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Mycoplasma hyopneumoniae infections in peri-weaned and post-weaned pigs in Belgium and The Netherlands: Prevalence and associations with climatic conditions

F.A.C.J. Vangroenweghe [a,*], G.G. Labarque [b], S. Piepers [c], K. Strutzberg-Minder [d], D. Maes [c]

[a] *Elanco Animal Health Benelux, Antwerp, Belgium*
[b] *Elanco Animal Health France, Neuilly sur Seine, France*
[c] *Faculty of Veterinary Medicine, Department of Reproduction, Obstetrics, and Herd Health, Ghent University, Merelbeke, Belgium*
[d] *IVD, Hannover, Germany*

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ABSTRACT

Mycoplasma hyopneumoniae (*M. hyo*) is an important pathogen in modern intensive pig farming in Europe. The objectives of the present study were (1) to use the tracheobronchial swab (TBS) technique to obtain data on the distribution of *M. hyo* infections in recently weaned pigs in Belgium and The Netherlands, and (2) to look for associations between infection prevalence and specific climatic conditions. One hundred and seventy-six pig herds were randomly selected and 30 piglets sampled on each farm: 18 at 3–5 weeks of age and 12 at 6–11 weeks. Mucus collected from the tracheobronchial bifurcation and suspended in saline was subjected to PCR analysis for *M. hyo*.

In 27% of herds ($n = 44$) at least one piglet tested positive for *M. hyo* at 3–5 weeks of age, and 29% ($n = 47$) at 6–11 weeks of age. The individual animal prevalence at the two ages was 7.1% and 10.9%, respectively. The probability of 3–5 week old piglets being *M. hyo*-positive was negatively associated with the precipitation rate (odds ratio [OR] = 0.971) during the week preceding the sampling. In the older post-weaning group, the odds of being *M. hyo*-positive at piglet level were significantly affected by season (OR of detection during autumn compared to summer 20.9). Thus, under Belgian and Dutch field conditions, piglets may be infected with *M. hyo* very early in life, with prevalence increasing further during the post-weaning period.

Introduction

Mycoplasma hyopneumoniae (*M. hyo*), one of the main pathogens associated with enzootic pneumonia and the porcine respiratory disease complex (PRDC), is an important pathogen in modern intensive pig farming in Europe (Maes et al., 2008; Sibila et al., 2009). Economic losses associated with mycoplasmal infections are related to a chronic, non-productive cough, reduced growth rate, poorer feed conversion, increased medication use and a higher susceptibility to secondary pathogens such as *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* (Sibila et al., 2009).

Diagnosis of mycoplasmal infections can be undertaken using a variety of approaches (Sibila et al., 2009), including clinical signs, slaughterhouse checks of affected lungs (Fraile et al., 2010; Meyns et al., 2011), serological examination of relevant animal groups (Fraile et al., 2010; Meyns et al., 2011), direct identification of the pathogen through bacteriological culture (Marois et al., 2007) or PCR techniques (Calsamiglia et al., 1999; Marois et al., 2010). Various sampling sites have been used, including nasal swabs (Calsamiglia and Pijoan, 2000; Fano et al., 2007; Villarreal et al., 2010), tonsil scrapings (Fablet et al., 2010) and bronchoalveolar lavage (BAL) fluids (Meyns et al., 2004, 2006; Fablet et al., 2010; Villarreal et al., 2011; Vranckx et al., 2012). Recently, the tracheobronchial swab (TBS) technique has been developed and validated for use in pigs (Fablet et al., 2010).

Numerous studies, using various sampling sites, have shown that suckling piglets can be infected by their dam (Calsamiglia and Pijoan, 2000; Fano et al., 2007; Sibila et al., 2007a; Nathues et al., 2010; Villarreal et al., 2010; Fablet et al., 2012; Segalés et al., 2012) and that further spread of infection occurs after weaning (Meyns et al., 2004, 2006; Villarreal et al., 2011). In Belgium and The Netherlands, limited data are available on the prevalence of *M. hyo* around the time of weaning and during the immediate post-weaning period. As part of a larger study, Villarreal et al. (2010) studied *M. hyo* prevalence in six herds from Belgium and six from The Netherlands, with coughing in grower-finisher pigs (a clinical sign associated with *M. hyo*). In Belgium 4/6 herds and 3.3% of tested piglets were positive whereas in The Netherlands the figures were 5/6 herds and

* Corresponding author. Tel.: +32 477 558 562.
E-mail address: vangroenweghefr@elanco.com (F.A.C.J. Vangroenweghe).

