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1 **Control of *Mycoplasma hyopneumoniae* infections in pigs**

2
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24 **Abstract**

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

46 **Keywords:** *Mycoplasma hyopneumoniae*, control, medication, vaccination, eradication

47 **1. Introduction**

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

54 *M. hyopneumoniae* is extremely difficult to isolate because of its slow growth and
55 interference with other swine mycoplasmas. The organism is primarily found on the
56 mucosal surface of the trachea, bronchi, and bronchioles (Blanchard et al., 1992), and
57 adherence of *M. hyopneumoniae* to the ciliated epithelium is a prerequisite for initiation of
58 the infection. Zhang et al. (1995) identified the P97 protein to be a ciliary adhesin. Other
59 adhesins may include a glycoprotein of 110 kDa (Chen et al., 1998), a P159 protein that is
60 post-translationally cleaved in proteins of 27, 51 and 110 kDa (Burnett et al., 2006) and a
61 146 kDa protein (Stakenborg et al., 2006). *M. hyopneumoniae* affects the mucosal
62 clearance system by disrupting the cilia on the epithelial surface and, additionally, the
63 organism modulates the immune system of the respiratory tract (Thacker, 2006).
64 Therefore, *M. hyopneumoniae* predisposes animals to concurrent infections with other
65 respiratory pathogens including bacteria, parasites and viruses. Limited information is
66 available about the mechanisms which trigger the cilia damage. *M. hyopneumoniae* may
67 increase the intracellular free calcium concentration in ciliated epithelial cells, which can
68 induce loss of cilia (Park et al., 2002). A 54 kDa membrane protein of *M. hyopneumoniae*
69 induced cytopathogenic effects in human lung fibroblast cell lines (Geary and Walczak,

70 1985). Epithelial cell damage may also be caused by the mildly toxic by-products of
71 mycoplasma metabolism, such as hydrogen peroxide and superoxide radicals (Razin et al.,
72 1998).

73 Chronic, non-productive coughing, the main clinical sign, appears 10-16 days after
74 experimental infection, but this period can vary greatly under field conditions.
75 Macroscopic lesions, consisting of purple to grey areas of pulmonary consolidation, are
76 mainly found bilaterally in the apical, cardiac, intermediate and the anterior parts of the
77 diaphragmatic lobes. Recovering lesions consist of interlobular scar retractions, and in case
78 of a pure *M. hyopneumoniae* infection macroscopic lesions are resolved 12-14 weeks after
79 infection.

80 Clinical signs and lesions can lead to a tentative diagnosis, but laboratory testing is
81 necessary for a conclusive diagnosis (Thacker, 2004). Although culturing of the organism
82 is described as the gold standard, it is not used for routine diagnosis. The organism can be
83 detected by immunofluorescence testing, but this test has limited sensitivity. Serology can
84 be used to show presence of the organism at a herd level, but is not suited for diagnosis on
85 individual animals (Sørensen et al., 1997). At present, (nested) Polymerase Chain Reaction
86 (PCR) testing is seen as the most sensitive tool to detect the infection (Calsamiglia et al.,
87 1999).

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

93

94 **2. Optimization of management practices and housing conditions**

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

104 *2.1. Production system*

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

114 Early weaning (< 3 weeks) can reduce transmission of *M. hyopneumoniae* organisms from
115 the sow to the offspring, but it is not allowed to be applied systematically in the EU. Parity

116 segregation has been used in large production systems as a means to control several
117 diseases in the breeding herd, including *M. hyopneumoniae* infections. Gilts and also their
118 offspring are kept separated from the sows until they reach their second gestation. By that
119 time, they are expected to have acquired the desired immune status and to pose no
120 destabilization risk for the herd anymore (Hoy et al., 1986; Joo, 2003).

121 *2.2. Purchase of animals*

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

131 *2.3. Animal stocking density*

132 Decreasing animal density during the different production stages has been shown to reduce
133 the level of respiratory disease (Pointon et al., 1985). Crowding may lead to increased
134 transmission of pathogens, and to stress reactions, making the pigs more susceptible to
135 infectious diseases. Too low stocking densities are financially not justified. Therefore, it is
136 important to find a reasonable compromise between stocking densities that are appropriate
137 for the health of the pigs and those that maximize the returns on the building's cost. In

138 general, the stocking density for finishing pigs on fully slatted floors should equal or
139 exceed 0.7 m² per pig (Madec, 1984).

140 2.4. Herd size

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

154 2.5. Prevention of other diseases

155 Appropriate parasite control measures are necessary to avoid any lung damage caused by
156 migrating *Ascaris suum* larvae. Lung damage may also arise from infections with other
157 respiratory pathogens including bacteria and viruses. Porcine reproductive and respiratory
158 syndrome virus (PRRSV), influenza viruses, porcine circovirus type 2 (PCV-2) and
159 porcine respiratory corona virus (PRCV), in combination with *M. hyopneumoniae*, are
160 considered to play an important role in the development of the porcine respiratory disease

161 complex (PRDC). The impact of these infections can be reduced by means of medication,
162 vaccination or by appropriate management practices.

