Acclimation strategies in gilts to control *Mycoplasma hyopneumoniae* infection

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Abstract

Mycoplasma hyopneumoniae (M. hyopneumoniae) is the primary causative agent of enzootic pneumoniae (EP), one of the most economically important infectious disease for the swine industry worldwide. *M. hyopneumoniae* transmission occurs mainly by direct contact (nose-to-nose) between infected to susceptible pigs as well as from infected dams to their offspring (sow-to-piglet). Since disease severity has been correlated with *M. hyopneumoniae* prevalence at weaning in some studies, and gilts are considered the main bacterial shedders, an effective gilt acclimation program should help controlling *M. hyopneumoniae* in swine farms. The present review summarizes the different *M. hyopneumoniae* monitoring strategies of incoming gilts and recipient herd and proposes a farm classification according to their health statuses. The medication and vaccination programs against *M. hyopneumoniae* most used in replacement gilts are reviewed as well. Gilt replacement acclimation against *M. hyopneumoniae* in Europe and North America indicates that vaccination is the main strategy used, but there is a current trend in US to deliberately expose gilts to the pathogen.

Keywords: *Mycoplasma hyopneumoniae*, gilt acclimation, adaptation strategies, Europe, North America

1. Introduction

Mycoplasma hyopneumoniae (M. hyopneumoniae) is the causative agent of mycoplasmal pneumonia (MP), an important porcine respiratory disease. This infectious process is frequently complicated by other respiratory bacteria (such as *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and others) causing a more severe chronic and economically important disease known as enzootic pneumonia (EP). In addition to bacterial complication, viral pathogens like *Porcine reproductive and respiratory syndrome virus*, *Porcine circovirus 2* and *Swine influenza virus* can aggravate the disease scenario; this viral-bacteria complex is clinically referred as porcine respiratory disease complex (PRDC) (Thacker and Minion, 2012). Despite all efforts implemented to reduce the economic impact caused by *M. hyopneumoniae* (vaccination and antimicrobial treatments together with improvement of management practices), EP and PRDC still cause great concern in the swine industry worldwide.

EP mainly affects growing and finishing pigs and it is characterized by dry, non-productive cough, reduction in growth rate, and increased feed conversion ratio. The severity of the disease is dependent on the presence of co-infections and environmental conditions (Maes et al., 1996) and on the virulence and number of *M. hyopneumoniae* strains involved (Vicca et al., 2003; Woolley et al., 2012; Michiels et al., 2017). *M. hyopneumoniae* is mostly transmitted by direct contact (nose-to-nose) between pigs, horizontally from infected to susceptible/naïve pigs (Morris et al., 1995) as well as from dam to their offspring (Sibila et al., 2008; Nathues et al., 2014; Pieters et al., 2014). Other putative indirect transmission routes are aerosol and fomites. Whereas the aerosol transmission has been experimentally proved (Fano et al., 2005; Otake et al., 2010), transmission by fomites has not been clearly demonstrated and it can be potentially prevented by basic biosecurity practices (Batista et al., 2004; Pitkin et al., 2011).