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7.8% of piglets. Vranckx et al. (2012) studied four pig herds with known respiratory problems due to early infection (before week 15) with *M. hyo*; in these herds the percentage of pigs testing positive on qPCR increased from 35% at 6 weeks to 96% at 26 weeks of age.

In enzootic pneumonia studies, the risk of infection with *M. hyo* has been shown to be associated with several risk factors, such as distance to non-SPF (specific pathogen free) herds, herd size, density of pig population in the specific area (Stärk et al., 1992), specific season for start of growing cycle, presence of breeding stock in the herd, lack of an *M. hyo* vaccination programme (Ostanello et al., 2007), period of the year (temperature and sunlight) (Dee et al., 2010) and climatological parameters (average daily temperature and average daily rainfall) (Segalés et al., 2012). A recent study has shown that rainfall and temperature may have a significant impact on *M. hyo* infection dynamics (Segalés et al., 2012). In that investigation, the weekly precipitation rate had a positive association (odds ratio [OR] = 1.31) with the probability of being *M. hyo*-positive (nPCR on nasal swabs) at a pig level, while weekly temperature had a negative association (OR = 0.89) with the probability of being *M. hyo*-negative (Segalés et al., 2012).

The present study was designed to investigate the association between the prevalence of early *M. hyo* infections (as detected using TBS) in Belgian and Dutch pig herds (without clinical respiratory disease) and climatic conditions across the year.

Materials and methods

Selection of study herds

The study was conducted in Belgium and The Netherlands between April 2011 and March 2012. Closed pig herds were randomly selected through local veterinary practices in both countries. The inclusion criteria of the study herds were at least 200 sows in the herd, preferably two age groups in nursery pigs (3–5 weeks of age and 6–11 weeks) in each herd, no specific clinical signs of respiratory disease (such as coughing or sneezing), observed for at least 4 months before sampling in all age categories (peri-weaned, post-weaned and fattening pigs where present) and no use of antimicrobials active against *M. hyo* in piglets <3 weeks of age or during the post-weaning period.

Batch-management systems were used in many of the herds, varying from 2-week to 5-week systems. Each herd operated one specific batch-management system with the week number (2–3–4–5) indicating the interval between weaning groups. Vaccination status against *M. hyo* was not taken into account as a selection criterion. However, in many herds (80% in Belgium, 35% in The Netherlands) piglets were vaccinated between 1 and 3 weeks of age using a *M. hyopneumoniae* bacterin.

In total, 176 closed pig herds were included in the study, consisting of 73 Belgian and 103 Dutch herds. The timing of sampling of each farm across the year is given in Table 1. A standard sampling protocol of 30 pigs per herd was applied (based on 18 in the peri-weaning period at 3–5 weeks of age, and 12 in the later post-weaning period; i.e. 6–11 weeks of age). In 13 farms, piglets of 3–5 weeks of age were not available for sampling, whereas in 14 other farms, piglets of 6–11 weeks of age were not available, both due to the specific batch-management system or multi-site production. The piglets within each herd were selected randomly from as many different pens in the nursery as possible. Sampling was always performed by the same trained veterinarian.

Tracheobronchial swab sampling procedure

TBS samples were obtained following restraint of the piglets with a nose snare, and subsequent use of a mouth opener. The aspiration tube used (CH12 × 50 cm, Medinorm) was inserted through the mouth and glottis down to the tracheobronchial bifurcation where mucus was collected through gentle swab movement. The tip of the swab was collected in a sterile 10 mL polystyrene tube (MLS), mixed with 1 mL sterile saline and kept at 3–5 °C until analysis within 48 h of sampling.

Table 1
Distribution of herds during subsequent seasons of the year, and between Belgium and The Netherlands.

Season	Belgium	The Netherlands	Total
S1	14	33	47
S2	21	27	48
S3	6	19	25
S4	31	25	56
Total	*73*	*103*	*176*

S1, winter; S2, spring; S3, summer and S4, autumn.

