



# Effects of pre-farrowing sow vaccination against *Mycoplasma hyopneumoniae* on offspring colonisation and lung lesions

Ioannis Arsenakis,<sup>1</sup> Annelies Michiels,<sup>1</sup> Gabriele Schagemann,<sup>2</sup> Charles Oliver Gomez-Duran,<sup>2</sup> Filip Boyen,<sup>3</sup> Freddy Haesebrouck,<sup>3</sup> Dominiek G D Maes<sup>1</sup>

This study investigated *Mycoplasma hyopneumoniae* colonisation and lung lesions at slaughter in pigs from vaccinated (V) and non-vaccinated (NV) sows, in two herds (A and B). In each herd, two sow batches were V against *M. hyopneumoniae* with a commercial bacterin at six and three weeks before farrowing and two sow batches remained NV. From each sow batch, laryngeal swabs were collected from the litters of five primiparous sows at weaning and seven days post-weaning. All samples were tested for *M. hyopneumoniae* by nested PCR. In total, 488 piglets were sampled. At slaughter, the extent of *Mycoplasma*-like pneumonia lesions (lung lesion score (LLS)) was assessed. The colonisation rates with *M. hyopneumoniae* at weaning and seven days post-weaning were (V-A=14.2, NV-A=20.0 (P=0.225); V-B=0.9, NV-B=0.8 (P=0.948)) and (V-A=0.8, NV-A=7.0 (P=0.039); V-B=1.8, NV-B=2.5 (P=0.738)), respectively. The average LLS (in per cent) was V-A=15.5, NV-A=26.4 (P=0.021); V-B=9.7, NV-B=8.4 (P=0.541). In conclusion, in herd A, with a substantially higher level of piglet colonisation at weaning than herd B, offspring from V sows had a significantly lower colonisation rate seven days post-weaning and a significantly lower LLS at slaughter compared with the offspring of the NV sows. This implies that sow vaccination might be useful for control of *M. hyopneumoniae* infections, although significant results may not be achieved at all times (such as in herd B).

## Introduction

*Mycoplasma hyopneumoniae* is the causative agent of enzootic pneumonia (EP) and one of the most prevalent and important agents associated with the porcine respiratory disease complex.<sup>1</sup> Infections with *M. hyopneumoniae* occur in almost all swine-producing areas around the world, causing major economic losses to the swine industry.<sup>2</sup> Vaccination is one of the ways to control *M. hyopneumoniae* infections in pigs.<sup>2</sup> The most common vaccination strategy in practice is vaccination

of the piglets during suckling or at weaning. Single vaccination or double vaccination strategies are used, and in general vaccination results in significant improvements of clinical signs and lung lesions, lower performance losses and less antimicrobial use for treating *M. hyopneumoniae* infections.<sup>3</sup>

Concerning the breeding sow population, in some herds the gilts are vaccinated against *M. hyopneumoniae* during the quarantine period upon purchase, before moving them to the sow breeding facilities.<sup>4</sup> This practice is part of the gilt acclimatisation protocols applied in *M. hyopneumoniae*-positive herds. It aims to avoid the destabilisation of the breeding stock immunity by decreasing the bacterial load and the clinical signs in the vaccinated gilts.<sup>5,6</sup> Vaccination of gestating sows against *M. hyopneumoniae* is not frequently practised under field conditions.<sup>7</sup> Nevertheless, breeding sows are responsible for maintaining *M. hyopneumoniae* infections within the herds<sup>8</sup> and the percentage of piglets colonised with *M. hyopneumoniae* at weaning may be indicative of the number of sows shedding the pathogen during the suckling period.<sup>9</sup> Additionally, it has been shown that low parity sows are more likely to

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<sup>1</sup>Unit Porcine Health Management, Department of Reproduction, Obstetrics & Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

<sup>2</sup>Boehringer Ingelheim Animal Health GmbH, Ingelheim, Germany

<sup>3</sup>Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

E-mail for correspondence: ioannis.arsenakis@UGent.be

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transmit the pathogen to their piglets<sup>10 11</sup> and that they shed more *M. hyopneumoniae* organisms<sup>11 12</sup> compared with older sows. Finally, some studies suggest that in some herds the level of piglet colonisation with *M. hyopneumoniae* at weaning could be a predictor of the extent of *Mycoplasma*-like lung lesions at slaughter.<sup>7 13</sup>

Thus, it is interesting to investigate whether vaccinating sows during gestation could decrease the percentage of their offspring that is found to be colonised with *M. hyopneumoniae* at weaning. In addition, it is not known whether this possible beneficial effect can still be observed at later time points, for example, in the nursery units when piglets from different sows are mixed together, or even at slaughter age.