Different studies showed that disease severity in growing pigs is correlated with *M. hyopneumoniae* prevalence of piglet colonization at weaning (Fano et al., 2007; Sibila et al., 2008). However, other studies could not show this association (Vranckx et la, 2012b). This prevalence can be influenced by different factors such as housing and management conditions of the production system as well as dam parity, piglet's age at weaning and replacement rate (Nathues et al., 2013, 2014). Since newborn piglets are *M. hyopneumoniae* free, the most logical source of infection is the dam at the time of farrowing or during the lactation period (Sibila et al., 2007). Some authors suggested this transmission could be influenced by the dam's parity (Calsamiglia and Pijoan, 2000; Fano et al., 2006). Indeed, bacterial shedding of gilts or young sows seems to be higher than that of older parity sows (Boonsoongnern et al., 2012). Therefore, the first farrowing is considered a critical moment at which *M. hyopneumoniae* excretion should have ceased (Pieters and Fano, 2016). These latter data together with a low transmission rate (reproduction ratio [R_n] varies among 1.16-1.28 and 0.56-0.71 under experimental and field conditions, respectively) (Meyns et al., 2006; Villarreal et al., 2009; Roos et al., 2016) and the persistence of infection in pigs (up to 214 days post infection, dpi) (Pieters et al., 2009) imply the need of performing an effective gilt acclimation process. This effective acclimatization protocol would reduce *M. hyopneumoniae* shedding at first farrowing (Pieters and Fano, 2016) and, consequently, would decrease pre-weaning prevalence, subsequent spread of the pathogen to growing pigs, and putative respiratory problems in fattening animals (Fano et al., 2007; Sibila et al., 2008). Therefore, assuming that gilt population are crucial in the spread of the infection, the purpose of this review was to summarize different management practices, antimicrobial treatments and vaccination protocols in replacement gilts to control *M. hyopneumoniae* infections in pig herds.

2. *M. hyopneumoniae* health status

2.1. *Monitoring and diagnosis*

One of the main risks for *M. hyopneumoniae* colonization in piglets at weaning is a high gilt replacement rate (Nathues et al., 2013). Therefore, the first step to perform an appropriate adaptation of future replacements to *M. hyopneumoniae* is monitoring the health status of the recipient breeding herd, as well as incoming gilts to detect potential disease/infection indicators. In case of *M. hyopneumoniae* infection suspicion, a definitive diagnosis should be performed.

Monitoring of *M. hyopneumoniae* associated disease is sometimes challenging as the infection can take a clinical or subclinical course (Table 1). In clinical cases, the observation of signs (dry, non-productive coughing) and lung lesions (pulmonary craneo-ventral consolidation) are indicative, but not exclusive of *M. hyopneumoniae*. In subclinical infections, animals can display *M. hyopneumoniae*-like lung lesions without any evidence of coughing (Maes et al., 1996). Therefore, clinical diagnosis should be confirmed by additional laboratory tests (Table 1).

The most commonly used herd monitoring method is *M. hyopneumoniae* antibody detection by ELISA. It provides evidence of exposure to *M. hyopneumoniae* without differentiating maternally derived antibodies, or antibodies elicited by infection, and/or vaccination (Bandrick et al., 2011; Thacker and Minion, 2012). Moreover, absence of antibodies (seronegative animals) may not be equivalent to a *M. hyopneumoniae* free status in early infection scenarios, suggesting that antibody and pathogen detection combined is the main goal for *M. hyopneumoniae* final diagnosis.

Different laboratory techniques have been described to confirm the presence of *M. hyopneumoniae* (Table 1). The most useful technique to detect *M. hyopneumoniae* is

polymerase chain reaction (PCR), as it can be performed using different respiratory tract samples. Up to now, there is no consensus on which type of sample from the porcine respiratory tract is the most suitable to detect bacterial DNA in live pigs. To confirm *M. hyopneumoniae* free status of live animals or to determine the involvement of such pathogen in an outbreak, the desired sample should be collected from the lower respiratory tract (i.e. laryngeal or tracheo-bronchial swabs or tracheo-bronchial lavage fluids), where *M. hyopneumoniae* colonization of respiratory cilia occurs (Fablet et al., 2010; Pieters et al., 2017). In dead animals, the sample of preference is lung tissue or bronchial swab.

2.2. *Recipient herd and incoming replacement classification regarding M. hyopneumoniae health status*

Once the *M. hyopneumoniae* health status of the recipient herds and the incoming gilts has been assessed, farms and incoming replacement could be classified into negative, provisional negative and positive according the following criteria (summarized in Table 2):

Negative herds/replacement. Clinical signs and lung lesions associated with *M. hyopneumoniae* are not present and serology and detection of pathogen in lung by PCR are negative. This type of breeding and fattening farms is the less frequent one in the current swine production in Europe (Garza-Moreno et al., 2017). Nevertheless, *M. hyopneumoniae* negative farms are increasingly common among gilt producers, genetic companies, high health farms and in certain countries such as United States (US), where a trend for *M. hyopneumoniae* elimination is growing (Maria Pieters, personal communication).

