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

Review

Current perspectives on the diagnosis and epidemiology of *Mycoplasma hyopneumoniae* infection

Marina Sibila [a,*], Maria Pieters [b], Thomas Molitor [b], Dominiek Maes [c], Freddy Haesebrouck [c], Joaquim Segalés [a,d]

[a] *Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[b] *College of Veterinary Medicine, University of Minnesota, 1988 Fitch Avenue, St. Paul, MN 55108, USA*
[c] *Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
[d] *Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*

Abstract

Mycoplasma hyopneumoniae is the principal aetiological agent of enzootic pneumonia (EP), a chronic respiratory disease that affects mainly finishing pigs. Although major efforts to control *M. hyopneumoniae* infection and its detrimental effects have been made, significant economic losses in pig production worldwide due to EP continue. *M. hyopneumoniae* is typically introduced into pig herds by the purchase of subclinically infected animals or, less frequently, through airborne transmission over short distances. Once in the herd, *M. hyopneumoniae* may be transmitted by direct contact from infected sows to their offspring or between pen mates.

The 'gold standard' technique used to diagnose *M. hyopneumoniae* infection, bacteriological culture, is laborious and is seldom used routinely. Enzyme-linked immunosorbent assay and polymerase chain reaction detection methods, in addition to post-mortem inspection in the form of abattoir surveillance or field necropsy, are the techniques most frequently used to investigate the potential involvement of *M. hyopneumoniae* in porcine respiratory disease. Such techniques have been used to monitor the incidence of *M. hyopneumoniae* infection in herds both clinically and subclinically affected by EP, in vaccinated and non-vaccinated herds and under different production and management conditions. Differences in the clinical course of EP at farm level and in the efficacy of *M. hyopneumoniae* vaccination suggest that the transmission and virulence characteristics of different field isolates of *M. hyopneumoniae* may vary. This paper reviews the current state of knowledge of the epidemiology of *M. hyopneumoniae* infection including its transmission, infection and seroconversion dynamics and also compares the various epidemiological tools used to monitor EP.
© 2008 Elsevier Ltd. All rights reserved.

Keywords: *Mycoplasma hyopneumoniae*; Diagnosis; Epidemiology; Transmission; Pig

Introduction

Mycoplasma hyopneumoniae is the principal aetiological agent responsible for enzootic pneumonia (EP) in pigs. Other pathogens such as *Pasteurella multocida*, *Actinobacillus pleuroneumoniae*, *Mycoplasma hyorhinis*, *Streptococcus suis*, *Haemophilus parasuis*, *Bordetella bronchiseptica* and *Arcanobacterium pyogenes* are also frequently involved

(Thacker, 2006). The disease is characterised by high morbidity and low mortality and although pigs of all ages are susceptible to *M. hyopneumoniae* infection, EP is usually not observed in animals younger than 6 weeks of age. The prevalence of EP is particularly high in animals of mid-finishing to slaughter age and the severity of clinical signs is dictated by the strain of *M. hyopneumoniae* involved, infection pressure, the presence of secondary infections and by environmental conditions. When *M. hyopneumoniae* infection is not complicated by concomitant pathogens, the disease can take a subclinical course with mild clinical signs consisting of chronic, non-productive

cough, reduced rate of average daily weight gain (ADWG) and reduced feed conversion efficiency. When secondary pathogens are involved, clinical signs include laboured breathing and pyrexia, and deaths may occur (Maes et al., 1996).

M. hyopneumoniae is intimately involved in the pathogenesis of porcine respiratory disease complex (PRDC), a disease involving both bacterial (those potentially involved in EP listed above) and viral (porcine reproductive and respiratory syndrome virus [PRRSV], porcine circovirus type 2, Aujeszky's disease virus, swine influenza viruses [SIVs] and porcine respiratory coronavirus) pathogens. Porcine respiratory disease complex typically affects finishing pigs of between 14 and 20 weeks of age and is characterised clinically by depressed growth rate and feed conversion efficiency and by anorexia, fever, cough, and dyspnoea. This disease has been referred to as the '18 week wall' given its higher prevalence in pigs of this age (Dee, 1996).

Although improved management methods and the judicious use of medication and vaccination have greatly alleviated the detrimental effects of EP on herd health and on carcass quality, EP-associated economic losses remain important within pig production worldwide (Thacker, 2006). These losses are mainly due to decreased ADWG, increased feed conversion ratio, increased medication costs and, in some cases, to higher mortality rates (Maes et al., 1998). No recent estimates of the financial losses attributable to EP are available and these are likely to vary considerably between herds (Maes et al., 1998).

A sound knowledge of the routes of transmission of *M. hyopneumoniae* and of the other pathogens associated with EP is necessary to control the disease as well as to understand the factors that influence the pathogenesis. In the next sections we review current knowledge of *M. hyopneumoniae* transmission and seroconversion dynamics in different swine production systems and compare the different epidemiological tools used to monitor EP. The existence of *M. hyopneumoniae* strains with different virulence characteristics and the molecular techniques available to detect them are also discussed.

Epidemiological and diagnostic tools to assess *M. hyopneumoniae* infection

The investigation and control of infectious disease is critically dependent on the availability of appropriate diagnostic tools. Several diagnostic methodologies are used to monitor *M. hyopneumoniae* infection.

Clinical signs

The main clinical sign of EP is the gradual onset of a chronic, non-productive cough, particularly in pigs at the finishing stage of the production cycle. Co-infection with the additional pathogens detailed previously results in fever, anorexia and laboured breathing. The onset of coughing, although gradual, can be inconsistent and of variable intensity depending on the infecting dose of *M. hyopneumoniae*. To identify pigs with non-productive coughs, animals need to be observed over a considerable time-span and should be encouraged to move. Quantifying the number of coughing pigs in a given period of time (the 'coughing score') has been used in transmission (Meyns et al., 2006; Marois et al., 2007) and pathogenesis studies (Morris et al., 1995a; Vicca et al., 2003) and has also been used in the assessment of the efficacy of *M. hyopneumoniae* vaccines under both natural (Maes et al., 1999; Moreau et al., 2004) and experimental (Thacker et al., 2000) conditions. However, given the lack of diagnostic specificity of coughing and that subclinically affected pigs would not display it, additional diagnostic modalities are required.

Abattoir surveillance

The assessment of respiratory disease within a pig herd by lung 'lesion scoring' at abattoir inspection is frequently used to estimate the incidence of EP and its impact on carcass market price. It has been estimated that the lungs of at least 30 animals should be examined to provide a reliable measure of the prevalence and severity of the pneumonia at herd level (Davies et al., 1995). Such surveillance may also be useful in detecting subclinical disease which can adversely affect production during the fattening period. However EP lesions are not pathognomonic of *M. hyopneumoniae* infection as other organisms such as SIV can produce similar lesions (Thacker et al., 2001).

Retrospective evaluation of the prevalence of EP in a herd by abattoir surveillance is limited in that this approach only identifies chronic lung lesions at the end of the production period and does not provide information regarding the ongoing respiratory health of the pigs during fattening (Noyes et al., 1990). Similarly, the presence of additional bacterial pathogens such as *A. pleuropneumoniae* can cause severe pleuritis that mask EP lesions. Lesion resolution may lead to false-negative results or to an equivocal diagnosis of early mycoplasmosis (Sørensen et al., 1997). The scoring systems used most frequently in EP abattoir surveillance are summarised in Table 1. The subjectivity involved in the visual estimation of the proportion of lung consolidated and the lack of diagnostic specificity of these lesions, limit abattoir surveillance as a diagnostic approach and, therefore, the use of additional confirmatory methods is needed.