Different sampling methods have been applied for the in vivo detection of *M. hyopneumoniae* by PCR, such as nasal swabs, laryngeal swabs or tracheo-bronchial swabs and lavage.<sup>14 15</sup> Pieters and others<sup>15</sup> demonstrated that laryngeal swabs are superior to the more commonly used nasal swabs and tracheo-bronchial lavage fluid in detecting the pathogen, at least during the early stages of EP. The use of oral fluids is another, very easy way of sampling, which is commonly applied for the diagnosis of other pathogens such as porcine reproductive and respiratory syndrome virus and porcine circovirus type 2.<sup>16</sup> For the detection of *M. hyopneumoniae*, with the exception of the experimental study of Pieters and others<sup>15</sup> and the field study of Hernandez-Garcia and others<sup>17</sup> where a low sensitivity was observed, there are no other studies using oral fluid samples in post-weaned piglets. Therefore, it is interesting to investigate whether under field conditions the detection of *M. hyopneumoniae* in oral fluid samples obtained from post-weaned piglets could be further optimised.

This study was conducted in two Belgian herds having piglets colonised with *M. hyopneumoniae* at weaning. Its objective was to investigate the effect of vaccinating gestating sows on the detection rates of *M. hyopneumoniae* at weaning and seven days after weaning, as well as on the prevalence and extent of *Mycoplasma*-like lung lesions at slaughter. For both sows and pigs, laryngeal swabs constituted the primary diagnostic procedure for detecting *M. hyopneumoniae*. As a secondary diagnostic procedure, oral fluids were used to detect *M. hyopneumoniae* in nursery pens.

## Materials and methods

### Herd description

The study was conducted between November 2015 and August 2016 in two farrow-to-finish herds: one two-site (herd A) and one single-site (herd B). Herd A was managed by all-in/all-out (AIAO) across all production periods and operated a four-week batch production system, while herd B only applied AIAO in the farrowing and the nursery units, and operated a two-week batch production system. The pigs of herd A were weaned at 21 days of age, while those of herd B were weaned at 28 days of age. A description

of both herds, together with the health management practices applied, is presented in [table 1](#).

Both herds were included in the study on the basis of a 10.0 per cent minimum prevalence of colonisation with *M. hyopneumoniae* at weaning. This cut-off point was chosen in order to match as much as possible with the prevalence of early piglet colonisation reported in other studies.<sup>8 18 19</sup> Before the onset of the study, a 10.0 per cent prevalence rate was detected in each of the herds A and B by testing laryngeal swabs from 50 randomly selected pigs per herd. The pigs were sampled on transfer to the nursery units and the swabs were tested for *M. hyopneumoniae* by nested PCR (n-PCR<sup>20</sup>).

### Study population and experimental design

From four consecutive farrowing batches of sows in each herd, two batches were vaccinated (V) against *M. hyopneumoniae* and two remained non-vaccinated (NV). Vaccination was applied in an alternating way, so that one NV batch was followed by a V batch. In the V batches, all gilts and sows received twice a commercial one-dose bacterin (1 ml; Ingelvac MycoFLEX, Boehringer Ingelheim) at six and three weeks before the expected farrowing date. The bacterin was administered intramuscularly and its use was extra-label. In each herd, the different farrowing batches participating in the study were named as follows: batch a (first batch, NV), batch b (second batch, V), batch c (third batch, NV) and batch d (fourth batch, V). There were no obvious clinical adverse effects observed in the vaccinated sows during the period that the study was conducted, such as oedema and/or erythematous skin lesions, reduction of feed intake and vomiting. In addition, neither the numbers of total and liveborn piglets/litter were affected, nor abortions were recorded in each of herds A and B.

The current experimental design of using NV and V batches alternately one after the other was chosen over an experimental design that would have two consecutive NV batches followed by two V batches or *vice versa* in order to account for seasonal variations in the percentage of sows and piglets colonised with *M. hyopneumoniae*, as well as in the prevalence of *Mycoplasma*-like lung lesions at slaughter. In fact, several publications have indicated a seasonal variation in the detection of piglets colonised with *M. hyopneumoniae* either by nPCR<sup>21</sup> or real-time PCR<sup>22</sup> as well as in the prevalence of macroscopic pneumonic lesions at slaughter.<sup>23</sup> An additional reason for using the current experimental design was to reduce as much as possible the contact between the non-vaccinated and vaccinated sows, and their piglets, compared with the more conventional randomised complete block design of having fixed batches, where half of the sows would be non-vaccinated and half vaccinated. In both herds selected in the present study, the decision of whether to begin with a V or a NV batch was made by random order.

