res. 61, 1384–1389.

Thacker, E., Thacker, B., Wolff, T., 2004. Efficacy of a chlortetracycline feed additive in reducing pneumonia and clinical signs induced by experimental *Mycoplasma hyopneumoniae* challenge. J. Swine Health Prod. 14, 140–144.

Thacker, E., Thacker, B., Young, Th., Halbur, P., 2000c. Effect of vaccination on the potentiation of porcine reproductive and respiratory syndrome virus (PRRSV)-induced pneumonia by *Mycoplasma hyopneumoniae*. Vaccine 18, 1244–1252.

Vasconcelos, A.T., Ferreira, H.B., Bizarro, C.V., Bonatto, S.L., Carvalho, M.O., Pinto, P.M., Almeida, D.F., Almeida, R., ALves-Filho, L., Assunçao, E.N., Azevedo, V.A., Bogo, M.R., Brigido, M.M., Brocchi, M., Helio, A.B., Camargo, A.A., Camargo, S.S., Carepo, M.S., Carraro, D.C., de Mattos Cascardo, J.C., Castro, L.A., Cavalcanti, G., Chemale, G., Collevatti, R.G., Cunha, C.W., Dallagiovanna, B., Dambrós, B.P., Dellagostin, O.A., Falcão, C., Fantinatti-Garboggini, F., Felipe, M.S.S., Fiorentin, L., Franco, G.R., Freitas, N.S.A., Frías, D., Grangeiro, T.B., Grisard, E.C., Guimarães, C.T., Hungria, M., Jardim, S.N., Krieger, M.A., Laurino, J.P., Lima, L.F.A., Lopes, M.I., Loreto, E.L.S., Madeira, H.M.F., Manfio, G.P., Maranhão, A.Q., Martinkovics, C.T., Medeiros, S.R.B., Moreira, M.A.M., Neiva, M., Ramalho-Neto, C.O., Nicolás, M.F., Oliveira, S.C., Paixão, R.F.C., Pedrosa, F.O., Pena, S.D.J., Pereira, M., Pereira-Ferrari, L., Piffer, I., Pinto, L.S., Potrich, D.P., Salim, A.C.M., Santos, F.R., Schmitt, R., Schneider, M.P.C., Schrank, A., Schrank, I.S., Schuck, A.F., Seuanez, H.N., Silva, D.W., Silva, R., Silva, S.C., Soares, C.M.A., Souza, K.R.L., Souza, R.C., Staats, C.C., Steffens, M.B.R., Teixeira, S.M.R., Urmenyi, T.P., Vainstein, M.H., Zuccherato, L.W., Simpson, A.J.H., Zaha, A., 2005. Swine and poultry pathogens: the complete genome sequences of two strains of *Mycoplasma hyopneumoniae* and a strain of *Mycoplasma synoviae*. J. Bacteriol. 187, 5568–5577.

Vicca, J., Maes, D., Jonker, L., de Kruif, A., Haesebrouck, F., 2005. The efficacy of tylosin premix for the treatment and control of *Mycoplasma hyopneumoniae* infections. Vet. Rec. 156, 606–610.

Vicca, J., 2005. Virulence and antimicrobial susceptibility of *Mycoplasma hyopneumoniae* isolates from pigs. PhD thesis, Faculty of Veterinary Medicine, Ghent University, ISBN 90-5864-086-8, 219 pp.

Vicca, J., Stakenborg, T., Maes, D., Butaye, P., Peeters, J., de Kruif, A., Haesebrouck, F., 2004. *In vitro* susceptibilities of *Mycoplasma hyopneumoniae* field isolates. Antimicrob. Agents Chemother. 48, 4470–4472.

Vicca, J., Thermote, L., Maes, D., D'Hooghe, I., Peeters, J., Haesebrouck, F., de Kruif, A., 2002. Patterns of *Mycoplasma hyopneumoniae* infection in closed pig herds using serology and nested PCR on nasal samples. J. Vet. Med. B 49, 349–353.

Whittlestone, P., 1990. Control of Enzootic Pneumonia Infection in Pigs. Zentralblatt (Suppl.) 20. Gustav Fischer Verlag, Stuttgart, New York, pp. 254–259.

Zhang, Q., Young, T.F., Ross, R.F., 1995. Identification and characterization of *Mycoplasma hyopneumoniae* adhesins. Infect. Immun. 62, 1616–1622.

Zimmerman, W., Odermatt, W., Tschudi, P., 1989. Enzootische Pneumonie (EP): die Teilsanierung EP-reinfizierter Schweinezuchtbetriebe als Alternative zur Totalsanierung. Schweiz. Arch. Tierheilk. 131, 179–191.