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Acclimation strategies in gilts to control *Mycoplasma hyopneumoniae* infection

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Highlights

- *M. hyopneumoniae* monitoring should be performed in incoming gilts and recipient herd.
- Gilt acclimation against *M. hyopneumoniae* aids to maintain farm health stability.
- Vaccination is the main strategy used to acclimate gilts in Europe and North America.

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).

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

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

3. Prevention and control

3.1. *Vaccination*

Vaccination against *M. hyopneumoniae* is the most commonly used strategy to control its associated diseases in worldwide swine production systems (Maes et al., 2017). Most commercial vaccines against *M. hyopneumoniae* are inactivated whole-cell 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, 2012), it is able to reduce the number of microorganisms in the swine respiratory tract (Vranckx et al., 2012a; Woolley et al., 2012).

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

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

The number of required vaccine doses, application timing and its benefits are not standardized for sows and gilts. Nowadays, single vaccination is more frequently used due to the ease of implementation in farm management practices. Nevertheless, multiple-dose vaccination against *M. hyopneumoniae* could elicit a booster effect of the consecutive vaccine doses. The potential benefits of applying multiple vaccine doses in terms of reduction of shedding have not been yet investigated.

3.2. *Medication*

Since protection against *M. hyopneumoniae* infection and associated diseases conferred by commercial vaccines is not complete, antimicrobial treatments are frequently required in commercial swine farms to control disease outcome.

Mycoplasmas lack a cell wall, thus *M. hyopneumoniae* is resistant to β-lactam antibiotics. Nevertheless, several antibiotic classes are effective in reducing the incidence and severity of *M. hyopneumoniae* compatible lung lesions. Most commonly used antibiotics are macrolides, lincosamides, tetracycline, and fluoroquinolones, among others (Thacker and Minion, 2012). The route of administration can be parenteral or mixed in feed / water depending on antibiotic choice.

Medication is currently used with different purposes. Parenteral medication is used to treat animals suffering from severe clinical signs, normally associated with EP and PRDC. Under field conditions medication is also commonly used to control *M. hyopneumoniae* infection by means of minimizing pathogen transmission. Medication of sows prior to farrowing could be utilized as an attempt to decrease the bacterial shedding to the offspring (Thacker and Minion, 2012; Holst et al., 2015). Nevertheless, it has been shown that antibacterial treatments do not eliminate the bacterium from the host, and shedding of *M. hyopneumoniae* can be detected in pigs after medication

programs (Overesch and Kuhnert, 2017). Therefore, the use of antimicrobials should be limited and only justified in specific situations to avoid the development of antimicrobial resistance (Lee et al., 2013).

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 controlled exposure has been attempted to expose the gilts by administering (intra-tracheally) lung tissue homogenate containing *M. hyopneumoniae* (Fano and Payne, 2015; Centeno et al., 2016) to individual gilts or groups of them (via aerosol), since the success of exposure is higher when these controlled procedures are used (Sponheim A., 2017). Finally, according to aforementioned studies, overall, the verification of gilt acclimation process is minimally performed in North American farms.

4. Conclusion

M. hyopneumoniae is a respiratory pathogen that causes important economic losses to the swine industry worldwide. A proper gilt acclimation against *M. hyopneumoniae* prior entrance into a recipient breeding farm could maintain the farm health stability and control respiratory disease caused by this pathogen. Gilt replacement acclimation procedures against *M. hyopneumoniae* in Europe and North America showed that vaccination is the main strategy used, but there is a current trend in the US to deliberately expose gilts to the pathogen. Further investigations are needed to identify the ideal gilt acclimation protocol taking into account that these strategies must be based on incoming and recipient herd health statuses and the characteristics of each farm.

Conflict of interest

The authors declare no conflict of interest.

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References

Alarcon, P., Wieland, B., Mateus, A.L.P., Dewberry, C., 2014. Pig farmers' perceptions, attitudes, influences and management of information in the decision-making process for disease control. Prev. Vet. Med. 116, 223–242.

