Received: 28 June 2021 | Revised: 4 October 2021 | Accepted: 28 November 2021

DOI: 10.1002/vetr.1317

Vet Record

ORIGINAL RESEARCH

Cross-sectional study on the in-herd prevalence of *Mycoplasma hyopneumoniae* at different stages of pig production

Pauline Deffner[1] | **Roland Maurer**[1] | **Vojislav Cvjetković**[2] | **Wolfgang Sipos**[3] | **Roman Krejci**[4] | **Mathias Ritzmann**[1] | **Matthias Eddicks**[1]

[1] Clinic for Swine, Centre for Clinical Veterinary Medicine, LMU Munich, Oberschleißheim, Germany

[2] Ceva Tiergesundheit, Duesseldorf, Germany

[3] Clinical Department for Farm Animals and Herd Management, University of Veterinary Medicine Vienna, Vienna, Austria

[4] Ceva Santé Animale, Libourne, France

Correspondence
Pauline Deffner, Clinic for Swine, Centre for Clinical Veterinary Medicine, LMU Munich, Sonnenstraße 16, 85764 Oberschleißheim, Germany.
Email: pauline.deffner@lmu.de

Funding information
Ceva Tiergesundheit GmbH; Ceva Santé Animale

Abstract

Background: A cross-sectional study was carried out to assess the prevalence of *Mycoplasma hyopneumoniae* infections before vaccination in 3-week-old piglets and to gain information about infection dynamics.

Methods: In 13 German and three Austrian farms with a known history of enzootic pneumonia, 790 piglets and 158 sows were sampled (blood samples, tracheobronchial swabs [TBS] [piglets], laryngeal swabs [LS] [sows]), and 525 pen-based oral fluids (OFs) were collected in growing and fattening pigs. Laboratory diagnostics included enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (PCR) analyses.

Results: Antibodies to *M. hyopneumoniae* were present in 87.5 per cent of all herds. Seroprevalence ranged from 0.0 to 100.0 per cent and 0.0 to 88.0 per cent in sows and piglets, respectively. *M. hyopneumoniae*-deoxyribonucleic acid (DNA) was present in 3.8 and 0.4 per cent of LS and TBS, respectively. Gilts had a 10.9 times higher chance being *M. hyopneumoniae* PCR-positive than older sows. In 75.0 per cent of all farms, *M. hyopneumoniae*-DNA was present in OFs. Detection rate was significantly higher in OFs of 20-week-old than in younger pigs ($p < 0.001$).

Conclusion: Results indicate that *M. hyopneumoniae* infections of the lower respiratory tract in piglets are rare but highlight the role of gilts in maintaining infection in the herd. Collecting OFs seems promising for surveillance, if coughing occurs simultaneously.

INTRODUCTION

Mycoplasma hyopneumoniae is known as the aetiological agent of enzootic pneumonia (EP) in pigs and is considered as an important pathogen in the development of the porcine respiratory disease complex.[1,2] The consequences for EP-affected growing and fattening farms are reduced growth performance and feed conversion rate as well as the need for antibiotic treatment, making *M. hyopneumoniae* an economically relevant pathogen.[3–5]

The transmission of the pathogen occurs horizontally and vertically within and between farms. The possibility of airborne spread up to 9.2 km was proven by Otake and others.[6] Consequently, a high pig density in the surrounding area was described as a risk factor for farrow-to-finish farms to be seropositive for *M. hyopneumoniae*.[7] However, in-herd transmission via direct nasal contact between individuals seems to be the most important route for the spread of *M. hyopneumoniae*.[8,9] Several in-herd risk factors that increased the chance for infection of suckling pigs,[10] growing pigs, or sows[11–13] were identified. Particularly, dams play a decisive role in maintaining *M. hyopneumoniae* in pig populations due to the possibility of vertical transmission from sows to their offspring.[14] Since piglets can become infected within the first days of life,[15] colonised piglets at the time of weaning are considered responsible for further spread of the bacterium in later stages of pig production.[16,17]

Accordingly, Pieters and others[18] demonstrated that vertical transmission of *M. hyopneumoniae* from dams to their offspring represents a risk factor for the colonisation of piglets at weaning age. Furthermore, every additional day of suckling period was identified as risk factor for the occurrence, high seroprevalence and clinical signs of EP in fattening pigs.[19] The impact of vertical transmission of *M. hyopneumoniae* becomes clearly recognisable within a study by Sibila and others,[20] when a 50 per cent lower lung-lesion score was present in pigs from vaccinated sows as compared to unvaccinated sows, where piglets remained unvaccinated against *M. hyopneumoniae*.

In the literature, different methods concerning the evaluation of infections in suckling piglets are used. Mostly, polymerase chain reaction (PCR)-testing from different sample types is described as a cheap, fast and sensitive way of DNA detection. However, there is a lack of information about the infectivity of the detected material,[21] and cross-reactions with other *Mycoplasma* species such as *Mycoplasma hyorhinis* or *Mycoplasma flocculare* might occur.[22] Additionally, the great genetic variability of *M. hyopneumoniae* strains circulating in the swine population[23–27] affects the sensitivity of some PCRs. Strait and others[22] demonstrated that some *M. hyopneumoniae*-specific real-time PCRs did not detect all strains of their 36-isolate *M. hyopneumoniae* collection. Thus, to minimise these drawbacks, there is the need to use the most appropriate sample material and optimal sampling site for detection of *M. hyopneumoniae* in the corresponding age groups to gain valid information.

Nasal swabs are often used because of the easy handling. Moreover, gained results might be a good indicator whether and to what extent EP-lesions occur at slaughter.[17,20] However, in several studies, samples from the lower respiratory tract revealed a higher sensitivity than swabs from the nasal cavity.[8,28–31] Furthermore, the lower respiratory tract represents the natural site of *M. hyopneumoniae* multiplication,[32] whereas the nasal mucosa may only be transiently colonised before *M. hyopneumoniae* reaches the lower respiratory tract.[33] Particularly, tracheobronchial swabbing seems to be suitable for *M. hyopneumoniae* detection, since sensitivity of detection by PCR turned out to be at least as high as that of tracheobronchial washing.[28] Moreover, tracheobronchial swabs (TBS) are less time-consuming and cost-intensive. Whether laryngeal swabs (LS) or TBSs are superior for *M. hyopneumoniae* detection is still under discussion.[34–36] However, sampling individual animals remain time-consuming which is why sampling by pen-based oral fluids (OFs) is increasingly practised.[37]

As vaccination normally aims to immunise individuals before exposure to the corresponding pathogen to reach optimal vaccine efficacy, we evaluated the infection status of suckling piglets at the age of approximately 3 weeks directly before vaccination against *M. hyopneumoniae* in order to figure out the occurrence of pre-vaccinated *M. hyopneumoniae* positive piglets. Furthermore, the relation between the infection status of sows and piglets was investigated, and OFs from downstream productions steps were collected to gain information on infection patterns in growing and fattening pigs.