142 *Provisional negative herds/replacement*. *M. hyopneumoniae*-like clinical signs and
143 lung lesions are not observed but animals are seropositive and PCR negative. The
144 presence of antibodies against *M. hyopneumoniae* provides evidence of exposure to the
145 pathogen by prior infections and/or vaccination against it. This type of farms (PCR
146 negative and seropositive) is frequently found in US since they are applying vaccination
147 against *M. hyopneumoniae* (Maria Pieters, personal communication).

148 *Positive herds/replacement*. These farms can be classified into subclinical infected
149 or clinical affected. Subclinical infected farms can be differentiated in two different
150 categories (I and II) according to the presence of ELISA antibodies against *M.*
151 *hyopneumoniae*, the detection of the pathogen by PCR and the presence of lung lesions
152 attributed to *M. hyopneumoniae* (Table 2). In category I, lung lesions associated to *M.*
153 *hyopneumoniae* are not observed, the detection of antibodies depends on the disease
154 phase (in early stages might not be detected) but the presence of the pathogen is
155 confirmed. Animals from herds included in category II do not show clinical signs
156 compatible with *M. hyopneumoniae* but have *M. hyopneumoniae*-like lung lesions,
157 antibodies against the pathogen might be detected and the presence of *M.*
158 *hyopneumoniae* is confirmed by PCR. Finally, in clinical affected farms, infected pigs
159 also display signs and lung lesions associated to *M. hyopneumoniae*.

160

161 **3. Prevention and control**

162 **3.1. *Vaccination***

163 Vaccination against *M. hyopneumoniae* is the most commonly used strategy to
164 control its associated diseases in worldwide swine production systems (Maes et al.,
165 2017). Most commercial vaccines against *M. hyopneumoniae* are inactivated whole-cell
166 preparations or bacterins, combined with an adjuvant to induce a stronger immune

response (Haesebrouck et al., 2004). Administration route of these commercial vaccines is mainly intramuscular and the volume per dose can vary according to the vaccine used (Table 3). Besides bacterins, attenuated vaccines against *M. hyopneumoniae* are also available in Mexico and China (Feng et al., 2013).

An alternative to commercial vaccines may be autogenous vaccines, based on isolated strains from the affected farm. These vaccines are not frequently used because of the difficulty to isolate *M. hyopneumoniae* strains and the apparent lack of vaccine safety and efficacy data. Although information is limited, a single study has compared the efficacy of immunization with homologous and heterologous strains against an experimental infection and no significant differences in protection were observed (Villarreal et al., 2012). Further investigation on new vaccines, as recombinant subunit or attenuated vaccines, is required to provide an effective and total protection against *M. hyopneumoniae* (Simionatto et al., 2013).

Different vaccination schedules against *M. hyopneumoniae* have been implemented depending on the type of herd, production system, infection dynamics, and number of doses administered (Haesebrouck et al., 2004). Commercial vaccines are most frequently applied to piglets, prior to or after weaning (Alarcon et al., 2014). Additionally, previous studies have shown that the weaning process does not significantly affect vaccination efficacy (Arsenakis et al., 2016), although numerical differences in terms of performance among vaccinated and non-vaccinated groups were detected (Arsenakis et al., 2017). Piglet vaccination efficacy has been widely demonstrated by reduction of clinical signs and prevalence and severity of lung lesions, improvement of production parameters, decrease of treatment costs and, in some cases, lower mortality rates (Maes et al., 1996). Although vaccination against *M. hyopneumoniae* does not prevent infection (Pieters et al., 2010; Villarreal et al., 2011,

192 2012), it is able to reduce the number of microorganisms in the swine respiratory tract
193 (Vranckx et al., 2012a; Woolley et al., 2012).