Alfonso, A., Geiger, J., Feixes, C., Fonz, J., Torremorell, M., 2004. Mycoplasma hyopneumoniae and PRRSV elimination in a 1700 sows multi-site system. In: Proceedings of the 18th IPVS Congress, Hamburg, Germany, pp. 174.

Arsenakis, I., Michiels, A., Sacristán, R.D.P., Boyen, F., Haesebrouck, F., Maes, D., 2017. Mycoplasma hyopneumoniae vaccination at or shortly before weaning under field conditions : a randomised efficacy trial. Vet. Rec. 181, 19.

Arsenakis, I., Panzavolta, L., Michiels, A., Del Pozo Sacristán, R., Boyen, F., Haesebrouck, F., Maes, D., 2016. Efficacy of Mycoplasma hyopneumoniae vaccination before and at weaning against experimental challenge infection in pigs. BMC Vet. Res. 12, 63.

Bandrick, M., Pieters, M., Pijoan, C., Baidoo, S.K., Molitor, T.W., 2011. Effect of cross-fostering on transfer of maternal immunity to Mycoplasma hyopneumoniae to piglets. Vet. Rec. 168, 100.

Bargen, L.E., 2004. A system response to an outbreak of enzootic pneumonia in grow / finish pigs. Can. Vet. Journal 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.

Boonsoongnern, A., Jirawattanapong, P., Lertwatcharasarakul, P., 2012. The Prevalence of Mycoplasma hyopneumoniae in Commercial Suckling Pigs in Thailand. World J Vaccines 2, 161–163.

Calsamiglia, M., Pijoan, C., 2000. Colonisation state and colostral immunity to

Mycoplasma hyopneumoniae of different parity sows. Vet. Rec. 146, 530–2.

Centeno, N., Chévez, J., Fano, E., 2016. Mexican swine industry on Mycoplasma hyopneumoniae gilts acclimatation. In: Proceedings of the 24th IPVS Congress, Dublin, Ireland, 31, 2013.

Dalquist, L., 2014. Mycoplasma hyopneumoniae acclimation: Overcoming challenges in the field. In:Allen D. Leman Swine Conference, St. Paul, MN.

Fablet, C., Marois, C., Kobisch, M., Madec, F., Rose, N., 2010. Estimation of the sensitivity of four sampling methods for Mycoplasma hyopneumoniae detection in live pigs using a Bayesian approach. Vet. Microbiol. 143, 238–245.

Fano, E., Payne, B., 2015. Mycoplasma hyopneumoniae gilt acclimation and sow herd stability : Essentials to the systematic control - approach, In: AASV Annual Meeting, Orlando, Florida, pp. 175–178.

Fano, E., Pijoan, C., Dee, S., 2005. Evaluation of the aerosol transmission of a mixed infection of Mycoplasma hyopneumoniae and porcine reproductive and respiratory syndrome virus. Vet. Rec. 157, 105–108.

Fano, E., Pijoan, C., Dee, S., Deen, J., 2007. Effect of Mycoplasma hyopneumoniae colonization at weaning on disease severity in growing pigs. Can. J. Vet. Res. 71, 195–200.

Fano, E., Pijoan, C., Dee, S., Torremorell, M., 2006. Assessment of the effect of sow parity on the prevalence of Mycoplasma hyopneumoniae in piglets at weaning. In: Proceedings of the 19th IPVS Congress, Copenhagen, Denmark, pp. 96.

Feng, Z., Wei, Y., Li, G., Lu, X., Wan, X., Pharr, G.T., Wang, Z., Kong, M., Gan, Y., Bai, F., Liu, M., Xiong, Q., Wu, X., Shao, G., 2013. Development and validation of an attenuated Mycoplasma hyopneumoniae aerosol vaccine. Vet. Microbiol. 167, 417–424.