163 2.6. Biosecurity measures

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

173 2.7. Improvement of housing conditions

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

181

182 3. Antimicrobial medication

183 To control and treat respiratory disease including *M. hyopneumoniae* infections in pigs,
184 tetracyclines and macrolides are most frequently used. Also, other potentially active
185 antimicrobials against *M. hyopneumoniae* include lincosamides, pleuromutilins,
186 fluoroquinolones, florfenicol, aminoglycosides and aminocyclitols. Fluoroquinolones and
187 aminoglycosides have mycoplasmacidal effects. Since the organism lacks a cell wall, it is
188 insensitive to β -lactamic antibiotics such as penicillins and cephalosporins.

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

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

201 The control of the disease by medication can be approached by strategic administration of
202 antimicrobials. The product is added for about 1-3 weeks, commencing approximately 1
203 week prior to the expected time of disease onset. Such medication regimen can limit the
204 severe consequences of the disease and the infection load (Thacker et al., 2004), but it does
205 not prevent pigs from becoming infected with *M. hyopneumoniae*. Treatment and control of

206 enzootic pneumonia outbreaks may be disappointing because the symptoms may reappear
207 after cessation of the therapy. Pulse medication in which medication is provided
208 intermittently during critical production stages of the pigs, can also be used (Le Grand and
209 Kobish, 1996). Pulse medication during extended periods of time as well as continuous
210 medication during one or more production stages should be discouraged because of both
211 the increased risk of antimicrobial resistance development and the possible risk for
212 antimicrobial residues in the pig carcasses at slaughter.

213 In endemically infected farms, strategic medication of the reproductive herd is sometimes
214 practiced as an attempt to decrease the bacterial shedding from sows to the newly
215 introduced gilts. Antimicrobial medication of recently weaned pigs has been shown to
216 reduce the number of *M. hyopneumoniae* organisms in the respiratory tract (Vicca et al.,
217 2005; Thacker et al., 2006), but further research is necessary to quantify the shedding of *M.*
218 *hyopneumoniae* organisms in sows receiving antimicrobial medication.

219

220 **4. Vaccination**

221 *4.1. Commercial vaccines*

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

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

241

242 *4.2. Mechanisms of protection after vaccination*

243 The exact mechanisms of protection are not yet fully understood. Intramuscular injection of
244 a commercial bacterin (Thacker et al., 2000a) has been shown to induce the secretion of
245 specific antibodies in the serum and the respiratory tract, as well as the generation of IFN- γ
246 secreting cells in blood. Based on the findings of this study, the authors suggested that both
247 local mucosal antibodies and systemic cell-mediated immune (CMI) responses are
248 important for protection. However, the correlation of local *M. hyopneumoniae*-specific
249 antibody production and protection against pneumonia remains unclear. Djordjevic et al.
250 (1997) and Thacker et al. (1998) found that antibody concentrations in respiratory tract
251 washings did not correlate with protection against pneumonia.

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

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

283

284 4.3. Vaccination strategies

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

290 Different vaccination strategies have been adopted, depending on the type of herd, the
291 production system and management practices, the infection pattern and the preferences of
292 the pig producer. Moreover, under field conditions, optimal vaccination strategies must
293 balance the advantage of delayed vaccination with the need to induce immunity before
294 exposure to pathogens. Since infections with *M. hyopneumoniae* may already occur during
295 the first weeks of life (Vicca et al., 2002; Sibila et al., 2007b), vaccination of piglets is most
296 commonly used. Its efficacy has been demonstrated by means of numerous studies under

297 experimental as well as field conditions (Jensen et al., 2002). Vaccination of suckling
298 piglets (early vaccination; < 4 weeks of age) is more common in single-site herds, whereas
299 vaccination of nursery/early fattening pigs (late vaccination; between 4 and 10 weeks) is
300 more often practiced in three-site systems where late infections are more common.

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

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

320 role in the influence vaccination has on the clinical outcome of this viral infection
321 (Opriessnig et al., 2006).