Bacteriological culture

The isolation of *M. hyopneumoniae* from affected lungs by bacteriological culture is considered the 'gold standard' diagnostic technique (Thacker, 2006) but isolation of the pathogen requires specialised Friis medium. Sørensen et al. (1997) compared the detection of *M. hyopneumoniae* by culture, immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA) and by a polymerase

Table 1
Summary of scoring systems used in the abattoir surveillance of enzootic pneumonia

Reference	Scoring unit	Multiplying factor: Relative weight of each lobe							Maximum total score
		Apical		Middle		Diaphragmatic		Accessory	
		Right	Left	Right	Left	Right	Left		
Hannan et al. (1982)	1–5 points per lobe	5/7	5/7	5/7	5/7	5/19	5/19	5/8	35
Madec and Kobisch (1982)	1–4 points per lobe depending on percentage of lobe affected	0 points = no lesions; 1 point = <25% of lobe; 2 points = 25–49%; 3 points = 50–74%; and 4 points >75%. Points for each of the seven lobes.							28
Morrison et al. (1985)	Percentage of lobe affected	10	10	7	7	30	30	6	100
Straw et al. (1986)	Percentage of lobe affected	10	10	10	10	25	25	10	100
Christensen et al. (1999)	Percentage of lobe affected	7	4	15	9	35	25	5	100

chain reaction (PCR) method and found culture the most sensitive technique particularly at the later stages of EP when fewer mycoplasma organisms were present. However, *M. hyopneumoniae* culture is laborious, time-consuming (isolation from field samples requires 4–8 weeks) and frequently culture media can become overgrown with *M. hyorhinis* or *M. flocculare* (Maes et al., 1996).

Serological detection of M. hyopneumoniae infection

Serological tests are commonly used to monitor the health status of pig herds. Detection of antibodies to *M. hyopneumoniae* can be accomplished by ELISA and, less frequently, by complement fixation test. Antibody profiling of pig herds requires the simultaneous testing of groups of animals of different ages (transversal study) or the testing of one group of animals throughout the production cycle (longitudinal study) by ELISA. The ELISA in this context is a rapid, inexpensive and easily automated method that provides useful information on the presence of maternally-derived and acquired antibodies, as well as on the time required for animals to seroconvert. A blocking ELISA (IDEI, *Mycoplasma hyopneumoniae* EIA kit, Oxoid) and two indirect ELISA tests (HerdCheck, IDEXX and Tween 20-ELISA) are the most frequently used serological tests to detect antibodies to *M. hyopneumoniae*. Comparative studies have reported differing specificities and sensitivities between these kits (Pijoan, 1994; Strait et al., 2004; Ameri-Mahabadi et al., 2005; Erlandson, 2005). When discrepancies in serological test results are identified, a Western blot immunoassay (WBI) targeting different *M. hyopneumoniae* antigens can be used as a confirmatory test (Ameri et al., 2006). The utility of antibody profiling can be hindered by: variation in ELISA results depending on the test used (Ameri-Mahabadi et al., 2005; Erlandson, 2005); the inability of serology to differentiate natural infection from vaccination; lack of correlation between different measures of antibody titre; variations in the detection of antibodies to different strains of *M. hyopneumoniae* (Strait et al., 2004); significant variability in the time taken by animals to seroconvert.

Seroconversion under natural conditions is slower than in experimental studies. Although antibodies were detected 2–4 weeks after intratracheal infection of pigs with *M. hyo-pneumoniae* (Sheldrake et al., 1990; Kobisch et al., 1993). Sørensen et al. (1997) reported seroconversion as early as 8 days post-infection (PI) in a proportion of pigs infected by aerosol, although the remaining animals required five further weeks to seroconvert. Under natural conditions, Morris et al. (1995b) detected seroconversion in a herd 3 weeks after contact exposure, with titres peaking around 11 weeks after exposure. In two other studies carried out under field conditions, seroconversion occurred mainly in grower-finishing units (8–24 weeks of age), between 6 and 9 weeks PI (Andreasen et al., 2000; Leon et al., 2001). The delay in seroconversion associated with infection with this pathogen may partly be due to the fact that *M. hyopneumoniae* attaches to the ciliated respiratory epithelium and does not invade the pulmonary tissue to the same extent as other pathogens. This may result in slower presentation of mycoplasma antigens to the host. Of further note is the fact that there is no correlation between antibody titres and protection against the infection (Maes et al., 1996).

Detection of M. hyopneumoniae antigen

Although immunohistochemistry (IHC) and IFA specifically detect *M. hyopneumoniae* in lung tissue sections or smears, these techniques have the drawback that a diagnosis can only be made post-mortem. Moreover, only a small lung sample is tested when using these techniques, increasing the risk of a false-negative result if airway is not included in the sample (Cai et al., 2007). Furthermore, test sensitivity may be compromised by improper tissue processing. The quality of the antibodies used in these methods may also limit specific detection given that polyclonal antibodies may link with other, closely related, *Mycoplasma* spp. (Cheikh Saad Bouh et al., 2003).

Although IHC using monoclonal or polyclonal antibodies specific to *M. hyopneumoniae* on formalin-fixed, paraffin-embedded lung sections allows the pathologist to correlate the presence of mycoplasma antigen with EP lesions (Sarradell et al., 2003; Rodriguez et al., 2004), the technique is not routinely used in diagnostic laboratories due to its limited sensitivity. Direct (Kobisch et al., 1978) and indirect IFAs (Piffer and Ross, 1985) have been used to localise *M. hyopneumoniae* antigen in

porcine lungs. The IFA has also been compared with PCR as a diagnostic technique in pathogenesis and transmission studies (Sørensen et al., 1997; Vicca et al., 2003; Meyns et al., 2006). A positive IFA test is associated with the more acute stage of EP when the mycoplasmal load is high, in contrast with the low sensitivity of this assay in more chronic disease when lower numbers of mycoplasma organisms are likely to be present (Ross, 1999). A further limitation of the IFA is that in the frozen tissue sections used, artefactual tissue fragmentation may obscure morphological features.

Molecular detection methods

In situ hybridisation

In situ hybridization (ISH) has been used to detect and specifically locate *M. hyopneumoniae* DNA in formalin-fixed, paraffin-embedded lung tissue of naturally (Kwon and Chae, 1999) and experimentally infected pigs (Kwon et al., 2002). This technique uses a digoxigenin-labelled specific probe targeting a repetitive sequence of the *M. hyopneumoniae* genome. A fluorescent oligonucleotide probe targeting 16S ribosomal DNA has also been used for species-specific identification of *M. hyopneumoniae*, *M. hyosynoviae*, and *M. hyorhinis* (Boye et al., 2001). Drawbacks of this method are that it requires post-mortem samples and it is relatively time-consuming and therefore not suitable for rapid diagnosis.

Polymerase chain reaction

Several PCR techniques for *M. hyopneumoniae* DNA detection in different sample types have been described (Table 2). These PCR methods are more rapid than bacteriological culture and are relatively inexpensive to perform (Calsamiglia et al., 1999a). However the confounding significance of sample contamination is much higher with PCR. Given that *M. hyopneumoniae* DNA from both live and dead organisms is amplified, the identification of PCR positive animals raises the question of whether such pigs have active infection or not. Since *M. hyopneumoniae* attaches to the ciliated epithelium of the airways, the best samples to detect *M. hyopneumoniae* by PCR are tracheo-bronchial swabs or bronchoalveolar lavage fluid (BALF). Tracheo-bronchial swabs and BALF are equally predictive of infection in both live (Marois et al., 2008) and dead (Kurth et al., 2002) experimentally infected pigs. The use of PCR to detect *M. hyopneumoniae* in lung tissue has produced variable results. Moorkamp et al. (2008) suggested that lung samples are more appropriate than BALF in cases of moderate to severe EP, whereas Kurth et al. (2002) found lung tissue unreliable in this context.