Garza-Moreno, L., Segalés, J., Pieters, M., Romagosa, A., Sibila, M., 2017. Survey on Mycoplasma hyopneumoniae gilt acclimation practices in Europe. Porcine Health Manag. 3, 21.

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.

Holst, S., Yeske, P., Pieters, M., 2015. Elimination of Mycoplasma hyopneumoniae from breed-to-wean farms: A review of current protocols with emphasis on herd closure and medication. J. Swine Health Prod. 23, 321–330.

Lee, C., Cho, I.H., Jeong, B.C., Lee, S.H., 2013. Strategies to Minimize Antibiotic Resistance. Int. J. Environ. Res. Public Heal. 10, 4274–4305.

Lorenzen, J., 2000. Eradication of Mycoplasma hyopneumoniae froman acutely infected Danish 2-site 390 sow herd without restocking. In: Proceedings of the 16th IPVS Congress, Melbourne, Australia, pp. 340.

Maes, D., Sibila, M., Kuhnert, P., Segalés, J., Haesebrouck, F., Pieters, M., 2017. Update on Mycoplasma hyopneumoniae infections in pigs: Knowledge gaps for improved disease control. Transbound Emerg Dis. doi:10.1111/tbed.12677

Maes, D., Segales, J., Meyns, T., Sibila, M., Pieters, M., Haesebrouck, F., 2008. Control of Mycoplasma hyopneumoniae infections in pigs. Vet. Microbiol. 126, 297–309.

Maes, D., Verdonck, M., Deluyker, H., de Kruif, A., 1996. Enzootic pneumonia in pigs. Vet. Q. 18, 104–109.

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., Dewulf, J., Vicca, J., Haesebrouck, F., Kruif, A. De, 2004.

Quantification of the spread of Mycoplasma hyopneumoniae in nursery pigs using transmission experiments. Prev. Vet. Med. 66, 265–275.

Michiels, A., Vranckx, K., Piepers, S., Del Pozo Sacristán, R., Arsenakis, I., Boyen, F., Haesebrouck, F., Maes, D., 2017. Impact of diversity of Mycoplasma hyopneumoniae strains on lung lesions in slaughter pigs. Vet. Res. 48, 2.

Morris, C.R., Gardner, I.A., Hietala, S.K., Carpenter, T.E., Anderson, R.J., Parker, K.M., 1995. Seroepidemiologic study of natural transmission of Mycoplasma hyopneumoniae in a swine herd. Prev. Vet. Med. 21, 323–337.

Nathues, H., Chang, Y.M., Wieland, B., Rechter, G., Spergser, J., Rosengarten, R., Kreienbrock, L., grosse Beilage, E., 2014. Herd-Level risk factors for the seropositivity to mycoplasma hyopneumoniae and the occurrence of enzootic pneumonia among fattening pigs in areas of endemic infection and high pig density. Transbound. Emerg. Dis. 61, 316–328.

Nathues, H., Doehring, S., Woeste, H., Fahrion, A.S., Doherr, M.G., grosse Beilage, E., 2013. Individual risk factors for Mycoplasma hyopneumoniae infections in suckling pigs at the age of weaning. Acta Vet. Scand. 55, 44.

Otake, S., Dee, S., Corzo, C., Oliveira, S., Deen, J., 2010. Long-distance airborne transport of infectious PRRSV and Mycoplasma hyopneumoniae from a swine population infected with multiple viral variants. Vet. Microbiol. 145, 198–208.

Overesch, G., Kuhnert, P., 2017. Persistence of Mycoplasma hyopneumoniae sequence types in spite of a control program for enzootic pneumonia in pigs. Prev. Vet. Med. 145, 67–72.

Pieters, M., Cline, G.S., Payne, B.J., Prado, C., Ertl, J.R., Rendahl, A.K., 2014. Intra-farm risk factors for Mycoplasma hyopneumoniae colonization at weaning age. Vet. Microbiol. 172, 575–580.