MATERIAL AND METHODS

The study was carried out between July 2018 and August 2019 in 13 German and three Austrian farms. The farms were spread across different federal states of Germany and Upper Austria. The study was approved by the governmental authorities of each federal state (Table S1 in the supporting material).

Study design

The present investigation was conducted on 16 farms as a cross-sectional study. At each farm, 10 sows and five corresponding littermates were sampled individually in the suckling period. OF samples from pigs at 6, 12, 16 and 20 weeks of age were collected on the same day. LS, TBS and OFs were examined by real-time PCR for *M. hyopneumoniae* DNA. Additionally, serum samples were examined for specific IgG-antibodies to *M. hyopneumoniae*. The examination of 10 sows and 50 piglets per farm allowed the detection of a 25 and five per cent prevalence of *M. hyopneumoniae* DNA with a 95 per cent confidence level, respectively.

Farms and animals

Farms were enrolled in the present study when the following criteria were fulfilled. Farms were farrow-to-finish farms (or farrow-to-wean farms with a one-on-one connected fattening farm) with one-shot vaccination against *M. hyopneumoniae* at the age of approximately 3 weeks, occurrence of coughing in the fattening unit and detection of EP-type lesions at slaughter or a known history (provided by the herd attending veterinarians) of EP on the farm. Moreover, lungs from a batch of slaughtered pigs of each farm were pre-checked before the decision to include the farm in the study. Participation in the study was voluntary, and participating farms are listed in Table 1. Based on the governmental restrictions, only clinically healthy sows and piglets were included in order to reduce the negative impact on the animals to a minimum. If possible, five gilts per herd were included, as gilts seem to have a higher chance to transmit *M. hyopneumoniae* to their offspring.[38,39] To ensure a random selection of sows, sampling was always started on the first farrowing pen on the right-hand side of the farrowing compartment proceeding counter-clockwise with the following pen. Selection of five piglets per litter was made irrespectively of gender by briefly fixing all piglets in the piglet nest using a drift board and picking five piglets randomly.

TABLE 1 Farm description and distribution of the farms on governmental districts including vaccination schemes of sows and piglets, farm-type, batch system/duration of suckling period, as well as raised enzootic pneumonia (EP)-Index at the slaughterhouse

Farm	Federal state	Type of farm	Sows	Vaccination (sows)	Vaccination (piglets)	Piglet age (weeks) at *M. hyo* vaccination	Batch system (weeks)/ suckling period (days)	EP Indices (0–28)
1	Bavaria	ff	180	Ery-PPV/IAV	PCV2/M. hyo	7	4/21	0.68
2	Bavaria	ff	180	APP/PRRSV/Ery-PPV	PCV2/M. hyo/PRRSV	3	5/28	1.27
3	North Rhine-Westphalia	1:1 connected	470	M. hyo/APP/PRRSV/IAV/Ery-PPV	PCV2/M. hyo/PRRSV	3	1.5/28	1.89
4	Lower Saxony	1:1 connected	400	PRRSV/PCV2/Ery-PPV/IAV	PCV2/M. hyo/PRRSV	3	2/21	1.84
5	Baden-Wuerttemberg	ff	360	PCV2/PRRSV/IAV/Ery-PPV	PCV2/M. hyo/PRRSV	3	1.5/28	3.43
6	Schleswig-Holstein	ff	160	M. hyo/PCV2/PRRSV/IAV/Ery-PPV	PCV2/M. hyo/PRRSV	4	1/28	1.2
7	Lower Saxony	ff	160	APP/PRRSV	PCV2/M. hyo	3	3/28	1.27
8	Baden-Wuerttemberg	ff	120	Ery-PPV	PCV2/M. hyo/PRRSV	3	3/28	3.15
9	North Rhine-Westphalia	ff	320	PRRSV/IAV/Ery-PPV	PCV2/M.hyo	4	2/21	0.15
10	Bavaria	ff	250	Ery-PPV	PCV2/M. hyo	4	1/28	2.55
11	North Rhine-Westphalia	ff	380	PRRSV/IAV/Ery-PPV	PCV2/M. hyo	4	1/28	3.64
12	North Rhine-Westphalia	ff	145	M. hyo/PCV2/PRRSV/IAV/Ery-PPV	PCV2/M. hyo/PRRSV	4	4/21	1.18
13	Upper Austria	ff	120	APP/M. hyo PCV2/PRRSV/Ery-PPV	PCV2/M. hyo/PRRSV	3	3/28	6.68
14	Upper Austria	ff	90	PCV2/Ery-PPV	PCV2/M. hyo	3	3/21	3.09
15	Upper Austria	ff	76	Ery-PPV	PCV2/M. hyo	3	3/28	2.47
16	Lower Saxony	ff	900	APP/M. hyo/PCV2/PRRSV/IAV/Ery-PPV	PCV2/M. hyo/PRRSV	3	1/28	1.65

Abbreviations: APP, *Actinobacillus pleuropneumoniae*; EP, enzootic pneumonia; Ery, *Erysipelothrix rhusiopathiae*; ff, farrow-to-finish; IAV, influenza-A-virus; *M. hyo, Mycoplasma hyopneumoniae*; PCV2, porcine cirovirus Type 2; PRRSV, porcine reproductive and respiratory syndrome virus; PPV, porcine parvovirus.

All samples were collected within 1 day, when the piglets were 21 ± 3 days old and before piglet vaccination against *M. hyopneumoniae*.

Sample collection

Blood samples were collected from both sows and piglets, whereas LS and TBS were obtained either from sows or piglets, respectively. Growing and fattening pigs were sampled by pen-based OFs. Required tools (scissors, mouth gags) were cleaned and disinfected after every single application using disinfectant wipes (Meliseptol HBV Tücher; B.Braun Melsungen AG, Germany).

From each sow, an LS was obtained by using a snare and a mouth gag to prevent biting and swallowing of the swab, as well as improving the visibility of the laryngeal region. When inserting the cotton swab (Eydam-Tupfersystem; Erich Eydam KG, Germany) into the larynx, a change of voice was evaluated as reaching the correct specimen sampling site. Samples were collected by rotating swab movements until cough was triggered. Afterwards, the tip of the swab (4–5 cm) was cut off with scissors, transferred to a sterile sample tube containing 1 ml phosphate buffered saline (PBS) (Gico PBS; pH 7.4, Gibco, Great Britain) and stored at −20°C until laboratory analysis.