194 Sow vaccination is less frequently applied, but gaining relevance every day (Bargen,
195 2004). Nevertheless, a limited number of vaccines are currently licensed for the
196 reproductive population (Table 3) and studies on their effect are scarce (Table 4). Dam
197 vaccination sought to decrease the infectious pressure, lowering bacterial load and,
198 consequently, transmission from sow to piglet (Vranckx et al., 2012b; Takeuti et al.,
199 2017), as well as conferring maternal immunity via colostrum (Bandrick et al., 2011).
200 Indeed, some studies have shown that sow vaccination prior to farrowing is able to
201 reduce dam-to-piglet transmission, the number of positive piglets from vaccinated sows
202 (Ruiz et al., 2003), and the EP lung lesions of them at abattoir (Sibila et al., 2008).

203 Gilt vaccination combined with optimal management strategies have also been
204 suggested to stimulate the immune response against a controlled exposure to *M.*
205 *hyopneumoniae* (Holst et al., 2015) or in endemically infected herds (Maes et al., 2008).
206 Additionally, gilt vaccination is recommended to homogenize immunity of the
207 replacement batch and avoid destabilization of recipient breeding herd (Bargen, 2004).
208 This is especially important when replacement is external and originates from *M.*
209 *hyopneumoniae* negative farms. In this situation, the introduction of negative
210 replacement stock into positive farms may contribute to the development of
211 subpopulations of non-infected pigs, increasing the risk of pathogen re-circulation and
212 its persistence in the farm (Takeuti et al., 2017).

213 The number of required vaccine doses, application timing and its benefits are not
214 standardized for sows and gilts. Nowadays, single vaccination is more frequently used
215 due to the ease of implementation in farm management practices. Nevertheless,
216 multiple-dose vaccination against *M. hyopneumoniae* could elicit a booster effect of the

217 consecutive vaccine doses. The potential benefits of applying multiple vaccine doses in
218 terms of reduction of shedding have not been yet investigated.
219
220 **3.2. *Medication***
221 Since protection against *M. hyopneumoniae* infection and associated diseases
222 conferred by commercial vaccines is not complete, antimicrobial treatments are
223 frequently required in commercial swine farms to control disease outcome.
224 *Mycoplasmas* lack a cell wall, thus *M. hyopneumoniae* is resistant to β-lactam
225 antibiotics. Nevertheless, several antibiotic classes are effective in reducing the
226 incidence and severity of *M. hyopneumoniae* compatible lung lesions. Most commonly
227 used antibiotics are macrolides, lincosamides, tetracycline, and fluoroquinolones,
228 among others (Thacker and Minion, 2012). The route of administration can be
229 parenteral or mixed in feed / water depending on antibiotic choice.
230 Medication is currently used with different purposes. Parenteral medication is used
231 to treat animals suffering from severe clinical signs, normally associated with EP and
232 PRDC. Under field conditions medication is also commonly used to control *M.*
233 *hyopneumoniae* infection by means of minimizing pathogen transmission. Medication
234 of sows prior to farrowing could be utilized as an attempt to decrease the bacterial
235 shedding to the offspring (Thacker and Minion, 2012; Holst et al., 2015). Nevertheless,
236 it has been shown that antibacterial treatments do not eliminate the bacterium from the
237 host, and shedding of *M. hyopneumoniae* can be detected in pigs after medication
238 programs (Overesch and Kuhnert, 2017). Therefore, the use of antimicrobials should be
239 limited and only justified in specific situations to avoid the development of
240 antimicrobial resistance (Lee et al., 2013).
241

3.3. *Acclimation scenarios in Europe and North America*

Different acclimation scenarios may be in place and should be managed according to health status of the recipient herds, as well as the replacement batch (Table 5). In addition, the different production systems, management practices, and acclimation strategies used could have an impact on the acclimation process performed. To understand these differences, available information about gilt acclimation strategies used in Europe and North America are detailed (Table 6).