Pieters, M., Daniels, J., Rovira, A., 2017. Comparison of sample types and diagnostic methods for in vivo detection of Mycoplasma hyopneumoniae during early stages of infection. Vet. Microbiol. 203, 103–109.

Pieters, M., Fano, E., 2016. *Mycoplasma hyopneumoniae* management in gilts. Vet. Rec. 178, 122.1-123.

Pieters, M., Fano, E., Pijoan, C., Dee, S., 2010. An experimental model to evaluate Mycoplasma hyopneumoniae transmission from asymptomatic carriers to unvaccinated and vaccinated sentinel pigs. Can. J. Vet. Res. 74, 157–160.

Pieters, M., Pijoan, C., Fano, E., Dee, S., 2009. An assessment of the duration of Mycoplasma hyopneumoniae infection in an experimentally infected population of pigs. Vet. Microbiol. 134, 261–266.

Pitkin, A., Otake, S., Dee, S., 2011. A one-night downtime period prevents the spread of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae* by personnel and fomites (boots and coveralls). J. Swine Health Prod. 19, 345–348.

Roos, L.R., Fano, E., Homwong, N., Payne, B., Pieters, M., 2016. A model to investigate the optimal seeder-to-naïve ratio for successful natural Mycoplasma hyopneumoniae gilt exposure prior to entering the breeding herd. Vet. Microbiol. 184, 51–58.

Ruiz, A.R., Utrera, V., Pijoan, C., 2003. Effect of Mycoplasma hyopneumoniae sow vaccination on piglet colonization at weaning. J. Swine Health Prod. 11, 131–135.

Schneider, P., 2006. Experiences with Mycoplasma hyopneumoniae and Transmissible Gastroenteritis eradication from sow herd. Proceedings of Allen D. Leman Swine Conference, St Paul, MN, pp. 82–86.

Sibila, M., Bernal, R., Torrents, D., Riera, P., Llopart, D., Calsamiglia, M., Segalés, J.,

2008. Effect of sow vaccination against Mycoplasma hyopneumoniae on sow and piglet colonization and seroconversion, and pig lung lesions at slaughter. Vet. Microbiol. 127, 165–170.

Sibila, M., Nofrarías, M., López-Soria, S., Segalés, J., Riera, P., Llopart, D., Calsamiglia, M., 2007. Exploratory field study on Mycoplasma hyopneumoniae infection in suckling pigs. Vet. Microbiol. 121, 352–356.

Simionatto, S., Marchioro, S.B., Maes, D., Dellagostin, O.A., 2013. Mycoplasma hyopneumoniae: From disease to vaccine development. Vet. Microbiol. 165, 234–242.

Sponheim A., 2017. A diagnostic Approach to Confirm Day Zero. In: Allen D. Leman Swine Conference, St. Paul, MN.

Takeuti, K.L., de Barcellos, D.E.S.N., de Lara, A.C., Kunrath, C.F., Pieters, M., 2017. Detection of Mycoplasma hyopneumoniae in naturally infected gilts over time. Vet. Microbiol. 203, 215–220.

Thacker, E.L., Minion, F.C., 2012. Mycoplasmosis, In: Zimmerman, J.J., Karriker, L.A., Schwartz, K.J (Eds), Diseases of Swine. 10th ed. Wiley-Blackwell, Oxford, UK, pp. 779-797.

Vicca, J., Stakenborg, T., Maes, D., Butaye, P., Peeters, J., De Kruif, A., Haesebrouck, F., 2003. Evaluation of virulence of Mycoplasma hyopneumoniae field isolates. Vet. Microbiol. 97, 177–190.

Villarreal, I., Maes, D., Meyns, T., Gebruers, F., Calus, D., Pasmans, F., Haesebrouck, F., 2009. Infection with a low virulent Mycoplasma hyopneumoniae isolate does not protect piglets against subsequent infection with a highly virulent M. hyopneumoniae isolate. Vaccine 27, 1875–1879.