From each piglet, a TBS was obtained. Therefore, a second person fixed the piglet beneath his/her arm and held the forelimbs with both hands cranial of the carpal joints in order to keep the animals as calm as possible for the sampling procedure. Further sampling was carried out as described previously.[28] Briefly, the sampling person placed a mouth gag between the upper and lower jaw to be able to collect the TBS using a sterile catheter (DCT-Nelaton Katheter 40 cm; servoprax GmbH, Germany). During inspiration, the catheter was inserted deeply into the trachea until reaching the bifurcatio tracheae. Triggering coughing was evaluated as successful sampling. Obtained TBSs were processed analogously to LS afterwards.

Serum samples were obtained directly after LS/TBS by venepuncture of vena jugularis dextra (sows) or vena cava cranialis (piglets). Blood was collected in Primavette V serum containers (KABE Labortechnik; Germany). Blood samples were allowed to clot and centrifuged on the day of collection or 1 day after at 1560 g for 10 minutes. Obtained serum was stored at −20°C until laboratory examination.

OF collection took place pen-wise via cotton ropes (OF Sample Collection Accessory Kit; IDEXX, USA). The ropes were placed in pens of pigs at the age of approximately 6, 12, 16 and 20 weeks. Ten cotton ropes (one rope for a maximum of 25 animals) were used per age group with the aim to sample at least 200 pigs per farm and group for *M. hyopneumoniae* DNA by PCR. Selection of pens for sampling was made randomly in the same way as in the farrowing site: when entering a finishing compartment, sampling was started at the first pen on the right-hand side and then continued counter-clockwise until the full sample size was reached. OF collection took place as described previously.[40] The collection period lasted 25–30 minutes, afterwards the lower, wet part of the rope was inserted into the supplied plastic bag and squeezed manually for releasing the OF. Sampled OFs were decanted into supplied 5 ml centrifuge tubes and were centrifuged at 1560 g for 10 minutes to separate feed particles and other contaminants from the liquid. They were stored at −80°C until laboratory examination.

Laboratory diagnostics

Examination of samples took place in commercial labs.

Serum was analysed by enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to *M. hyopneumoniae* using the ID Screen *M. hyopneumoniae* indirect ELISA (ID.vet Innovative Diagnostics; France). Samples were considered positive if the calculated sample to positive (s/p) ratio was greater than 0.4.

LS and TBS were analysed for the presence of *M. hyopneumoniae* DNA by PCR. TBSs were examined in pools of five. Individual swabs from positive pools were examined separately. LSs of sows were examined individually. Detection of *M. hyopneumoniae* DNA was performed by real-time PCR as described by Marois and others.[30] Commercially available master mix was used (GoTaq Probe qPCR Master Mix; PROMEGA Corporation, USA). Samples which did not generate a signal after 40 cycles were considered to be negative.

M hyopneumoniae DNA detection in OFs was performed by real-time PCR following the protocol described by Strait and others.[22] For DNA extraction, the QIAamp DNA Mini Kit (some samples) or QIAamp 96 DNA QIAcube HT Kit (more than 10–20 samples) (QIAGEN; Germany) was used. PCR was performed using RotorGene Probe real-time PCR Master Mix (QIAGEN; Germany). Samples which did not generate a signal after 40 cycles were considered to be negative.

Statistical analysis

All statistical calculations were performed using statistic software IBM SPSS Statistics version 24 for Microsoft Windows. For the statistical analysis, metric variables were tested for normal distribution. Student´s *t* test was performed in normally distributed data, or an ANOVA was carried out in case of more than two groups. Parametric tests (Mann–Whitney *U* test or Wilcoxon rank-sum test) were used in case of non-normal distribution. Dichotomous variables were analysed for possible associations by chi-squared test. In case of more than one independent variable associated with a dependent variable, a binary logistic regression was carried out.

TABLE 2 Percentage (number) of *M. hyopneumoniae* PCR-positive samples per farm

Farm	*M. hyo* vaccination sows		PCR		OF
			Sows	Piglets	6th–22nd week
1	No sow vaccination		0.0% (0/10)	0.0% (0/50)	2.8% (1/36)
2			0.0% (0/10)	0.0% (0/50)	19.4% (7/36)
4			0.0% (0/10)	0.0% (0/50)	21.6% (8/37)
5			0.0% (0/10)	0.0% (0/50)	27.0% (10/37)
7			0.0% (0/10)	6.0% (3/50)	2.6% (1/38)
8			10.0% (1/10)	0.0% (0/50)	18.8% (6/32)
9			10.0% (1/10)	0.0% (0/50)	5.9% (2/34)
10			20.0% (2/10)	0.0% (0/50)	0.0% (0/28)
11			0.0% (0/10)	0.0% (0/50)	44.4% (16/36)
14			0.0% (0/10)	0.0% (0/50)	0.0% (0/20)
15			0.0% (0/8)	0.0% (0/40)	0.0% (0/23)
∑no vacc		**68.8% (11/16)**	**3.7% (4/108)[a]**	**0.6% (3/540)[a]**	**14.0% (50/356)[a]**
3	Sow vaccination	Gilts	0.0% (0/10)	0.0% (0/50)	2.7% (1/37)
6		Gilts	0.0% (0/10)	0.0% (0/50)	12.1% (4/33)
12		+	0.0% (0/10)	0.0% (0/50)	0.0% (0/38)
13		Every rep cycle	0.0% (0/10)	0.0% (0/50)	30.4% (7/23)
16		Gilts	20.0% (2/10)	0.0% (0/50)	2.7% (1/37)
∑ vacc		**31.3% (5/16)**	**4.0% (2/50)[a]**	**0.0% (0/250)[a]**	**8.3% (14/169)[b]**
∑ all			**3.8% (6/158)**	**0.4% (3/790)**	**12.2% (64 /525)**

Abbreviations: *M. hyo*, *Mycoplasma hyopneumoniae*; OF, oral fluid; rep, reproduction; vacc, vaccination.
[a:b] $p = 0.059$.

RESULTS

In total, serum and LS/TBS samples from 158 sows and 790 piglets were obtained. Due to leakage during transport, two piglet serum samples could not be analysed. Additionally, 525 OFs were collected from growing and fattening pigs. All farms were stated as *M. hyopneumoniae*-positive either based on the results of ELISA or PCR. The individual farm-based qualitative PCR results are displayed in Table 2.