Ideally, a test to detect the presence of a pathogen in a living animal should be easy to perform, rapid, inexpensive, and should provide data of use in the implementation of control measures. Although the detection of *M. hyopneumoniae* in the nasal cavities of living pigs by PCR might theoretically fit these criteria, pigs inoculated with *M. hyopneumoniae* intratracheally were found to have low numbers of organisms in their upper respiratory tract and only shed the organism intermittently (Kurth et al., 2002; Ruiz et al., 2002; Pieters and Pijoan, 2006). However, the use of PCR to diagnose natural infection from nasal swabs was found reliable and an association was found between the detection of *M. hyopneumoniae* in the nasal cavities and bronchi with lesions of EP (Sibila et al., 2004a). Although the potential use of nasal swabs for nested

Table 2
Summary of reported PCR-based techniques used to detect *M. hyopneumoniae*

Reference	Type of PCR	Amplicon		Threshold of detection	Clinical samples tested
		Gene	Length (bp)		
Harasawa et al. (1991)	S	Repeated unknown sequence	520	5 ng or 1000 CFU/mL	None
Artiushin et al. (1993)	S	Unique hypothetical gene	456	1–10 pg of DNA	BALF, lung tissue
Stemke et al. (1994)	S	16 SrRNA	200	1000 genome	None
Mattsson et al. (1995)	S	16 SrRNA	649	5 CFU	Nasal swab
Blanchard et al. (1996)	S	Putative ABC transporter	1561	500 fg	Tracheo-bronchial lavage
Stärk et al. (1998)	N	MHYP1-03-950 repetitive element	808	1 cell/filter	Filtered air sample
Baumeister et al. (1998)	S	Not given	853	100 CFU/mL	BALF
Calsamiglia et al. (1999a)	N	16SrRNA	352	80 cells	Nasal swab
Verdin et al. (2000)	N	Putative ABC transporter	706	1 fg	Tracheo-bronchial lavage, nasal swab
Caron et al. (2000)	S	Intergenic sequence (p36)	948	50 pg	Lung tissue, tracheo-bronchial lavage
	S	Intergenic sequence (p46)	580	0.5 ng	Nasal swab
	M	p36 and p46	948 and 580	Not given	
Kurth et al. (2002)	N	Unique hypothetical gene	240	0-5-1 fg	Tracheo-bronchial brushes, BALF
Dubosson et al. (2004)	RT	MHYP1-03-950 repeated element	808	1 fg	Bronchial swabs
		I-141 fragment putative ABC transporter	706	1 fg	
Stakenborg et al. (2006b)	M	16SrRNA	1000	1 pg	None
Cai et al. (2007)	S	16SrRNA	649	0.18 CFU/g	Lung tissue

S, standard; N, nested; M, multiplex; RT, real time; BALF, bronchoalveolar lavage fluid.

PCR (nPCR) testing for *M. hyopneumoniae* in live pigs has been demonstrated (Sibila et al., 2007b), the procedure is currently considered more useful for the monitoring of infection at a herd rather than at an individual animal level (Otagiri et al., 2005).

The detection of *M. hyopneumoniae* by PCR provides a more precise method of determining when animals become infected that using time for seroconversion to occur, as this may vary considerably (Morris et al., 1995b; Andreasen et al., 2000; Leon et al., 2001).

Molecular typing techniques

Variations in the clinical course of EP (Vicca et al., 2002) and inconsistencies in the efficacy of vaccination have raised suspicions that *M. hyopneumoniae* isolates vary in virulence. Differences at the antigenic (Assuncao et al., 2005), chromosomal (Frey et al., 1992; Stakenborg et al., 2005), genomic (Lin, 2001; Minion et al., 2004; Madsen et al., 2007; Mayor et al., 2007a) and proteomic (Calus et al., 2007) levels have been reported between *M. hyopneumoniae* isolates and, finally Vicca et al. (2003) demonstrated differing virulence characteristics between such isolates. Differences in adhesion (de Castro et al., 2006) and transmission (Meyns et al., 2004; Marois et al., 2007) of the organism have been suggested but not clearly demonstrated.

From an epidemiological perspective, typing *M. hyopneumoniae* isolates would facilitate the understanding of the transmission of *M. hyopneumoniae* isolates within and between herds. Recently described molecular typing techniques (Table 3) suggest that one strain of *M. hyopneumoniae* infects a given herd, whereas the strains involved in different outbreaks vary (Stakenborg et al., 2005; Mayor et al., 2007b).

Transmission

Transmission under field conditions

M. hyopneumoniae may be introduced into a herd in two main ways: by direct transmission following the introduction of purchased, subclinically infected replacement gilts or other pigs; and by airborne transmission. The role of fomites is thought to be minimal (Batista et al., 2004).

Direct transmission

Once in the herd, *M. hyopneumoniae* is transmitted between animals in aerosolised droplets generated by coughing and sneezing or may spread through direct contact. Infection may spread horizontally from infected to naïve pigs (Morris et al., 1995b) or vertically from sows to their piglets (Maes et al., 1996).

Vertical transmission

Infected gilts and sows can transmit *M. hyopneumoniae* to newly introduced gilts, including vaccinated animals (Pieters et al., 2006). In addition to the introduction of such animals, the transmission dynamics also includes the ongoing infection of piglets by sows. In particular low parity sows or gilts have low levels of antibodies and excrete more mycoplasma organisms than do older sows (Maes et al., 1996; Fano et al., 2006). However, based on a nPCR technique, it has been indicated that breeding sows from their second to seventh parity can remain persistently infected with *M. hyopneumoniae* (Calsamiglia and Pijoan, 2000). More research is required to determine how sow parity affects the shedding of *M. hyopneumoniae* and the development of disease in their offspring

Horizontal transmission

Horizontal transmission of infection may occur between pen mates or, in continuous flow production systems, from older to younger animals. Moreover, airborne transmission of infection can occur between different barns or units within a herd. Once established, *M. hyopneumoniae* infection may persist in the respiratory tract of adult animals for up to 185 days (Fano et al., 2005a). Persistently infected pigs typically have subclinical disease, are difficult to detect using currently available diagnostic tools and remain carriers, capable of transmitting the pathogen to susceptible animals (Ruiz et al., 2002; Pijoan, 2005).

Horizontal transmission of *M. hyopneumoniae* between wild boar and domestic pigs has recently been suggested in France (Marois et al., 2006) and Slovenia (Vengust et al., 2006). In the former study, *M. hyopneumoniae* DNA was detected by PCR in lung homogenates of 9% of the wild boars tested. Although preliminary, these findings suggest that wild boar are a potential reservoir of *M. hyopneumoniae* infection for domestic swine or vice versa.