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

329 In contrast with the numerous studies that investigated the efficacy of vaccination of
330 piglets, only a few studies have assessed the effects of sow vaccination. Vaccination of
331 sows at the end of gestation aims to both reduce the shedding of *M. hyopneumoniae* from
332 the sow to the offspring and to protect the piglets against infection via maternally-derived
333 immunity. It has been shown that vaccinating sows 5 and 3 weeks before farrowing was
334 associated with a lower number of positive piglets at weaning using nested PCR on nasal
335 swabs, both in farrow-to-finish operations and multi-site production systems (Sibila et al.,
336 2006). However, maternally derived antibodies only provide partial protection against
337 lesion development following challenge infection, and under experimental conditions, they
338 provide limited to no effect on colonization of *M. hyopneumoniae* (Thacker et al., 2000b).
339 The role of antigen-specific maternally derived immune cells in protection against *M.*
340 *hyopneumoniae* is not known. Bandrick et al. (2006) showed *in vivo* response by delayed-
341 type hypersensitivity and *in vitro* proliferation of maternally derived cells when newborn
342 piglets were stimulated with *M. hyopneumoniae* antigen. Since piglets from vaccinated

343 sows can still be infected, additional measures to control *M. hyopneumoniae* during the
344 nursery and finishing phases may be warranted. Further research is needed to investigate
345 the effects of sow vaccination on infection levels in pigs in different production systems.

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

350

351 4.4. Factors influencing the efficacy of vaccination

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

358 The existence of high proteomic heterogeneity between *M. hyopneumoniae* isolates from
359 different herds has been clearly demonstrated (Calus et al., 2007). It remains to be
360 investigated however whether this high protein variability may account partially for
361 reduced efficacy of vaccination as observed in some herds.

362 Under experimental conditions, PRRSV infection or administration of a modified live virus
363 (MLV) PRRS vaccine (US strain) at the time of *M. hyopneumoniae* vaccination appeared to
364 significantly reduce the efficacy of the *M. hyopneumoniae* vaccine (Thacker et al., 2000c).

365 On the contrary, administration of a MLV PRRS vaccine to pigs one week prior to

366 vaccination with *M. hyopneumoniae* vaccine did not interfere with vaccine efficacy or
367 immune responses to *M. hyopneumoniae* infection (Boettcher et al., 2002). It is likely that
368 also other infections or factors *e.g.* mycotoxins affecting the health and/or immune system
369 of the pig at the moment of vaccination may interfere with vaccine efficacy.

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

384

385 *4.5. Experimental vaccines*

386 Investigation of new vaccines is actively occurring, including the use of aerosol and feed-
387 based vaccines as well as subunit and DNA vaccines (Fagan et al., 2001; Lin et al., 2003;
388 Murphy et al., 1993). Intradermal vaccination with a commercial bacterin has been shown

389 to be efficacious (Jones et al., 2004). If *M. hyopneumoniae* vaccines could be delivered to
390 the animals via aerosols or via the feed, this would provide an easy means for mass
391 vaccination since it would substantially reduce labor costs and it would also be better for
392 the welfare of the pigs as well as for stimulating a mucosal immune response at the
393 respiratory tract. However, aerosol vaccination given 3 times with 2 weeks interval
394 provided insufficient protection, in contrast with the intramuscular application of the same
395 commercial vaccine which was efficacious (Murphy et al., 1993). On the other hand, Lin
396 et al. (2003) showed that an oral micro-spheres experimental vaccine based on the PRIT-5
397 *M. hyopneumoniae* strain and prepared by a co-spray drying method significantly reduced
398 pneumonia lesions following challenge infection with *M. hyopneumoniae* in pigs.

399 King et al. (1996) found only minimal and non-significant protection in a pig challenge
400 infection model using a recombinant subunit vaccine based on the P97 adhesin of *M.*
401 *hyopneumoniae*. Intranasal immunization of pigs with the attenuated *Erysipelothrix*
402 *rhusiopathiae* YS-19 strain expressing a recombinant protein of *M. hyopneumoniae* P97
403 adhesin significantly reduced the severity of pneumonic lung lesions following challenge
404 infection (Shimoji et al., 2003). However, apparently significant humoral and cell-
405 mediated immune responses were not observed in the immunized pigs. Okamba et al.
406 (2007) showed that a replication-defective adenovirus expressing the C-terminal portion of
407 *M. hyopneumoniae*-P97 adhesin applied intranasally and intramuscularly in BALB/c mice,
408 induced significant immune responses. Also several experimental DNA-vaccines have
409 been developed and tested for immune responses in mice or pigs. Significant immune
410 responses with DNA-vaccines were elicited in mice, based on the expression of a heat
411 shock protein gene P42 (Chen et al., 2003) or a ribonucleotide reductase R2 subunit gene

412 fragment of *M. hyopneumoniae* (Chen et al., 2006). The studies suggest that these vaccines
413 may represent new strategies for controlling *M. hyopneumoniae* infections in pigs, but they
414 need to be validated in pigs under experimental and practical circumstances.