Villarreal, I., Maes, D., Vranckx, K., Calus, D., Pasmans, F., Haesebrouck, F., 2011.

Effect of vaccination of pigs against experimental infection with high and low virulence Mycoplasma hyopneumoniae strains. Vaccine 29, 1731–1735.

Villarreal, I., Vranckx, K., Calus, D., Pasmans, F., Haesebrouck, F., Maes, D., 2012. Effect of challenge of pigs previously immunised with inactivated vaccines containing homologous and heterologous Mycoplasma hyopneumoniae strains. BMC Vet. Res. 8, 2.

Vranckx, K., Maes, D., Marchioro, S.B., Villarreal, I., Chiers, K., Pasmans, F., Haesebrouck, F., 2012a. Vaccination reduces macrophage infiltration in bronchus-associated lymphoid tissue in pigs infected with a highly virulent Mycoplasma hyopneumoniae strain. BMC Vet. Res. 8, 24.

Vranckx, K., Maes, D., Sacristán, R.D.P., Pasmans, F., Haesebrouck, F., 2012b. A longitudinal study of the diversity and dynamics of Mycoplasma hyopneumoniae infections in pig herds. Vet. Microbiol. 156, 315–321.

Woolley, L.K., Fell, S., Gonsalves, J.R., Walker, M.J., Djordjevic, S.P., Jenkins, C., Eamens, G.J., 2012. Evaluation of clinical, histological and immunological changes and qPCR detection of Mycoplasma hyopneumoniae in tissues during the early stages of mycoplasmal pneumonia in pigs after experimental challenge with two field isolates. Vet. Microbiol. 161, 186–195.

Yeske, P., 2007. Mycoplasma eradication strategies. In: AASV Anual Meeting, Orlando, Florida, pp. 367–370.

Table 1. Different strategies used for monitoring *M. hyopneumoniae* and disease diagnosis.

	Monitoring strategy	Parameters	Samples	Advantages	Disadvantages
Observational diagnosis / Monitoring	Clinical examination	Presence of dry and non-productive cough	None	First indication of respiratory problems Assessment of EP by coughing index	Not exclusive of *M. hyopneumoniae* Not able to detect subclinical infected pigs Observed several weeks post infection
	Lung examination	Macroscopic lesions: Visual observation of CVPC	Entire lungs	Monitoring the respiratory disease under field conditions by lung lesion scoring systems at abattoir Identification of clinical and subclinical farms	Post mortem diagnosis Not exclusive of *M. hyopneumoniae* Do not provide information regarding respiratory clinical disease in real time
		Microscopic lesions: Broncho-interstitial pneumonia with bronchus-associated lymphoid tissue hyperplasia	Lung tissue		
Laboratory diagnosis / Conclusive diagnosis	Bacterial isolation	Detection of the pathogen	Lung tissue	Traditional as "gold standard" technique	Growth and time requirements (4-8 w) Low isolation rate Frequently contaminated by other *Mycoplasma spp.*
	Immunofluorescence (IF)	Detection of *M. hyopneumoniae* antigen	Lung tissue	Detection and localization of *M. hyopneumoniae in situ* Highly specific	Post mortem diagnosis Limited sensitivity Histologic sections are required
	Immunohistochemistry (IHC)				
	In situ hybridization (ISH)	Detection of *M. hyopneumoniae* nucleic acid			
	Polymerase chain reaction (PCR)	Detection of *M. hyopneumoniae* nucleic acid	Respiratory tract samples	Different samples can be used [a] No cross-reaction with other pathogens High sensitivity	Detection of DNA from live and dead mycoplasmas
	ELISA	Detection of antibody response against *M. hyopneumoniae*	Serum	Most frequent technique for herd monitoring. Easy and quick to perform	Potential cross-reactions with other *Mycoplasma spp.* Delayed seroconversion (6-9w) No differentiation between natural infection and vaccine antibodies

EP: Enzootic pneumoniae; CVPC: Cranio-ventral pulmonary consolidation; w: weeks; [a] nasal, laryngeal and bronchial swabs, broncho-alveolar lavage fluid and lung tissue.