Airborne transmission

Although the main source of transmission between pigs is considered to be direct contact with subclinically infected carrier animals, the spread of *M. hyopneumoniae* infection by aerosol has recently gained more significance (Desrosiers, 2004). Airborne particles containing the microorganism are generated by sneezing and coughing, and are also exhaled by infected pigs (Stärk, 1999). Goodwin (1985) suggested that aerosol transmission between farms may occur and that the risk of a herd becoming infected with *M. hyopneumoniae* was inversely related to the proximity of other pig farms. Zhuang et al. (2002) found that a pig herd's risk of becoming infected was closely associated with pig density in the area and with the distance to neighbouring farms. The minimum distance between pig farms to theoretically avoid airborne transmission has been calculated to be at least 3 km. In Denmark, Thomsen et al. (1992) proposed airborne transmission as a major source of *M. hyopneumoniae* infection in mycoplasma-free SPF pig herds

Table 3
Summary of molecular techniques used to genotype *M. hyopneumoniae* field strains (adapted from Stakenborg, 2005)

Target of technique	Methodology	Technique(s) used	Reference	Amplicon	Reproducibility	Discriminatory power	Ease of performance	Time required (days)	Ease of interpretation	Cost-efficient
Entire genome	Restriction and electrophoresis	FIGE	Frey et al. (1992)	Eco RI	++	+	−	2–3	+	+
		PFGE	Blank and Stemke (2000) Stakenborg et al. (2005)	ApaI, Sal I, ApaL, Asp718	++	++	−	2–3	+	+
	Restriction and hybridisation	REA and DNA specific probe	Ferrell et al. (1989) Harasawa et al. (1995)	IS-like Unknown repetitive sequence	±	±	++	1	−−	−
	Restriction and PCR	AFLP	Kokotovic et al. (1999) Stakenborg et al. (2006a)	Restriction enzymes	+	++	±	2	−	+
	PCR	RAPD (AP-PCR)	Artiushin and Minion (1996) Vicca et al. (2003) Stakenborg et al. (2006a)	OPA-3 primer	−	++	++	<1	±	−
Specific DNA fragment	PCR	PCR	Hsu et al. (1997), Lin (2001)	P97	++	−	++	<1	+	−
	PCR of repetitive elements	VNTR	Stakenborg et al. (2006a) de Castro et al. (2006)	P97 VNTR genes	++	±	++	<1	+	−
	PCR and restriction	PCR-RFLP	Stakenborg et al. (2006a)	P146	++	±	++	<1	+	±
	PCR and electrophoresis	PCR-DGGE	McAuliffe et al. (2005)	16 SrRNA						
	PCR and sequencing	PCR-seq	Wilton et al. (1998)	P97	+	++	+	1	+	+
			Mayor et al. (2007a)	p146						
			Mrazek (2006)	mnSSR	NA	++	+	1	+	+
		MLST	Mayor et al. (2007b)	*adk, rpoB, tpiA*	++	++	±	2	+	+
	PCR and hybridisation	Microarray	Madsen et al. (2007)	125–350 bp PCR products	++	++	−	1	−	−

PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA; VNTR, variable number tandem repeats; AFLP, amplified fragment length polymorphism; RLFP, restriction fragment length polymorphism; PFGE, pulsed-field gel electrophoresis; DGGE, denaturing gradient gel electrophoresis; Seq, sequencing; REA, restriction endonuclease analysis; AP-PCR, arbitrarily primed PCR; FIGE, field-inversed gel electrophoresis; mnSSR, mononucleotide simple sequence repeats; MLST, multi locus sequence typing; NA, not available; ++, very high; +, high; ±, moderate; −, low; −−, very low.

and indicated that the risk of these herds becoming infected was greater when neighbouring infected herds were larger.

Other transmission routes

Apart from direct contact and airborne transmission, indirect transmission of infection through fomites has also been suggested (Goodwin, 1985). However an investigation by Batista et al. (2004) concluded that when standard biosecurity protocols were followed by farm personnel in weekly contact with infected pigs, *M. hyopneumoniae* was not transmitted to naïve animals.

Transmission under experimental conditions

Experimental studies have been used to study *M. hyopneumoniae* transmission and the infection dynamics between pig populations of varying infection status. To evaluate and quantify the transmission of *M. hyopneumoniae* to nursery pigs under standardised experimental conditions, Meyns et al. (2004) used an adjusted reproduction ratio (R_n). This R_n value expressed the mean number of secondary infections caused by one infectious piglet during a nursery period of 6 weeks where the virulence of the isolate was taken into account (Vicca et al.,

2003). The R_n values for the high and low virulent isolates were 1.47 and 0.85, respectively, although this difference was not statistically significant. These results imply that one piglet infected before weaning is able to infect one to four pen mates during the nursery period. Under the experimental conditions of this study (Meyns et al., 2004), the spread of *M. hyopneumoniae* between animals was slow. Other experimental studies have demonstrated transmission of *M. hyopneumoniae* between pen mates by 7 (Marois et al., 2007) and 28 (Fano et al., 2005b) days PI. Fano et al. (2005a) reported directly and indirectly in-contact animals seroconverting 5 and 6 weeks later than experimentally inoculated pigs.

Meyns et al. (2006) demonstrated that vaccination, with a commercially available vaccine, did not significantly reduce *M. hyopneumoniae* transmission with R_n values of 2.38 and 3.51 in vaccinated and non-vaccinated animals, respectively. This finding confirms what has been observed in several field studies, that the number of vaccinated seropositive pigs gradually increases towards the end of the finishing period, indicating that *M. hyopneumoniae* can still circulate in vaccinated animals (Maes et al., 1999).

Few experimental studies have focused on the airborne transmission of *M. hyopneumoniae*. Fano et al. (2005b) reported the transmission of *M. hyopneumoniae* via aerosol to sentinel pigs located in a trailer 6 m from pigs experimentally co-infected with *M. hyopneumoniae* and PRRSV. Three weeks after exposure, 4/10 sentinel animals were positive for *M. hyopneumoniae* by nPCR in nasal and bronchial swabs and exhibited microscopic lesions suggestive of EP. Cardona et al. (2005) detected *M. hyopneumoniae* DNA in air samples 1, 75 and 150 m from where the microorganism was aerosolised. Although the movement of *M. hyopneumoniae* by aerosol was demonstrated in this study, the capacity of this aerosol to successfully infect pigs was not.

Ruiz et al. (2002) demonstrated different infection patterns among pigs sired by different boars suggesting a possible genetic component to the susceptibility of animals to *M. hyopneumoniae* infection. However, the small sample of animals studied and the fact that the role of sow genetics was not assessed, limited the conclusions that could be drawn.

Herd epidemiology: Infection and seroconversion dynamics

The clinical outcome of *M. hyopneumoniae* infection is dependent on environmental and management conditions and on the production system in operation. Among the various management conditions, all-in–all-out (AIAO) procedures may help in reducing the prevalence and severity of EP lesions (Maes et al., 1996). In farrow-to-finish (FF) systems, in which piglets remain until slaughter, transmission of infection from sows to piglets and from older to younger pigs occurs. In contrast, in multi-site (MS) systems the stages of pig production are physically separated from each other and the 'flow'

of animals is unidirectional (Harris and Alexander, 1999). In both systems the reproductive herd comprises animals of different ages and of different physiologic status (i.e. breeding, gestation, farrowing and lactation) and new animals are introduced to this group on an ongoing basis.

There is little information on the prevalence of *M. hyopneumoniae*-infected sows or their potential to shed bacteria and infect their offspring. Calsamiglia and Pijoan (2000) reported that between 24% and 56% of non-vaccinated sows were positive for *M. hyopneumoniae* by nPCR in a MS system. However, Ruiz et al. (2003) and Sibila et al. (2008) found a lower percentage (between 0% and 10.5%) of *M. hyopneumoniae*-infected sows at farrowing in both MS and FF systems. These findings also suggested that the prevalence of *M. hyopneumoniae* infection in sows was not related to the production system in operation.