Serology

Antibodies to *M. hyopneumoniae* were present in 87.5 per cent (14/16) of the sampled herds in at least one serum sample (sow or piglet). The seroprevalence within the herds ranged from 0 to 100 per cent in sows and from 0 to 88 per cent in piglets. The number of seropositive piglets was significantly higher on farms with sow vaccination against *M. hyopneumoniae* (59.6 vs. 34.0 per cent, $p < 0.001$). In addition, s/p ratios of piglets from vaccinated sows were higher than those of piglets from unvaccinated ($p < 0.001$). There was no statistically significant difference between the s/p ratios of vaccinated and unvaccinated sows. The s/p ratios of sows (median: 0.41, min: 0.00, max: 2.00) and piglets (median: 0.31, min: 0.02, max: 2.28) correlated significantly ($p < 0.001$, $\varrho = 0.782$). At the individual farm level, correlations between sows and piglets were detected in all except for three farms (18.8 per cent).

PCR

In total, 158 LS of sows, 788 TBS of piglets and 525 OFs from growing and fattening pigs were examined for the presence of *M. hyopneumoniae* DNA by real-time PCR. In 13 of 16 farms (81.3 per cent) at least one sample (LS, TBS or OF) was positive for *M. hyopneumoniae* DNA (Table 2).

At the farm level, *M. hyopneumoniae* DNA was present in 25 per cent (4/16) of all farms in LS of sows and in 6.3 per cent (1/16) of TBS of piglets. Concerning individual pigs, six of 158 (3.8 per cent) LS (mean cycle threshold [ct] value: 32.8; min: 28.0; max: 36.0, standard deviation [SD]: 2.7) and three of 788 (0.4 per cent) TBS (mean ct-value: 38.0, min: 37.0, max: 39.0; SD: 1.0) were *M. hyopneumoniae* DNA-positive. Five of the six *M. hyopneumoniae* DNA-positive LS originated from gilts (83.3 per cent), whereas only one of the positive LS belonged to an older sow ($p = 0.008$, sixth parity). Thus, gilts had a 10.9 (confidence interval [CI]: 1.4–20.8) times higher chance to be *M. hyopneumoniae* PCR-positive than older sows (second to 12th parity, mean: 4.68, SD: 2.33). An association between the PCR results of LS and TBS could not be shown.

In total, 12 of 16 farms (75 per cent) had at least one *M. hyopneumoniae* DNA-positive OF in one of the four sampled age groups. The detection rate within the 12 positive herds was 12.2 per cent (CI: 9.0–15.0) over all age groups. The mean ct-value of *M. hyopneumoniae* positive OFs was 33.8 (min: 27.9, max: 39.7, SD: 2.3). The rate of detection was significantly higher in the 20th week of life (34.3 per cent; CI: 26.0–43.0) than in

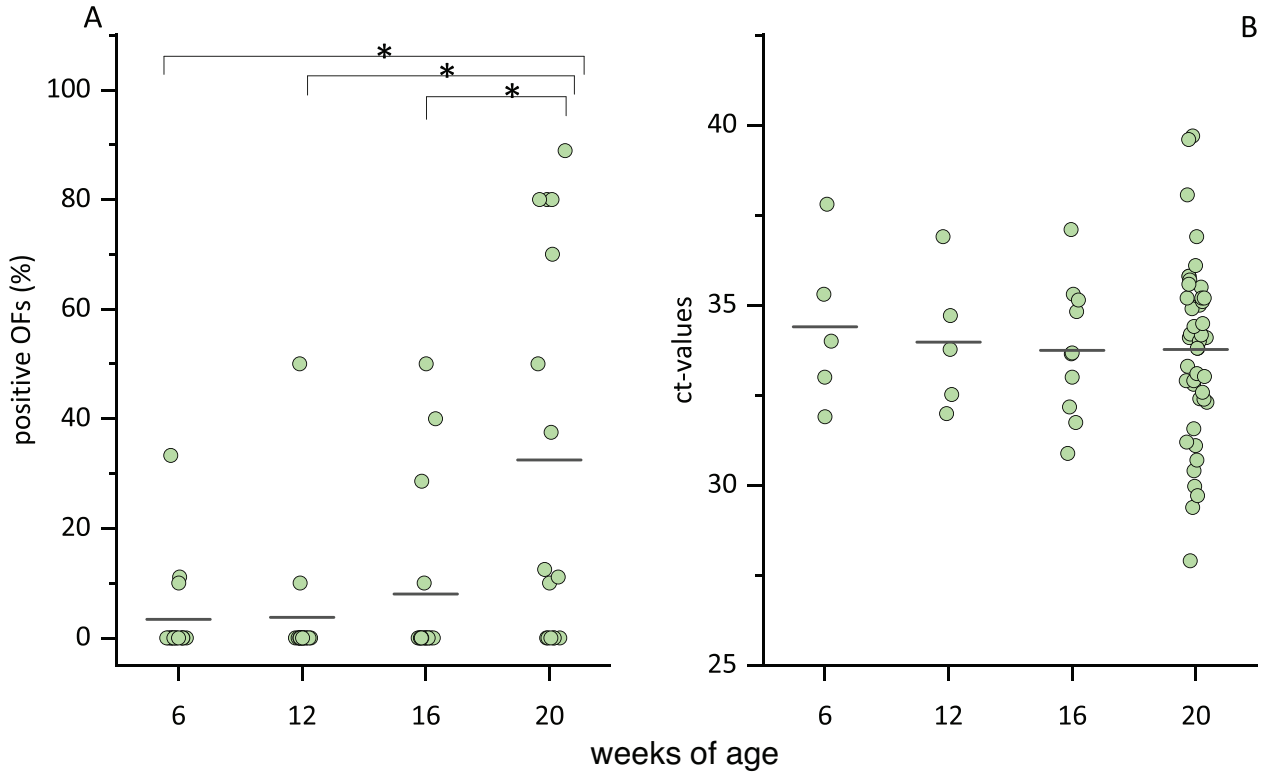

FIGURE 1 (A) Percentage of positive oral fluids (OFs) per farm ($n = 16$) and sampling age. Each bullet represents one farm. (B) Distribution of cycle threshold (ct)-values of positive OFs ($n = 64$) in the four sampled age groups. Each bullet represents one *M. hyopneumoniae* DNA-positive OF (samples with ct < 40 were considered to be positive). $^{*}p < 0.001$; horizontal lines in the scatter plot are marking the average value

the 16th (7.6 per cent; CI: 3.0–12.0), 12th (3.7 per cent; CI: 0.0–7.0) and sixth (3.8 per cent; CI: 0.0–7.0) week of life (Figure 1; $p < 0.001$). No association was present concerning the age at sampling and the ct-values of positive OFs. Farms with 21 days of suckling period (31.5 per cent) had a significantly smaller number of positive OFs (6.6 per cent; CI: 3.3–10.4) in rearing and fattening pigs than farms (68.5 per cent) with a suckling period of 28 days (15.2 per cent; CI: 11.4–19.2) ($p = 0.004$). Additionally, the detection rate of *M. hyopneumoniae* in OFs tended to be lower if vaccination of sows against *M. hyopneumoniae* was performed (Table 2; $p = 0.059$).