Table 2. Proposed farm classification according to *M. hyopneumoniae* health status.

	Classification	Clinical signs	Lung lesions	ELISA result[a]	PCR result
	Negative	Not observed	Not observed	Negative	Negative
	Provisional negative	Not observed	Not observed	Positive	Negative
Positive	**Subclinical infected I**	Not observed	Not observed	Positive/Negative	Positive
	Subclinical infected II	Not observed	Observed	Positive/Negative	Positive
	Clinical affected	Observed	Observed	Positive/Negative	Positive

[a]ELISA results (negative/positive) could depend on infection pattern in the farm and sampling time point.

Table 3. Summary of product characteristics of currently used vaccines against *M. hyopneumoniae* in Europe and North America.

Manufacturing company	Vaccine name	Antigen	Adjuvant	Licensed for	Dosage and route of application	Vaccination		Onset of immunity	Duration of immunity
						Schedule	Age		
Boehringer Ingelheim	Ingelvac Mycoflex®	Strain J	Carbomer	Pigs	1 ml, IM	Single	3w	2w	26w
				Sows	1 ml, IM	Single	Semiannually	2w	26w
Ceva	Hyogen®	Strain 2940	Mineral oil + LPS J5	Pigs	2 ml, IM	Single	3w	3w	26w
Elanco	Stellamune® One	Strain NL1042	Mineral oil + lecithin	Pigs	2 ml, IM	Single	3d/3w	18d/3w	26w/23w
	Stellamune®Mycoplasma			Pigs	2 ml, IM	Double	1w + 3w	2w	22w
Hipra	Mypravac® Suis	Strain J	Levamisol + carbomer	Pigs	2 ml, IM	Double	1w + 3w	NA	6m
MSD/ Merck Animal Health	M + PAC®	Strain J	Mineral oil + $Al(OH)_3$	Pigs	2 ml, IM	Single	3w	21d	6m
					1 ml, IM	Double	1w + 3w		
	Porcilis® Mhyo	Strain 11	Tocopherol	Pigs	2 ml, IM	Double	>1w + 3w	2w	20w
	Porcilis® Mhyo ID ONCE		Tocopherol	Pigs	0.2 ml, ID	Single	3w	3w	22w
	Porcilis® PCV-Mhyo[a]	Strain J	Mineral oil + $Al(OH)_3$	Pigs	2 ml, IM	Single	3w	4w	21w
Zoetis	Respisure®	Strain NL1042	Mineral oil	Pigs	2 ml, IM	Double	1w + 3w	3w	23w
				Dams	2 ml, IM	Double	2w BF[c]	NA	NA
				PS	2 ml, IM	Double	6w + 2w BF[c]	NA	NA
				Boars	2 ml, IM	Double	Semiannually	NA	NA
	Respisure-ONE®			Pigs	2 ml, IM	Single	>1d	18d	25w
				Dams	2 ml, IM	Single	Semiannually	NA	NA
	Suvaxyn® MH-One[a]/Mono	Strain P-5722-3	Mineral oil + Carbomer	Pigs	2 ml, IM	Single	>1w	2w	6m
	Suvaxyn® Mhyo		Carbomer	Pigs	2 ml, IM	Double	1w + 3w	NA	NA
	Suvaxyn® Circo+MH[b]		Squalene + oil-in-water emulsion	Pigs	2 ml, IM	Single	3w	3w	23w
	Suvaxyn® MHYO-PARASUIS[c]		Carbomer	Pigs	2 ml, IM	Double	>7d + 2w	1w	6m
AviMex	VaxSafe® MHP	LKR	-	Pigs	1ml, IN	Single	3d	-	-