In MS systems, piglets may become infected prior to weaning (Goodwin et al., 1965; Calsamiglia and Pijoan, 2000). Calsamiglia and Pijoan (2000) reported between 7.7% and 9.6% of pigs infected by 17 days of age, Ruiz et al. (2003) reported between 5.5% and 13.2% of piglets infected on a breeding unit at 19 days of age, and Sibila et al. (2007b) found between 0% and 6.4% of pigs from 1 to 3 weeks of age, infected. Fano et al. (2007) reported much higher infection rates of up to 51.8% in piglets at 17 days of age. Pijoan (2005) hypothesised that the prevalence of porcine mycoplasma infections in segregated production systems varies according to the prevalence of *M. hyopneumoniae* infection among pigs at weaning. Moreover, prevalence of *M. hyopneumoniae* nasal infection in recently weaned pigs has been suggested as a potential indicator of shedding by sows (Ruiz et al., 2003) and a possible predictor of the severity of EP in older animals (Fano et al., 2007; Sibila et al., 2007a,b).

Although animals can be infected early in life in both FF and MS systems, different infection and disease pathogenesis patterns have been reported (Sibila et al., 2004b). While in FF operations, infection of piglets at the nursery stage tends to be high and the percentage of infected pigs increases progressively with advancing age, this percentage in MS systems tends to decrease with age at the nursery stage and then to increase abruptly at fattening (Sibila et al., 2004b), a situation mirrored in herds with PRDC (Dee, 1996).

Detection of *M. hyopneumoniae* infection in a herd does not necessarily imply that this pathogen is the main causative agent of any existing respiratory problem. Sibila et al. (2004b) identified farms with subclinical or clinical EP depending on their *M. hyopneumoniae* nPCR profile. A farm was considered to have subclinical EP when the proportion of infected pigs at different ages was low even when coughing was present. However herds with a high and increasing proportion of *M. hyopneumoniae*-infected pigs with concomitant coughing were considered clinically affected.

Several epidemiological studies describe *M. hyopneumoniae* seroconversion dynamics (Calsamiglia et al., 1999b; Leon et al., 2001; Vicca et al., 2002; Sibila et al., 2004b; Vigre et al., 2004). Under field conditions, there is a progressive decrease in the numbers of pigs with maternal antibodies followed by a slow increase in the numbers of seropositive animals towards the finishing stage of production (Calsamiglia et al., 1999b). Although the median half-life of maternal antibody against *M. hyopneumoniae* is approximately 16 days (Morris et al., 1994), they may persist for 9 weeks when initial titres are high (Wallgren et al., 1998). Persistence of maternal antibody to *M. hyopneumoniae* in piglets is also dictated by the level of antibody in the sow's serum (Wallgren et al., 1998). Within a variable time-span after maternal antibodies wane, pigs seroconvert to *M. hyopneumoniae* (Sørensen et al., 1997; Vigre et al., 2004) although the waning of maternal antibodies may not be as significant in *M. hyopneumoniae*-vaccinated herds (Sibila et al., 2004b). The fact that the number of seropositive animals gradually increases towards the end of the fattening period in both vaccinated and non-vaccinated herds suggests that antibodies induced by either natural infection or vaccination do not prevent further infection (Maes et al., 1998; Maes et al., 1999).

Discussion

Although major efforts to control *M. hyopneumoniae* infection and its detrimental effects have been made, significant economic losses in pig production worldwide due to EP remain (Thacker, 2006). Effective control requires a number of actions including establishing and monitoring the extent of disease, reducing transmission of infection and developing an appropriate vaccination strategy.

Establishing and monitoring the extent of EP in pig herds now typically utilises ELISA, PCR and abattoir surveillance methods. Necropsy is also useful, particularly when accompanying PRDC is suspected. Given that each of these diagnostic techniques has the limitations outlined previously, their use in combination is required to optimise diagnostic accuracy.

Reducing *M. hyopneumoniae* transmission between animals requires particular attention to critical transmission points. At weaning, *M. hyopneumoniae*-infected piglets may transmit the agent to nursery and finishing sites. Also, by the time animals are moved from the nursery to the finishing units, many animals have low levels of maternal antibodies (Leon et al., 2001) and are thus more susceptible to infection. Furthermore, the possibility of the airborne transmission of *M. hyopneumoniae* between farms or between production units within a herd should also be considered.

The development of an appropriate vaccination strategy may involve vaccination of piglets, sows, or both, against *M. hyopneumoniae*. Although currently available vaccines reduce the extent of EP lesions in vaccinated piglets, the vaccine does not protect animals against infection (Haesebrouck et al., 2004). Sow vaccination can control EP by transferring colostral-derived immunity to the piglets

and thus reducing vertical transmission (Sibila et al., 2007a). Further studies will be required to determine the relative effectiveness of these strategies and in particular to look at the protective efficacy of vaccination against potentially more virulent field isolates.

Experimental transmission models are useful in estimating the effects of treatment and control measures on the spread of *M. hyopneumoniae* infection but the results of such work must be treated with a degree of caution as the infecting dose in experimental situations is likely to be larger than under field conditions. Such larger challenge doses may explain the more rapid seroconversion observed in experimentally relative to naturally infected animals (Fano et al., 2005a).

Although *M. hyopneumoniae* is usually considered primarily a respiratory pathogen, recent studies have reported the isolation of this agent from the liver, spleen and kidneys of experimentally (Le Carrou et al., 2006) and in-contact infected pigs (Marois et al., 2007). This finding suggests that *M. hyopneumoniae* infection is not always restricted to the respiratory tract and merits further investigation.

Conclusions

The effective control of *M. hyopneumoniae*-infection and of consequent EP in pig herds requires a number of actions including the establishment and monitoring of the extent of the disease, the use of techniques such as the molecular typing of isolates to analyse infection transmission patterns and the design of effective vaccines and vaccination strategies.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

References

Ameri, M., Zhou, E.M., Hsu, W.H., 2006. Western blot immunoassay as a confirmatory test for the presence of anti-*Mycoplasma hyopneumoniae* antibodies in swine serum. Journal of Veterinary Diagnostic Investigation 18, 198–201.

Ameri-Mahabadi, M., Zhou, E.M., Hsu, W.H., 2005. Comparison of two swine *Mycoplasma hyopneumoniae* enzyme-linked immunosorbent assays for detection of antibodies from vaccinated pigs and field serum samples. Journal of Veterinary Diagnostic Investigation 17, 61–64.

Andreasen, M., Nielsen, J.P., Baekbo, P., Willeberg, P., Bøtner, A., 2000. A longitudinal study of serological patterns of respiratory infections in

nine infected Danish swine herds. Preventive Veterinary Medicine 45, 221–235.

Artiushin, S., Minion, F.C., 1996. Arbitrarily primed PCR analysis of *Mycoplasma hyopneumoniae* field isolates demonstrates genetic heterogeneity. International Journal of Systematic Bacteriology 46, 324–328.

Artiushin, S., Stipkovits, L., Minion, F.C., 1993. Development of polymerase chain reaction primers to detect *Mycoplasma hyopneumoniae*. Molecular Cell Probes 7, 381–385.