Lung scoring

Lung scores were evaluated for each farm at the slaughterhouse before sampling according to Cvjetković and others,[41] where 0–4 scoring points per lung lobe are achievable (total maximum 28 scoring points). The average time gap between raising the score and visiting the farm was 76 days (min: 20, max: 192, SD: 59.0); mean EP score was 2.3 (min: 0.2; max: 6.7; SD: 1.6). There was no correlation between the number of *M. hyopneumoniae*-positive LS or TBS per farm and the EP score. However, lower ct-values in OFs correlated with higher EP scores raised at slaughter ($p = 0.034$; $\varrho = -0.266$). Furthermore, significantly higher EP scores were present in herds with a 28-day suckling period (median: 2.6, SD: 1.6) compared to farms with a 21-day suckling period (median: 1.2, SD: 0.7; $p = 0.013$).

DISCUSSION

Piglets that become infected with *M. hyopneumoniae* within the suckling period are considered to be mainly responsible for the spread of the pathogen within the growing and fattening units.[42] Previous studies on the occurrence of *M. hyopneumoniae* in suckling piglets report prevalence of at least 3.6 up to 14.1 per cent in European farms.[10,42–47] Thus, values obtained in these studies are much higher than the prevalence of 0.4 per cent in suckling piglets in the present study. Although participating farms were pre-selected based on the knowledge of present *M. hyopneumoniae* infections, there are still discrepancies to the study designs of previous examinations. Selection of tested animals and in particular the sample sites differ within former studies and compared with our investigation. Thus, in the present study, only healthy animals without signs of clinical disease were included, whereas in the study of Moorkamp and others[45] only animals showing coughing were selected for examination by tracheobronchial lavage. The selection of pre-diseased animals may explain 12.3 per cent of 3-week-old piglets being *M. hyopneumoniae* DNA-positive in this study, although TBS is considered equally sensitive as tracheobronchial lavage.[28] Likewise, Nathues and others[10] selected the weakest piglets per litter

for investigation by nasal swabs. Concerning nasal swabbing, this method has been widely used in previous studies to detect *M. hyopneumoniae*,[10,42–44] but might rather show the colonisation of the upper airways than the infection of the lower respiratory tract. In fact, the latter is known as multiplication site of *M. hyopneumoniae*,[32] and nasal cavities function as port of entry for *M. hyopneumoniae* before reaching the lower respiratory tract as target tissue. Thus, the authors assume that colonisation of upper airways with *M. hyopneumoniae* takes place in suckling piglets, but as it takes longer time to reach the target tissue, infections are therefore rare at the age of 3 weeks and tend to occur in downstream production stages, where also clinical presentation of EP mainly appears.[48] This is in line with a recent study that detected *M. hyopneumoniae* in only 1 per cent of TBS collected in pigs 28 days post-exposure to seeder pigs, while detection was up to 90 per cent 113 days post-exposure.[34] In addition, this might also explain the higher detection rates of *M. hyopneumoniae* in studies from Fablet and others[49] and Vangroenweghe and others[50] than in the present study, as they included piglets up to 4 and 5 weeks of age, respectively. However, determining an initial group colonisation in suckling piglets by nasal swabs is able to predict disease severity in later production stages and EP-like lesions at slaughter.[17] Unfortunately, we did not sample nasal swabs additionally to compare colonisation of the upper respiratory tract and infection of the lower respiratory tract. Due to the low overall prevalence of *M. hyopneumoniae* DNA in TBS of the piglets, we were not able to show any negative impact of infection of the lower respiratory tract of suckling piglets on the vaccination efficacy. However, the low rate of infections at the sampling time point might explain why vaccinations at 3 weeks of age can lead to significantly better performances in fattening pigs.[51] Therefore, it seems that the colonisation of the upper respiratory tract does not necessarily interfere with the success of vaccination.

Antibodies to *M. hyopneumoniae* were present in 87.5 per cent of all sampled herds in at least one serum sample (sow or piglet), which is in line with the endemic character in German pig herds.[52] Likewise, the detected significantly higher number of seropositive piglets as well as higher s/p ratios of piglets on farms vaccinating sows against *M. hyopneumoniae* is quite reasonable, since piglets take up maternal antibodies postpartum via colostrum,[42,53] and is also described in previous studies. Thus, significantly higher percentage of piglets turned out to be seropositive if sows were vaccinated twice before farrowing.[20,54] Additionally, high intra-farm correlation of s/p ratios of sows and piglets confirms the relationship between antibody titres in sows and piglets.[55,56] The lack of correlation concerning the s/p ratios in three of the 16 herds emphasises the meaning of external factors on the levels of maternal antibody titres in piglets,[53] as insufficient colostrum intake of single piglets (especially the smaller ones) in large litters[57] or reduced colostrum/milk production, for example, in the presence of postpartum dysgalactia syndrome in sows. Unfortunately, information about postpartal diseases in sows or litter size was not gathered in this study.

Although most PCR analyses gave negative results, and no relationship between *M. hyopneumoniae* colonisation of sows and piglets could be detected, the still existing statistical significance of gilts having a 10.9 times higher chance to be *M. hyopneumoniae* positive by LS than older sows highlights the importance of parity in the vertical transmission of *M. hyopneumoniae*. This is in line with previous findings, that bacterial shedding seems to be higher in gilts and young sows than in older parity sows.[38] All together, this emphasises the role of gilts in the infection process and the need for an appropriate gilt acclimatisation programme.[18,45] Fitting to that, herds vaccinating their sows against *M. hyopneumoniae*, either during gilts acclimatisation or at regular intervals before farrowing, tended ($p = 0.059$) to have fewer positive OFs in growing and fattening units than herds only vaccinating piglets. This is not surprising, since vaccination of sows can reduce the bacterial load and thus reduce vertical transmission.[58] Consistently, vaccination of sows before farrowing leads to fewer positive piglets at weaning than only vaccination of piglets[59] and to less EP lesions at slaughterhouse.[20]