3.3.1. European scenario

Information on gilt acclimation strategies for *M. hyopneumoniae* utilized in Europe is limited. Recently, Garza-Moreno *et al.* (2017) identified the current acclimation strategies used in this continent. In this investigation, information was collected by 321 questionnaires voluntarily responded by 108 veterinarians from 18 countries. The questionnaires were focused on the assessment of *M. hyopneumoniae* herd status, replacement health status, acclimation strategies and methods utilized to determine its effect.

This study showed that the most common replacement origin used in Europe was external and that most respondents knew *M. hyopneumoniae* health status of replacement on arrival, being in most of the cases seropositive. Nevertheless, only 28% of respondents verified this theoretical *M. hyopneumoniae* status, being ELISA, the most used technique (Garza-Moreno et al., 2017).

Replacement acclimation against *M. hyopneumoniae* was performed in most participating European farms. Although most farms have isolation units where to specifically acclimate replacement stock, several farms did not have those facilities or respondents did not answer the question. Independently of these sites, the most used strategy to acclimate gilt was vaccination alone (58%), being the number of doses most

frequently administered at acclimation one and two doses. Other acclimation strategy used in Europe was the combination of vaccination together with natural exposure to potentially infected animals. However, an effective exposure to *M. hyopneumoniae* is difficult to reach into a natural infection scenario. Finally, among respondents who performed the acclimation on gilts, only around 25% of them verified the effect of the process, being the combination of ELISA and PCR tests the most used strategy.

3.3.2. *North American scenario*

The importance of proper gilt acclimation to the incoming breeding herd against *M. hyopneumoniae* is paramount and highly recognized in the North American swine industry. This importance can be evidenced in the assessment of *M. hyopneumoniae* health status of the replacement and the existence of facilities for acclimatization against herd pathogens (gilt development units; GDUs). GDUs are utilized to allow ample time to incoming gilt to gradually adopt the health status of the recipient herd. According to previous studies based on questionnaires collected in US (Fano and Payne, 2015) and Mexico (Centeno et al., 2016), these acclimation facilities are in most of the cases continuous flow (72% and 75%, respectively) allowing an effective gilt exposure to *M. hyopneumoniae.*

Gilt vaccination in North American swine industry was also recognized as the most common practice used at acclimation (Fano and Payne, 2015; Centeno et al., 2016). Other methods as natural exposure to *M. hyopneumoniae,* alone or combined with vaccination, and contact with infected cull sows or/and piglets are also used to acclimate the gilts (Dalquist, 2014; Fano and Payne, 2015). Taking into account that pig-to-pig transmission of this bacterium has proven to be extremely slow (Meyns et al., 2004; Roos et al., 2016), the ratio of infected and naïve gilts as well as the time of exposure are crucial and should be considered to achieve an effective exposure. Recently, early

292 controlled exposure has been attempted to expose the gilts by administering (intra-
293 tracheally) lung tissue homogenate containing *M. hyopneumoniae* (Fano and Payne,
294 2015; Centeno et al., 2016) to individual gilts or groups of them (via aerosol), since the
295 success of exposure is higher when these controlled procedures are used (Sponheim A.,
296 2017). Finally, according to aforementioned studies, overall, the verification of gilt
297 acclimation process is minimally performed in North American farms.
298
299 **4. Conclusion**
300 *M. hyopneumoniae* is a respiratory pathogen that causes important economic losses
301 to the swine industry worldwide. A proper gilt acclimation against *M. hyopneumoniae*
302 prior entrance into a recipient breeding farm could maintain the farm health stability and
303 control respiratory disease caused by this pathogen. Gilt replacement acclimation
304 procedures against *M. hyopneumoniae* in Europe and North America showed that
305 vaccination is the main strategy used, but there is a current trend in the US to
306 deliberately expose gilts to the pathogen. Further investigations are needed to identify
307 the ideal gilt acclimation protocol taking into account that these strategies must be based
308 on incoming and recipient herd health statuses and the characteristics of each farm.
309
310 **Conflict of interest**
311 The authors declare no conflict of interest.
312
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