Assuncao, P., De la Fe, C., Ramirez, A.S., Gonzalez Llamazares, O., Poveda, J.B., 2005. Protein and antigenic variability among *Mycoplasma hyopneumoniae* strains by SDS-PAGE and immunoblot. Veterinary Research Communication 29, 563–574.

Batista, L., Pijoan, C., Ruiz, A., 2004. Assessment of transmission of *Mycoplasma hyopneumoniae* by personnel. Journal of Swine Health and Production 12, 75–77.

Baumeister, A.K., Runge, M., Ganter, M., Feenstra, A.A., Delbeck, F., Kirchhoff, H., 1998. Detection of *Mycoplasma hyopneumoniae* in bronchoalveolar lavage fluids of pigs by PCR. Journal of Clinical Microbiology 36, 1984–1988.

Blanchard, B., Kobisch, M., Bove, J.M., Saillard, C., 1996. Polymerase chain reaction for *Mycoplasma hyopneumoniae* detection in tracheobronchiolar washings from pigs. Molecular Cell Probes 10, 15–22.

Blank, W.A., Stemke, G.W., 2000. A physical and genetic map of the *Mycoplasma hyopneumoniae* strain J genome. Canadian Journal of Microbiology 46, 832–840.

Boye, M., Jensen, T.K., Ahrens, P., Hagedorn-Olsen, T., Friis, N.F., 2001. In situ hybridisation for identification and differentiation of *Mycoplasma hyopneumoniae*, *Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis* in formalin-fixed porcine tissue sections. Apmis 109, 656–664.

Cai, H.Y., van Dreumel, T., McEwen, B., Hornby, G., Bell-Rogers, P., McRaild, P., Josephson, G., Maxie, G., 2007. Application and field validation of a PCR assay for the detection of *Mycoplasma hyopneumoniae* from swine lung tissue samples. Journal of Veterinary Diagnostic Investigation 19, 91–95.

Calsamiglia, M., Pijoan, C., 2000. Colonisation state and colostral immunity to *Mycoplasma hyopneumoniae* of different parity sows. Veterinary Record 146, 530–532.

Calsamiglia, M., Pijoan, C., Trigo, A., 1999a. Application of a nested polymerase chain reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. Journal of Swine Health and Production 7, 263–268.

Calsamiglia, M., Pijoan, C., Bosch, G.J., 1999b. Profiling *Mycoplasma hyopneumoniae* in farms using serology and a nested PCR technique. Journal of Swine Health and Production 7, 263–268.

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

Cardona, A.C., Pijoan, C., Dee, S.A., 2005. Assessing *Mycoplasma hyopneumoniae* aerosol movement at several distances. Veterinary Record 156, 91–92.

Caron, J., Ouardani, M., Dea, S., 2000. Diagnosis and differentiation of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infections in pigs by PCR amplification of the p36 and p46 genes. Journal of Clinical Microbiology 38, 1390–1396.

Cheikh Saad Bouh, K., Shareck, F., Dea, S., 2003. Monoclonal antibodies to *Escherichia coli*-expressed P46 and P65 membranous proteins for specific immunodetection of *Mycoplasma hyopneumoniae* in lungs of infected pigs. Clinical and Diagnostic Laboratory Immunology 10, 459–468.

Christensen, G., Sørensen, V., Mousing, J., 1999. Diseases of respiratory system. In: Straw, B.E., D'Allaire, S., Mengeling, W., Taylor, D.J. (Eds.), Diseases of Swine. Iowa University Press, Ames, Iowa, pp. 913–940.

Davies, P.R., Bahnson, P.B., Grass, J.J., Marsh, W.E., Dial, G.D., 1995. Comparison of methods for measurement of enzootic pneumonia lesions in pigs. American Journal of Veterinary Research 56, 9–14.

de Castro, L.A., Pedroso, T.R., Kuchiishi, S.S., Ramenzoni, M., Kich, J.D., Zaha, A., Vainstein, M.H., Ferreira, H.B., 2006. Variable number of tandem amino acid repeats in adhesion-related CDS products in *Mycoplasma hyopneumoniae* strains. Veterinary Microbiology 116, 258–269.

Dee, S., 1996. The porcine respiratory disease complex: Are subpopulations important ? Swine Health and Production 4, 147–149.

Desrosiers, R., 2004. Epidemiology, diagnosis and control of swine diseases. In: American Association of Swine Veterinarians Annual Meeting, Des Moines, Iowa. pp. 9–37.

Dubosson, C.R., Conzelmann, C., Miserez, R., Boerlin, P., Frey, J., Zimmermann, W., Hani, H., Kuhnert, P., 2004. Development of two real-time PCR assays for the detection of *Mycoplasma hyopneumoniae* in clinical samples. Veterinary Microbiology 102, 55–65.

Erlandson, K.R., 2005. Evaluation of three serum antibody enzyme-linked immunosorbent assays for *Mycoplasma hyopneumoniae*. Journal of Swine Health and Production 13, 198–203.

Fano, E., Pijoan, C., Dee, S., 2005a. Dynamics and persistence of *Mycoplasma hyopneumoniae* infection in pigs. Canadian Journal of Veterinary Research 69, 223–228.

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

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 International Pig Veterinary Society, Copenhagen, Denmark, July 2006. p. 96.

Fano, E., Pijoan, C., Dee, S., Deen, J., 2007. Effect of *Mycoplasma hyopneumoniae* colonization at weaning on disease severity in growing pigs. Canadian Journal of Veterinary Research 71, 195–200.

Ferrell, R.V., Heidari, M.B., Wise, K.S., McIntosh, M.A., 1989. A mycoplasma genetic element resembling prokaryotic insertion sequences. Molecular Microbiology 3, 957–967.

Frey, J., Haldimann, A., Nicolet, J., 1992. Chromosomal heterogeneity of various *Mycoplasma hyopneumoniae* field strains. International Journal of Systematic Bacteriology 42, 275–280.

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

Goodwin, R.F., Pomeroy, A.P., Whittlestone, P., 1965. Production of enzootic pneumonia with a Mycoplasma. Veterinary Record 77, 1247–1249.

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? Veterinary Microbiology 100, 255–268.

Hannan, P.C., Bhogal, B.S., Fish, J.P., 1982. Tylosin tartrate and tiamutilin effects on experimental piglet pneumonia induced with pneumonic pig lung homogenate containing mycoplasmas, bacteria and viruses. Research Veterinary Science 33, 76–88.

Harasawa, R., Koshimizu, K., Takeda, O., Uemori, T., Asada, K., Kato, I., 1991. Detection of *Mycoplasma hyopneumoniae* DNA by the polymerase chain reaction. Molecular Cell Probes 5, 103–109.

Harasawa, R., Asada, K., Kato, I., 1995. A novel repetitive sequence from *Mycoplasma hyopneumoniae*. Journal of Veterinary Medical Science 57, 557–558.

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

Hsu, T., Artiushin, S., Minion, F.C., 1997. Cloning and functional analysis of the P97 swine cilium adhesin gene of *Mycoplasma hyopneumoniae*. Journal of Bacteriology 179, 1317–1323.

Kobisch, M., Tillon, J., Vannier, P., Magneur, S., Morvan, P., 1978. Pneumonie enzootique à *Mycoplasma suipneumoniae* chez le porc: diagnostic rapide et recherches d'anticorps. Recueil de Médecine Vétérinaire 154, 847–852.

Kobisch, M., Blanchard, B., Le Potier, M.F., 1993. *Mycoplasma hyopneumoniae* infection in pigs: duration of the disease and resistance to reinfection. Veterinary Research 24, 67–77.