In addition, other potential factors influencing the transmission of *M. hyopneumoniae* from sows to their piglets were investigated in further studies. Thereby, the duration of the suckling period turned out to be an important parameter for colonisation at weaning.[18,43] We also detected significantly higher EP indices in herds with 28 days than with 21 days of suckling period, which reinforces the impact of weaning age and the position of dams as initiators for *M. hyopneumoniae* spreading, but we were not able to show this based on TBS-PCR results, which may be interpreted as a weakness of this detection method. However, although the use of OFs to detect *M. hyopneumoniae* is not considered very reliable, we detected a significantly larger number of positive OFs in rearing and fattening pigs if the suckling period lasted 28 instead of 21 days. Hernandez–Garcia and others[60] suggest a limited sensitivity of OFs for the detection of *M. hyopneumoniae*, as PCR of OFs occurred to be negative in pig groups that were previously *M. hyopneumoniae* positive by OFs. Due to performing of a cross-sectional study with one single farm visit without following up the same animals at different time points, this observation could not be confirmed in this study. Also, Pieters and others[36] identified OFs as irregular indicators of *M. hyopneumoniae* during early stages of infection. Nevertheless, due to the slow spread of *M. hyopneumoniae*,[61,62] clinical signs occur primarily in late growing and finishing pigs[63] and according to this infection dynamics, we detected significantly more *M. hyopneumoniae* positive OFs in pigs of 20 weeks of age than in younger ones. Additionally, high EP scores at slaughter correlated with low

ct-values in OFs. As the oral cavity is not the natural habitat of the infectious agent, it is conclusive that OFs are less suitable for the detection of early stages of *M. hyopneumoniae* infections. Nevertheless, they may be useful for detecting *M. hyopneumoniae* when clinical signs already occur (mainly growers and finishers), as it requires an active action of the host, such as coughing, to transport the pathogen from the lower respiratory tract into the oral cavity.[64] Consequently, using OFs might be a practical addition in diagnostics of *M. hyopneumoniae* infections, if coughing appears simultaneously. Additionally, the use of a larger number of cotton ropes in different age groups seems to be necessary to be able to make statements about pathogen circulation, especially because of observed inconsistencies in proof of *M. hyopneumoniae* in OFs from cotton ropes within one batch.

In conclusion, infection of piglets with *M. hyopneumoniae* was low in sampled farms. Nevertheless, gilts could be detected as most frequent shedders, highlighting their role in maintaining *M. hyopneumoniae* infections in the herd. In consistency with the predominant occurrence of coughing in fattening pigs in case of *M. hyopneumoniae* infections, the infectious agent was more frequently detected at late sampling time points in OFs. Therefore, the use of OFs in case of occurrence of clinical signs might be suitable as a practical sampling method at the end of fattening for surveillance purposes and genomic analyses of *M. hyopneumoniae* and should be further evaluated in future investigations.

ACKNOWLEDGEMENT

We would like to thank all the persons involved in the study, especially the farmers and the local veterinarians for their support.

Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest. Roman Krejci and Vojislav Cvjetković are the employees of Ceva Santé Animale and Ceva Tiergesundheit, respectively. Roman Krejci and Vojislav Cvjetkovic were not involved in the statistical analysis.

ETHICS STATEMENT

The study has been approved by the governmental authorities of each federal state involved. Table S1 in the supporting material contains a listing of all federal states involved, their responsible authorities and the corresponding reference numbers used to register the study.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: *study conception and design*: Matthias Eddicks, Roman Krejci and Vojislav Cvjetković. *Study coordination*: Pauline Deffner, Mathias Ritzmann, Vojislav Cvjetković and Wolfgang Sipos. *Data collection*: Pauline Deffner, Roland Maurer, Vojislav Cvjetković and Matthias Eddicks. *Analysis and interpretation of results*: Pauline Deffner and Matthias Eddicks. *Writing*: Pauline Deffner and Matthias Eddicks. *Editing*: Matthias Eddicks, Roland Maurer, Mathias Ritzmann, Wolfgang Sipos, Vojislav Cvjetković and Roman Krejci. *Supervision*: Matthias Eddicks and Mathias Ritzmann. All authors reviewed the results and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Thacker EL, Thacker BJ, Janke BH. Interaction between Mycoplasma hyopneumoniae and swine influenza virus. J Clin Microbiol. 2001;39;2525–30.
2. Opriessnig T, Giménez-Lirola LG, Halbur PG. Polymicrobial respiratory disease in pigs. Anim Health Res Rev. 2011;12;133–48.
3. Pagot E, Pommier P, Keïta A. Relationship between growth during the fattening period and lung lesions at slaughter in swine. Rev Med Vet. 2007;158;253–9.
4. Ferraz MES, Almeida HMS, Storino GY, Sonálio K, Souza MR, Moura CAA, et al. Lung consolidation caused by Mycoplasma hyopneumoniae has a negative effect on productive performance and economic revenue in finishing pigs. Prev Vet Med. 2020;182;105091.
5. Sibila M, Pieters M, Molitor T, Maes D, Haesebrouck F, Segalés J. Current perspectives on the diagnosis and epidemiology of Mycoplasma hyopneumoniae infection. Vet J. 2009;181;221–31.
6. Otake S, Dee S, Corzo C, Oliveira S, Deen J. Long-distance airborne transport of infectious PRRSV and Mycoplasma hyopneumoniae from a swine population infected with multiple viral variants. Vet Microbiol. 2010;145;198–208.
7. Maes D, Deluyker H, Verdonck M, Castryck F, Miry C, Vrijens B, et al. Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds. Vet Res. 2000;31;313–37.
8. Marois C, Le Carrou J, Kobisch M, Gautier-Bouchardon AV. Isolation of Mycoplasma hyopneumoniae from different sampling sites in experimentally infected and contact SPF piglets. Vet Microbiol. 2007;120;96–104.
9. Fano E, Pijoan C, Dee S. Dynamics and persistence of Mycoplasma hyopneumoniae infection in pigs. Can J Vet Res. 2005;69;223–8.
10. Nathues H, Woeste H, Doehring S, Fahrion AS, Doherr MG, Beilage EG. Herd specific risk factors for Mycoplasma hyopneumoniae infections in suckling pigs at the age of weaning. Acta Vet Scand. 2013;55;30–42.
11. Clark L, Scheidt A, Armstrong C. The effect of all-in/all-out management on pigs from a herd with enzootic pneumonia. Vet Med. 1991;86:946–8.
12. Amass S, Baysinger A. Swine disease transmission and prevention. In: Straw B, Zimmerman J, D'allaire S, Taylor D, editors. Diseases of swine. Iowa: Blackwell Pub.; 2006. p. 1075–98.
13. Pointon AM, Heap P, Mccloud P. Enzootic pneumonia of pigs in South Australia–factors relating to incidence of disease. Aust Vet J. 1985;62;98–101.
14. Calsamiglia M, Collins JE, Pijoan C. Correlation between the presence of enzootic pneumonia lesions and detection of Mycoplasma hyopneumoniae in bronchial swabs by PCR. Vet Microbiol. 2000;76;299–303.
15. Sibila M, Nofrarias M, Lopezsoria S, Segales J, Valero O, Espinal A, et al. Chronological study of Mycoplasma hyopneumoniae infection, seroconversion and associated lung lesions in vaccinated and non-vaccinated pigs. Vet Microbiol. 2007;122;97–107.