[a]In US, the adjuvant is Amphigen; [b]Combined with Porcine Circovirus type 2; [c]Combined with *Haemophilus parasuis*-named Suvaxyn RespiFend MH HPS in US; [c] Before farrowing; IM: Intramuscular; IN: Intranasal; ID: Intradermal; w: weeks; d: days; m: months; PS: Pregnant sows; NA: No information available; $Al(OH)_3$: hydroxide aluminum

Table 4. Dam vaccination schemes against *M. hyopneumoniae* and management proposed in the literature to decrease *M. hyopneumoniae* infectious pressure and transmission from dam to offspring.

Reference	Animal target	No. of doses	Vaccination timing
Sibila et al. (2008)	Sows	2	5 and 3 w pre-farrowing
Yeske (2007)	Gilts Breeding herd	2 1	1 and 3 w post entry to IU On a quarterly schedule after herd closure
Schneider (2006)	Breeding herd Sows	2	5 and 2 w prior the antimicrobial treatment 2 w prior farrowing
Alfonso et al. (2004)	Gilts Sows	3	55 and 220 d of age 15 d prior farrowing
Ruiz et al. (2003)	Sows	2	5 and 3w prior farrowing
Lorenzen (2000)	Breeding herd	2	1 w prior the antimicrobial treatment and 2 w later

Breeding herd includes sows and boars; w: weeks; d: days; IU: isolation units.

Table 5. Scenarios for gilt replacement introduction within breeding herd farms according to *M. hyopneumoniae* health status (adapted from Pieters and Fano, 2016).

		Recipient sows	
		Negative/Provisional negative	**Subclinical infected and clinical affected**
Incoming gilts	**Negative / Provisional negative**	Isolation period to warrant gilts are *M.hyopneumoniae* negative and any antibodies against the pathogen is detected	Gilt acclimation is required to expose incoming gilts to *M. hyopneumoniae* of recipient sows: • Entry into acclimation unit as early as possible • Exposure at least 210-240 before farrowing - Vaccination against *M. hyopneumoniae* to stimulate and homogenize the immune response • Identification of *M. hyopneumoniae* source ("shedders") • Verification of process: absence of clinical signs and confirmation of the non-shedding status of the replacement before farrowing.
	Subclinical infected and clinical affected	Gilt entrance should be avoided. If it is not possible, gilt entrance will be postponed until the infection is cleared: - Clinical signs are not present - *M.hyopneumoniae* shedding is ceased - Gilts no longer have antibodies against *M.hyopneumoniae*	Gilt acclimation is required to expose incoming gilts to *M. hyopneumoniae* strain of recipient sows: • Early entry into acclimation unit • Exposure at least 210-240 before farrowing - Vaccination against *M. hyopneumoniae* to homogenize the replacement immunity • Identification of *M. hyopneumoniae* source ("shedders") • Verification processes: - Confirmation of the recovery and non-shedding status of replacement before farrowing. - Prevention of introduction of new *M. hyopneumoniae* strains into the farm.

Table 6. Summary of main characteristics of gilt acclimation protocols against *M. hyopneumoniae* used in Europe and North America.

Country / Region		Monitoring of health status		Acclimation process		
		Recipient herd	Incoming gilts	Management	Main strategy	Verification
Europe[a]		Clinical signs + Lung lesions	ELISA (58% seropositive)	AIAO (44%)	Vaccination (58%)	24%
North America	US[b]	NA	NA	CF (72%)	Vaccination (93%)	20%
	Mexico[c]	Clinical signs	NA (90% seropositive)	CF (75%)	Vaccination (67%)	14%

[a] Garza-Moreno *et al*. (2017); [b] Fano and Payne (2015);[c] Centeno *et al*. (2016) NA: not available; AIAO: All in-all out; CF: Continuous flow.