Kokotovic, B., Friis, N.F., Jensen, J.S., Ahrens, P., 1999. Amplified-fragment length polymorphism fingerprinting of *Mycoplasma* species. Journal of Clinical Microbiology 37, 3300–3307.

Kurth, K.T., Hsu, T., Snook, E.R., Thacker, E.L., Thacker, B.J., Minion, F.C., 2002. Use of a *Mycoplasma hyopneumoniae* nested polymerase chain reaction test to determine the optimal sampling sites in swine. Journal of Veterinary Diagnostic Investigation 14, 463–469.

Kwon, D., Chae, C., 1999. Detection and localization of *Mycoplasma hyopneumoniae* DNA in lungs from naturally infected pigs by in situ hybridization using a digoxigenin-labeled probe. Veterinary Pathology 36, 308–313.

Kwon, D., Choi, C., Chae, C., 2002. Chronologic localization of *Mycoplasma hyopneumoniae* in experimentally infected pigs. Veterinary Pathology 39, 584–587.

Le Carrou, J., Laurentie, M., Kobisch, M., Gautier-Bouchardon, A.V., 2006. Persistence of *Mycoplasma hyopneumoniae* in experimentally infected pigs after marbofloxacin treatment and detection of mutations in the parC gene. Antimicrobial Agents and Chemotherapy 50, 1959–1966.

Leon, E.A., Madec, F., Taylor, N.M., Kobisch, M., 2001. Seroepidemiology of *Mycoplasma hyopneumoniae* in pigs from farrow-to-finish farms. Veterinary Microbiology 78, 331–341.

Lin, B.C., 2001. Interspecies differentiation of *Mycoplasma hyopneumoniae* field strains isolated in the United States. In: American Association of Swine Veterinarians Annual Meeting, Nashville, Tennessee. pp. 225–235.

Madec, F., Kobisch, M., 1982. Bilan lesionnel des pumons de porcs charcutiers a l'abattoir. Journées de la Recherche Porcine en France 14, 405–412.

Madsen, M.L., Oneal, M.J., Gardner, S.W., Strait, E.L., Nettleton, D., Thacker, E.L., Minion, F.C., 2007. Array-based genomic comparative hybridization analysis of field strains of *Mycoplasma hyopneumoniae*. Journal of Bacteriology 189, 7977–7982.

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

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

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

Marois, C., Tocqueville, V., Le Potier, M.F., Hars, J., Kobisch, M., 2006. Detection of *Mycoplasma hyopneumoniae* in French Wild Boars. In: Proceedings of the 19th International Pig Veterinary Society, Copenhagen, Denmark. p. 213.

Marois, C., Le Carrou, J., Kobisch, M., Gautier-Bouchardon, A.V., 2007. Isolation of *Mycoplasma hyopneumoniae* from different sampling sites in experimentally infected and contact SPF piglets. Veterinary Microbiology 120, 96–104.

Mattsson, J.G., Bergstrom, K., Wallgren, P., Johansson, K.E., 1995. Detection of *Mycoplasma hyopneumoniae* in nose swabs from pigs by in vitro amplification of the 16S rRNA gene. Journal of Clinical Microbiology 33, 893–897.

Mayor, D., Zeeh, F., Frey, J., Kuhnert, P., 2007a. Diversity of *Mycoplasma hyopneumoniae* in pig farms revealed by direct molecular typing of clinical material. Veterinary Research 38, 391–398.

Mayor, D., Jores, J., Korczak, B.M., Kuhnert, P., 2007b. Multilocus sequence typing (MLST) of *Mycoplasma hyopneumoniae*: A diverse pathogen with limited clonality. Veterinary Microbiology 127, 63–72.

McAuliffe, L., Ellis, R.J., Lawes, J.R., Ayling, R.D., Nicholas, R.A., 2005. 16S rDNA PCR and denaturing gradient gel electrophoresis; a single generic test for detecting and differentiating *Mycoplasma* species. Journal of Medical Microbiology 54, 731–739.

Meyns, T., Maes, D., Dewulf, J., Vicca, J., Haesebrouck, F., de Kruif, A., 2004. Quantification of the spread of *Mycoplasma hyopneumoniae* in nursery pigs using transmission experiments. Preventive Veterinary Medicine 66, 265–275.

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.

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

Moorkamp, L., Nathues, H., Spergser, J., Tegeler, R., Beilage, E.G., 2008. Detection of respiratory pathogens in porcine lung tissue and lavage fluid. Veterinary Journal 175, 273–275.

Moreau, I.A., Miller, G.Y., Bahnson, P.B., 2004. Effects of *Mycoplasma hyopneumoniae* vaccine on pigs naturally infected with *M. hyopneumoniae* and porcine reproductive and respiratory syndrome virus. Vaccine 22, 2328–2333.

Morris, C.L., Gardner, I.A., Hietala, S.K., Carpenter, T.E., Anderson, R.J., Parker, K.M., 1994. Persistence of passively acquired antibodies to *Mycoplasma hyopneumoniae* in a swine herd. Preventive Veterinary Medicine 21, 29–41.

Morris, C.R., Gardner, I.A., Hietala, S.K., Carpenter, T.E., 1995a. Enzootic pneumonia: comparison of cough and lung lesions as predictors of weight gain in swine. Canadian Journal of Veterinary Research 59, 197–204.

Morris, C.R., Gardner, I.A., Hietala, S.K., Carpenter, T.E., Anderson, R.J., Parker, K.M., 1995b. Seroepidemiology study of natural transmission of *Mycoplasma hyopneumoniae* in a swine herd. Preventive Veterinary Medicine 21, 323–337.

Morrison, R.B., Pijoan, C., Hilley, H.D., Rapp, V., 1985. Microorganisms associated with pneumonia in slaughter weight swine. Canadian Journal of Comparative Medicine 49, 129–137.

Mrazek, J., 2006. Analysis of distribution indicates diverse functions of simple sequence repeats in mycoplasma genomes. Molecular Biology and Evolution 23, 1370–1385.

Noyes, E., Feeney, D., Pijoan, C., 1990. Comparison of the effect of pneumonia detected during lifetime with pneumonia detected at slaughter on growth in swine. Journal of American Veterinary Medical Association 197, 1025–1029.

Otagiri, Y., Asai, T., Okada, M., Uto, T., Yazawa, S., Hirai, H., Shibata, I., Sato, S., 2005. Detection of *Mycoplasma hyopneumoniae* in lung and nasal swab samples from pigs by nested PCR and culture methods. The Journal of Veterinary Medical Science 67, 801–805.

Pieters, M., Pijoan, C., 2006. Detection of *Mycoplasma hyopneumoniae* DNA in experimentally infected pigs. In: Proceedings of the 19th International Pig Veterinary Society, Copenhagen, Denmark. p. 209.

Pieters, M., Fano, E., Pijoan, C., Dee, S., 2006. Transmission of *Mycoplasma hyopneumoniae* to vaccinated and unvaccinated replacement gilts from persistently infected pigs. In: Proceedings of the 19th International Pig Veterinary Society Copenhagen, Denmark. p. 102.

Piffer, I., Ross, R.F., 1985. Immunofluorescence technique for detection of *Mycoplasma hyopneumoniae* in swine lungs. Pesquisa Agropecuária Brasileira 20, 877–882.

Pijoan, C., 1994. Serology of *Mycoplasma hyopneumoniae*. In: Allen D. Leman Swine Conference, St. Paul, Minnesota. p. 8.