16. Calsamiglia M, Pijoan C. Colonisation state and colostral immunity to Mycoplasma hyopneumoniae of different parity sows. Vet Rec. 2000;146;530–2.
17. Fano E, Pijoan C, Dee S, Deen J. Effect of Mycoplasma hyopneumoniae colonization at weaning on disease severity in growing pigs. Can J Vet Res. 2007;71;195–200.
18. Pieters M, Cline GS, Payne BJ, Prado C, Ertl JR, Rendahl AK. Intra-farm risk factors for Mycoplasma hyopneumoniae colonization at weaning age. Vet Microbiol. 2014;172;575–80.
19. Nathues H, Chang YM, Wieland B, Rechter G, Spergser J, Rosengarten R, et al. 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. 2012;61:316–28.
20. Sibila M, Bernal R, Torrents D, Riera P, Llopart D, Calsamiglia M, et al. Effect of sow vaccination against Mycoplasma hyopneumoniae on sow and piglet colonization and seroconversion, and pig lung lesions at slaughter. Vet Microbiol. 2008;127;165–70.
21. Calsamiglia M, Pijoan C, Trigo A. Application of a nested polymerase chain reaction assay to detect Mycoplasma hyopneumoniae from nasal swabs. J Vet Diagn Invest. 1999;11;246–51.
22. Strait EL, Madsen ML, Minion FC, Christopher-Hennings J, Dammen M, Jones KR, et al. Real-time PCR assays to address genetic diversity among strains of Mycoplasma hyopneumoniae. J Clin Microbiol. 2008;46;2491–8.
23. Vicca J, Stakenborg T, Maes D, Butaye P, Peeters J, De Kruif A, et al. Evaluation of virulence of Mycoplasma hyopneumoniae field isolates. Vet Microbiol. 2003;97;177–90.
24. Stakenborg T, Vicca Jo, Butaye P, Maes D, Peeters J, de Kruif A, et al. The diversity of Mycoplasma hyopneumoniae within and between herds using pulsed-field gel electrophoresis. Vet Microbiol. 2005;109;29–36.
25. Charlebois A, Marois-Créhan C, Hélie P, Gagnon CA, Gottschalk M, Archambault M. Genetic diversity of Mycoplasma hyopneumoniae isolates of abattoir pigs. Vet Microbiol. 2014;168;348–56.
26. Mayor D, Zeeh F, Frey J, Kuhnert P. Diversity of Mycoplasma hyopneumoniae in pig farms revealed by direct molecular typing of clinical material. Vet Res. 2007;38;391–8.
27. Michiels A, Vranckx K, Piepers S, Del Pozo Sacristán R, Arsenakis I, Boyen F, et al. Impact of diversity of Mycoplasma hyopneumoniae strains on lung lesions in slaughter pigs. Vet Res. 2017;48;1–14.
28. Fablet C, Marois C, Kobisch M, Madec F, Rose N. Estimation of the sensitivity of four sampling methods for Mycoplasma hyopneumoniae detection in live pigs using a Bayesian approach. Vet Microbiol. 2010;143;238–45.
29. Kurth KT, Hsu T, Snook ER, Thacker EL, Thacker BJ, Minion FC. Use of a Mycoplasma hyopneumoniae nested polymerase chain reaction test to determine the optimal sampling sites in swine. J Vet Diagn Invest. 2002;14;463–9.
30. Marois C, Dory D, Fablet C, Madec F, Kobisch M. Development of a quantitative real-time TaqMan PCR assay for determination of the minimal dose of Mycoplasma hyopneumoniae strain 116 required to induce pneumonia in SPF pigs. J Appl Microbiol. 2010;108;1523–33.
31. Rawal G, Arruda P, Rademacher C. General overview of the detection of Mycoplasma hyopneumoniae DNA by quantitative polymerase chain reaction in diagnostic cases submitted to the Iowa State University Veterinary Diagnostic Laboratory from 2004 to 2016. J Swine Health Prod. 2018;26;309–15.
32. Blanchard B, Vena MM, Cavalier A, Lannic JLe, Gouranton J, Kobisch M. Electron microscopic observation of the respiratory tract of SPF piglets inoculated with Mycoplasma hyopneumoniae. Vet Microbiol. 1992;30;329–41.
33. Moiso N, Pieters M, Degano F, Vissio C, Camacho P, Estanguet A, et al. Detection of Mycoplasma hyopneumoniae in nasal and laryngeal swab specimens in endemically infected pig herds. Vet Rec. 2020;186;27.
34. Sponheim A, Alvarez J, Fano E, Schmaling E, Dee S, Hanson D, et al. Comparison of the sensitivity of laryngeal swabs and deep tracheal catheters for detection of Mycoplasma hyopneumoniae in experimentally and naturally infected pigs early and late after infection. Vet Microbiol. 2020;241;108500.
35. Betlach AM, Valeris-Chacin R, Singer RS, Allerson M, Pieters M. Natural transmission and detection of Mycoplasma hyopneumoniae in a naïve gilt population. Vet Microbiol. 2020;248;108819.
36. Pieters M, Daniels J, Rovira A. Comparison of sample types and diagnostic methods for in vivo detection of Mycoplasma hyopneumoniae during early stages of infection. Vet Microbiol. 2017;203;103–9.
37. Bjustrom-Kraft J, Christopher-Hennings J, Daly R. The use of oral fluid diagnostics in swine medicine. J Swine Health Prod. 2018;26;262–9.
38. Boonsoongnern A, Jirawattanapong P, Lertwatcharasarakul P, Phatthanakunanan S, Poolperm P, Urairong S, et al. The Prevalence of Mycoplasma hyopneumoniae in commercial suckling pigs in Thailand. World J Vaccines. 2012;2;161–3.
39. Fano E, Pijoan C, Dee S. 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 Congress; 16–19 July 2006, Copenhagen, Denmark.
40. Prickett JR, Kim W, Simer R. Oral-fluid samples for surveillance of commercial growing pigs for porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections. J Swine Health Prod. 2008;16;86–91.
41. Cvjetković V, Sipos S, Szabó I, Sipos W. Clinical efficacy of two vaccination strategies against Mycoplasma hyopneumoniae in a pig herd suffering from respiratory disease. Porcine Health Manag. 2018;4;19.
42. Sibila M, Nofrarías M, López-Soria S, Segalés J, Riera P, Llopart D, et al. Exploratory field study on Mycoplasma hyopneumoniae infection in suckling pigs. Vet Microbiol. 2007;121;352–6.
43. Nathues H, Doehring S, Woeste H, Fahrion AS, Doherr MG, Grosse Beilage E. Individual risk factors for Mycoplasma hyopneumoniae infections in suckling pigs at the age of weaning. Acta Vet Scand. 2013;55;44–55.
44. Villarreal I, Vranckx K, Duchateau L, Pasmans F, Haesebrouck F, Jensen JC, et al. Early Mycoplasma hyopneumoniae infections in European suckling pigs in herds with respiratory problems: detection rate and risk factors. Vet Med (Praha). 2010;55;318–24.
45. Moorkamp L, Hewicker-Trautwein M, Grosse Beilage E. Occurrence of Mycoplasma hyopneumoniae in coughing piglets (3–6 weeks of age) from 50 herds with a history of endemic respiratory disease. Transbound Emerg Dis. 2009;56;54–6.
46. Fablet C, Marois-Créhan C, Simon G, Grasland B, Jestin A, Kobisch M, et al. Infectious agents associated with respiratory diseases in 125 farrow-to-finish pig herds: a cross-sectional study. Vet Microbiol. 2012;157;152–63.
47. Vangroenweghe F, Karriker L, Main R, Christianson E, Marsteller T, Hammen K, et al. Assessment of litter prevalence of Mycoplasma hyopneumoniae in preweaned piglets utilizing an antemortem tracheobronchial mucus collection technique and a real-time polymerase chain reaction assay. J Vet Diagn Invest. 2015;27;606–10.
48. Maes D, Sibila M, Kuhnert P, Segalés J, Haesebrouck F, Pieters M. Update on Mycoplasma hyopneumoniae infections in pigs: knowledge gaps for improved disease control. Transbound Emerg Dis. 2018;65;110–24.
49. Fablet C, Marois C, Dorenlor V, Eono F, Eveno E, Jolly JP, et al. Bacterial pathogens associated with lung lesions in slaughter pigs from 125 herds. Res Vet Sci. 2012;93;627–30.
50. Vangroenweghe FACJ, Labarque GG, Piepers S, Strutzberg-Minder K, Maes D. Mycoplasma hyopneumoniae infections in peri-weaned and post-weaned pigs in Belgium and The Netherlands: prevalence and associations with climatic conditions. Vet J. 2015;205;93–7.
51. Sipos W, Dobrokes B, Meppiel L, Sailer J, Friedmann U, Cvjetković V. Association of lung score findings from slaughter pigs with their vaccination status against Mycoplasma hyopneumoniae and PCV2. Berl Munch Tierarztl Wochenschr. 2020;133;1–5.