Analysis of tracheobronchial swabs

The material collected by TBS was processed in an *M. hyo* p183 real-time-PCR (Strait et al., 2008). Briefly, nucleic acid was extracted from TBS using an RNA/DNA isolation kit (MagMAX Pathogen RNA/DNA Kit, Life Technologies) and an automated nucleic acid isolation processor (MagMAX Express 96 processor, Life Technologies) based on magnetic bead technology. One microlitre of TBS was centrifuged for 5 min at 16,000 *g*, the pellet suspended in 400 μL lysis buffer, and 400 μL of the suspension was used as the sample. If no pellet was observable, 300 μL of the TBS was used as the sample. Bead mix and lysis/binding solution were added and the mix transferred onto a 96-well plate in the processor. Nucleic acid isolation was performed according to the manufacturer's instructions.

The PCR results were reported as negative or positive for the presence of *M. hyo*. The detection limit for *M. hyo* reported by Strait et al. (2008) was from 10 ng/μL to 2.5 fg/μL. The detection limit for the PCR was validated for TBS spiked with dilutions of *M. hyo* strain J of at least 5 fg/μL.

Data categorization for seasonality

In order to assess associations between climatic parameters and *M. hyo* infection, herds were categorized into four groups based on the sampling season. Sampling was undertaken at the following rates in each season: S1 (winter), 47 herds, 1422 piglets; S2 (spring), 48 herds, 1334 piglets; S3 (summer), 25 herds, 723 piglets; and S4 (autumn), 56 herds, 1809 piglets.

Climatic data

Meteorological information with air temperatures including minimums and maximums (°C; T, T_{max} and T_{min}, respectively), relative humidity (percentage; RH), rainfall (L/m^2; P), wind direction (°; WD) and wind speed (m/s; WS) were recorded daily from April 2011 to March 2012 based on data from the local meteorological institute (KNMI, Koninklijk Nederlands Meteorologisch Instituut[1]) collected at a recording location (Valkenswaard, The Netherlands) central for the entire sampling area in both countries. In addition, a rolling average of the data from the last 1, 4 and 10 weeks before the day of sampling was calculated for all sampling days throughout the study period.

Piglet and farm prevalences and within-herd prevalence

Piglet and herd *M. hyo* prevalences were calculated both overall for the entire study period and per season (S1–S4). A farm was considered *M. hyo*-positive if at least one of the piglets sampled in the specified age category was positive for *M. hyo*. The within-herd prevalence was calculated as the percentage of *M. hyo*-positive herds per *M. hyo*-prevalence category, with categories assigned as follows: 0%, 1–10%, 11–20%, etc.

Statistical analysis

Logistic mixed regression models using first-order penalized quasi-likelihood algorithms were developed in MlWiN 2.02 (Centre of Multilevel Modeling). Herd was included as a random effect to correct for clustering of piglets within a herd. Initially, univariable associations were tested between the binary outcome variables: (1) presence of *M. hyo* at the piglet level at 3–5 weeks (0 = negative for *M. hyo* at 3–5 weeks; 1 = positive for *M. hyo* at 3–5 weeks) and (2) presence of *M. hyo* at the piglet level at 6–11 weeks (0 = negative for *M. hyo* at 6–11 weeks; 1 = positive for *M. hyo* at 6–11 weeks), and the independent variables season or the different climate parameters measured on the day of sampling (T, T_{min}, T_{max}, WD, WS, RH, and P), over the last week (w) (T_1w, T_{max}_1w, T_{min}_1w, WD_1w, WS_1w, RH_1w, and P_1w), the last 4 weeks (T_4w, T_{max}_4w, T_{min}_4w, WD_4w, WS_4w, RH_4w, and P_4w) and the last 10 weeks (T_10w, T_{max}_10w, T_{min}_10w, WD_10w, WS_10w, RH_10w, and P_10w) before sampling.

Statistical significance in this step was assessed at $P < 0.20$. Spearman correlation coefficients were calculated for the significant independent variables to avoid multicollinearity. If two independent variables had a correlation coefficient ≥ 0.6, only one was selected for further analysis based on biological relevance. In the third step, separate multivariate models were fitted for the two dependent variables.

[1] See: http://www.knmi.nl/klimatologie (accessed 16 March 2015).