Pijoan, C., 2005. A controversial view of *Mycoplasma hyopneumoniae* epidemiology. In: Allen D. Leman Swine Conference, St. Paul, Minnesota. pp. 114–116.

Rodriguez, F., Ramirez, G.A., Sarradell, J., Andrada, M., Lorenzo, H., 2004. Immunohistochemical labeling of cytokines in lung lesions of pigs naturally infected with *Mycoplasma hyopneumoniae*. Journal of Comparative Pathology 130, 306–312.

Ross, R.F., 1999. Mycoplasmal diseases. In: Straw, B.E., D'Allaire, S., Mengeling, W.L., Taylor, D.J. (Eds.), Diseases of Swine. Iowa State University Press, Ames, pp. 495–505.

Ruiz, A., Galina, L., Pijoan, C., 2002. *Mycoplasma hyopneumoniae* colonization of pigs sired by different boars. Canadian Journal of Veterinary Research 66, 79–85.

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

Sarradell, J., Andrada, M., Ramirez, A.S., Fernandez, A., Gomez-Villamandos, J.C., Jover, A., Lorenzo, H., Herraez, P., Rodriguez, F., 2003. A morphologic and immunohistochemical study of the bronchus-associated lymphoid tissue of pigs naturally infected with *Mycoplasma hyopneumoniae*. Veterinary Pathology 40, 395–404.

Sheldrake, R.F., Gardner, I.A., Saunders, M.M., Romalis, L.F., 1990. Serum antibody response to *Mycoplasma hyopneumoniae* measured by enzyme-linked immunosorbent assay after experimental and natural infection of pigs. Australian Veterinary Journal 67, 39–42.

Sibila, M., Calsamiglia, M., Segalés, J., Rosell, C., 2004a. Association between *Mycoplasma hyopneumoniae* at different respiratory sites and presence of histopathological lung lesions. Veterinary Record 155, 57–58.

Sibila, M., Calsamiglia, M., Vidal, D., Badiella, L., Aldaz, A., Jensen, J.C., 2004b. Dynamics of *Mycoplasma hyopneumoniae* infection in 12 farms with different production systems. Canadian Journal of Veterinary Research 68, 12–18.

Sibila, M., Nofrarias, M., Lopez-Soria, S., Segales, J., Valero, O., Espinal, A., Calsamiglia, M., 2007a. Chronological study of *Mycoplasma hyopneumoniae* infection, seroconversion and associated lung lesions in vaccinated and non-vaccinated pigs. Veterinary Microbiology 122, 97–107.

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

Sibila, M., Bernal, R., Torrents, D., Riera, P., Llopart, D., Calsamiglia, M., Segales, J., 2008. Effect of sow vaccination against *Mycoplasma hyopneumoniae* on sow and piglet colonization and seroconversion, and pig lung lesions at slaughter. Veterinary Microbiology 127, 165–170.

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

Stakenborg, T., 2005. Identification of mollicutes and characterization of *Mycoplasma hyopneumoniae* isolates. Ph.D. Thesis. University of Ghent, Ghent.

Stakenborg, T., Vicca, J., Butaye, P., Maes, D., Peeters, J., de Kruif, A., Haesebrouck, F., 2005. The diversity of *Mycoplasma hyopneumoniae* within and between herds using pulsed-field gel electrophoresis. Veterinary Microbiology 109, 29–36.

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

Stakenborg, T., Vicca, J., Butaye, P., Imberechts, H., Peeters, J., De Kruif, A., Haesebrouck, F., Maes, D., 2006b. A multiplex PCR to identify porcine mycoplasmas present in broth cultures. Veterinary Research Communication 30, 239–247.

Stärk, K.D., 1999. The role of infectious aerosols in disease transmission in pigs. Veterinary Journal 158, 164–181.

Stärk, K.D., Nicolet, J., Frey, J., 1998. Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. Applied and Environmental Microbiology 64, 543–548.

Stemke, G.W., Phan, R., Young, T.F., Ross, R.F., 1994. Differentiation of *Mycoplasma hyopneumoniae*, *M. flocculare*, and *M. hyorhinis* on the basis of amplification of a 16S rRNA gene sequence. American Journal of Veterinary Research 55, 81–84.

Strait, E.L., Erickson, B.Z., Thacker, E.L., 2004. Analysis of *Mycoplasma hyopneumoniae* field isolates. In: American Association of Swine Veterinarians Annual Meeting, Des Moines, Iowa. p. 95.

Straw, B.E., Backstrom, L., Leman, A.D., 1986. Examination of swine at slaughter. Part II. Findings at slaughter and their significance. Compendium on Continuing Education for the Practicing Veterinarian 8, 106–112.

Thacker, E.L., 2006. Mycoplasmal Disease. In: Straw, B.E., Zimmermann, J.J., D'Allaire, S., Taylor, D.J. (Eds.), Diseases of Swine. Iowa State University Press, Ames, pp. 701–717.

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

Thacker, E.L., Thacker, B.J., Janke, B.H., 2001. Interaction between *Mycoplasma hyopneumoniae* and swine influenza virus. Journal of Clinical Microbiology 39, 2525–2530.

Thomsen, B.L., Jorsal, S.E., Andersen, S., Willeberg, P., 1992. The Cox regression model applied to risk factors analyses of infection in the breeding and multiplying herds in Danish SPF systems. Preventive Veterinary Medicine 12, 287–297.

Vengust, G., Valencak, Z., Bidovec, A., 2006. A serological survey of selected pathogens in wild boar in Slovenia. Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health 53, 24–27.

Verdin, E., Saillard, C., Labbé, A., Bové, J.M., Kobisch, M., 2000. A nested PCR assay for the detection of *Mycoplasma hyopneumoniae* in tracheo-bronchiolar washing from pigs. Veterinary Microbiology 76, 31–40.

Vicca, J., Maes, D., Thermote, L., Peeters, J., Haesebrouck, F., de Kruif, A., 2002. Patterns of *Mycoplasma hyopneumoniae* infections in Belgian farrow-to-finish pig herds with diverging disease-course. Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health 49, 349–353.

Vicca, J., Stakenborg, T., Maes, D., Butaye, P., Peeters, J., de Kruif, A., Haesebrouck, F., 2003. Evaluation of virulence of *Mycoplasma hyopneumoniae* field isolates. Veterinary Microbiology 97, 177–190.

Vigre, H., Dohoo, I.R., Stryhn, H., Busch, M.E., 2004. Intra-unit correlations in seroconversion to *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* at different levels in Danish multi-site pig production facilities. Preventive Veterinary Medicine 63, 9–28.

Wallgren, P., Bolske, G., Gustafsson, S., Mattsson, S., Fossum, C., 1998. Humoral immune responses to *Mycoplasma hyopneumoniae* in sows and offspring following an outbreak of mycoplasmosis. Veterinary Microbiology 60, 193–205.

Wilton, J.L., Scarman, A.L., Walker, M.J., Djordjevic, S.P., 1998. Reiterated repeat region variability in the ciliary adhesin gene of *Mycoplasma hyopneumoniae*. Microbiology 144, 1931–1943.

Zhuang, Q., Wachmann, H., Mortensen, S., Barford, K., 2002. Incidence of *Actinobacillus pleuropneumoniae* serotype 2 and *Mycoplasma hyopneumoniae* infections in the Danish SPF herds and risk factors for infections. In: Proceedings of the 17th International Pig Veterinary Society, Ames, Iowa. p. 228.