52. Grosse Beilage E, Nathues H, Grummer B, Hartung J, Kamphues J, Kietzmann M, et al. Diagnostik, Prophylaxe und Therapie von Atemwegserkrankungen in Schweinebeständen. In: Grosse Beilage E, Wendt M, editors. Diagnostik und Gesundheitsmanagement im Schweinebestand. Stuttgart: Eugen Ulmer; 2013. p. 200–70.
53. Morris CR, Gardner IA, Hietala SK, Carpenter TE, Anderson RJ, Parker KM. Persistence of passively acquired antibodies to Mycoplasma hyopneumoniae in a swine herd. Prev Vet Med. 1994;21;29–41.
54. Arsenakis I, Michiels A, Schagemann G, Gomez-Duran CO, Boyen F, Haesebrouck F, et al. Effects of pre-farrowing sow vaccination against Mycoplasma hyopneumoniae on offspring colonisation and lung lesions. Vet Rec. 2019;184;222–31.
55. Leon EA, Madec F, Taylor NM, Kobisch M. Seroepidemiology of Mycoplasma hyopneumoniae in pigs from farrow-to-finish farms. Vet Microbiol. 2001;78;331–41.
56. Yagihashi T, Kazama S, Tajima M. Seroepidemiology of mycoplasmal pneumonia of swine in Japan as surveyed by an enzyme-linked immunosorbent assay. Vet Microbiol. 1993;34;155–66.
57. Rautiainen E, Wallgren P. Aspects of the transmission of protection against Mycoplasma hyopneumoniae from sow to offspring. J Vet Med B. 2001;48;55–65.
58. Takeuti KL, De Barcellos DESN, De Lara AC, Kunrath CF, Pieters M. Detection of Mycoplasma hyopneumoniae in naturally infected gilts over time. Vet Microbiol. 2017;203; 215–20.
59. Ruiz A, Utrera V, Pijoan C. Effect of Mycoplasma hyopneumoniae sow vaccination on piglet colonization at weaning. J Swine Health Prod. 2003;11;131–5.
60. Hernandez-Garcia J, Robben N, Magnée D, Eley T, Dennis I, Kayes SM, et al. The use of oral fluids to monitor key pathogens in porcine respiratory disease complex. Porcine Health Manag. 2017;3;7–19.
61. Meyns T, Dewulf J, De Kruif A, Calus D, Haesebrouck F, Maes D Comparison of transmission of Mycoplasma hyopneumoniae in vaccinated and non-vaccinated populations. Vaccine 2006;24;7081–6.
62. Villarreal I, Meyns T, Dewulf J, Vranckx K, Calus D, Pasmans F, et al. The effect of vaccination on the transmission of Mycoplasma hyopneumoniae in pigs under field conditions. Vet J. 2011;188;48–52.
63. Pieters M, Maes D. Mycoplasmosis. In: Zimmerman JJ, Karriker L, Ramirez A, Schwartz K, Stevenson G, Zhang J, editors. Diseases of swine. Hoboken, NJ: John Wiley & Sons; 2019. p. 863–83.
64. Hare R. The transmission of respiratory infections. Proc R Soc Med. 1964;57;221–30.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Deffner P, Maurer R, Cvjetković V, Sipos W, Krejci R, Ritzmann M, et al. Cross-sectional study on the in-herd prevalence of *Mycoplasma hyopneumoniae* at different stages of pig production. Vet Rec. 2022;e1317. https://doi.org/10.1002/vetr.1317