415 Further studies are necessary for improving vaccines and vaccination strategies. From an
416 immunological point of view, challenges include induction of immunity at the mucosal
417 level. For rational design of vaccines, a comprehensive understanding of the pathobiology
418 of *M. hyopneumoniae* infections and the molecular basis of pathogenicity of this micro-
419 organism is required. Bacterial genes and antigens involved in survival of the bacterium in
420 the host or that render the bacterium harmful to the host need to be identified. This may be
421 facilitated by the fact that the genome of 3 different *M. hyopneumoniae* isolates has
422 recently been sequenced (Minion et al., 2004; Vasconcelos et al., 2005).

423

424 **5. Preventive medication versus vaccination**

425 The use and efficacy of either vaccination or preventive (strategic) medication is frequently
426 discussed among swine veterinarians and the question always arises whether medication
427 and/or vaccination should be used. Antimicrobials can be used in a flexible way, they are
428 often effective against several (respiratory) pathogens and their administration is less labor-
429 intensive since in-feed or in-water medication is mostly used. Vaccination, on the other
430 hand, does not select for antimicrobial resistance in pathogenic bacteria and in bacteria
431 belonging to the microbiota of the animal. It also avoids risks for antimicrobial residues in
432 the pig carcasses at slaughter. While an immediate effect can be expected for antimicrobial
433 treatment, the effect of vaccination of young piglets will only be evident at herd level if it is
434 practiced for at least several months. Although vaccines are directed towards control of *M.*

435 *hyopneumoniae* infections, also other secondary bacterial infections (*Pasteurella multocida*,
436 *Actinobacillus pleuropneumoniae*) or lung lesions caused by these pathogens less
437 frequently occur after vaccination (Maes et al., 1998; 1999; Meyns et al., 2006).

438 Neither vaccination nor preventive medication can prevent infection and adherence of *M.*
439 *hyopneumoniae* to the ciliated cells of the respiratory tract (Le Grand and Kobisch 1996).

440 Finally, in case of high infection levels and/or in herds with poor management and housing
441 conditions, the use of antimicrobials may remain necessary or may confer additional
442 clinical and performance benefits in vaccinated herds (Mateusen et al., 2002).

443

444 **6. Eradication**

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

450 Currently, eradication of *M. hyopneumoniae* is mainly applied in Denmark, Finland and
451 Switzerland. In Switzerland, a total and partial sanitation programme was set up
452 (Zimmermann et al., 1989), which is nowadays known as “the Swiss system” of eradication
453 of infectious diseases. The total sanitation programme involved complete emptying of the
454 animal facilities by selling or culling all animals. The partial sanitation programme
455 included a piglet and gilt free (< 10 months) interval and the temporary feeding of a
456 medicated diet during 10-14 days. The feed contained either tiamulin (6 mg/kg body weight
457 (BW)) or a combination of chlortetracycline (20 mg/kg BW), tylosin (4 mg/kg BW) and

458 sulfadimidin (30 mg/kg BW). Once finished this medication period, the farm continues
459 with its normal animal flow. The partial sanitation programme is well suited for small
460 farms. In the US and Canada, large swine operations have developed a modified version of
461 the Swiss method. The program includes the features of the partial depopulation scheme
462 along with multiple vaccinations of the breeding herd and an offsite breeding program. The
463 method is usually combined with herd closure when PRRSV elimination is also targeted.
464 Another method used mainly in the US includes medicated early weaning (MEW) wherein
465 intensive medication of the sow during late gestation and immediately following parturition
466 as well as the newborn piglets is used to derive pigs free of *M. hyopneumoniae*. Piglets are
467 weaned at 5 days of age, placed in an isolated nursery on a separate site and medicated for
468 the first 10 days after weaning (Harris and Alexander, 1999). Modifications of the MEW
469 programme have been applied, based on postponing weaning until 21-25 days of age,
470 keeping sows and nursery pigs at the same site, vaccination strategies and/or combined with
471 medication of the sows and medications of the piglets before and after weaning (Clark et
472 al., 1994). It was concluded that isolating the pigs was as effective as medication and
473 vaccination protocols in controlling the transmission of the pathogens investigated,
474 including *M. hyopneumoniae*, *Bordetella bronchiseptica* and *P. multocida*. *M.*
475 *hyopneumoniae* was not detected when any MEW procedure was used.
476 Although several attempts have been made to eradicate *M. hyopneumoniae* from a herd, re-
477 infection frequently occurs (2.6-10% per year). The high re-infection rates may be due to
478 airborne transmission from infected neighbourhood herds, or to purchase of infected
479 animals that were tested negative using ELISA techniques on serum or colostrum samples.
480 Although ELISA has proved to be a very useful technique, pigs can be colonized with low

481 numbers of *M. hyopneumoniae* organisms in the lung without eliciting a serological
482 detectable response (Thacker, 2006). Other risk factors for the re-infection include being a
483 finishing farm, large mixed breeding-finishing farm, and parking site for pig transport
484 vehicles close to the farm.

485

486 **7. Conclusions**

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

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