On farrowing, from each NV or V batch of sows within each herd, five primiparous sows together with

	Herd A	Herd B
Number of sows	400	600
Breed of sows	Topigs 20	DanBred
Breed of boars (sperm)	Belgian Piétrain	Belgian Piétrain
Vaccination of the sows		
Atrophic rhinitis	Rhiniseng (Hipra) four weeks before farrowing	Porcilis AR-T (MSD) 4 weeks before farrowing
Parvovirus+ <i>Erysipelothrix rhusiopathiae</i>	Eryseng Parvo (Hipra) three weeks before breeding	Porcilis Ery-Parvo (MSD) 2 weeks before breeding
<i>Escherichia coli</i>	Suiseng (Hipra) three weeks before farrowing	Porcilis coli (MSD) 3 weeks before farrowing
PRRS(V)	Ingelvac MLV (Boehringer Ingelheim) eight weeks before farrowing	Ingelvac MLV (Boehringer Ingelheim) applied twice/year
Vaccination of the quarantined gilts		
PRRS(V)	Ingelvac MLV (Boehringer Ingelheim) four weeks after arrival	Ingelvac MLV (Boehringer Ingelheim) 2 weeks after arrival
PCV-2	Ingelvac Circoflex (Boehringer Ingelheim) twice three weeks apart	Ingelvac Circoflex (Boehringer Ingelheim) twice 3 weeks apart
Atrophic rhinitis	Rhiniseng (Hipra) twice four weeks apart	Porcilis AR-T (MSD) twice 6 weeks apart
Management of the piglets		
Days 2–4	Amoxicillin (intramuscular)	Amoxicillin (IM)
	Tail docking	Tail docking
	Castration	Castration
	Iron (Uniferon, Pharmacosmos)	Iron (Ferraject, Eurovet)
	Toltrazuril (Baycox, Bayer)	
Vaccinations	At weaning	N/A
PRRS(V)	Ingelvac MLV (Boehringer Ingelheim)	N/A
Mhvo and PCV-2	Ingelvac FLEXcombo (Boehringer Ingelheim)	N/A
Nursery (days 21–70)		
Medication	Amoxicillin days 21–28 (IF)	Zinc oxide after weaning for 14 days at 2500 ppm (IF)
Facilities	One unit with four compartments	one unit with 12 compartments
	12 pens/compartment	4 to 12 pens/compartment
	40 pigs/pen (0.26 m <sup>2</sup> /pig)	20 to 26 pigs/pen (0.24 to 0.27 m <sup>2</sup> /pig)
Ventilation	Conventional mechanical ventilation	conventional mechanical ventilation
Fattening unit (days 70–220)		
Medication	Flubendazole (days 70–75; IF), repeat every six weeks	Flubendazole (day 84 to 91 and day 110 to 117; IF)
Facilities	three fattening units, each having four compartments	two fattening units, each having 5 compartments
	14 or 16 pens/compartment	8 or 10 pens/compartment
	12 or 15 pigs/pen (0.75 m <sup>2</sup> /pig)	10 or 15 pigs/pen (0.75 m <sup>2</sup> /pig)
Ventilation	Mechanical door ventilation	natural ventilation

IF, in-feed medication; N/A, non-applicable; PRRS(V), porcine reproductive and respiratory syndrome virus; PCV-2, porcine circovirus type 2.

their litters were randomly selected. Primiparous sows were selected in order to increase the likelihood of detecting piglets colonised with *M. hyopneumoniae* at weaning. This decision was based on the fact that Fano and others<sup>11</sup> found higher colonisation rates in suckling piglets from primiparous and parity-two sows than their counterparts from older parity sows. The primiparous sows selected in the current study had at least 10 piglets, and no addition of piglets from other sows to their litters was allowed. Removal of excessive piglets to other sows occurred only in four of the selected primiparous sows ( $n=3$  for herd A and  $n=1$  for herd B), but at least 10 piglets remained with their mother. All piglet removals were applied at once, within the first two to three days after farrowing (no other piglet removals occurred later during the suckling period).

All piglets originating from the selected primiparous sows were ear-tagged at weaning in order to be identified throughout the whole production period until slaughter. In both herds, pigs housed within the same pen belonged to the same farrowing batch. At weaning, when moving pigs to the nursery units, they were partly regrouped according to pen size, weight

and sex, while on transfer to the fattening units, the pigs were regrouped according to pen size and weight. Within each nursery and fattening unit, the ear-tagged pigs were housed in different pens along with the rest of the pigs from the same farrowing batch. In herds A and B, the pigs of the different production batches participating in the study were distributed to three and two different fattening units, respectively (see online supplementary appendix 1). During the study, in both herds, all antimicrobial treatments were applied using  $\beta$ -lactam antibiotics. These antibiotics are not active against *M. hyopneumoniae* as the bacterium lacks a cell wall.

### Sample and data collection

All samplings were conducted by the first author. Laryngeal swabs were collected from the selected primiparous sows and their piglets by the introduction of sterile swabs into the mouth cavity until they reached the larynx, using a mouth gag and a laryngoscope.<sup>15</sup> The swabs used for sows were manufactured by Kruuse (product id: 290955, equivet uterine culture swab, length 760 mm, tip diameter 4 mm, Kruuse A/S,

Denmark), whereas a different type of swabs was used for their piglets (product id: 155C rayon, plain dry swab, length 65 mm, tip diameter 4 mm, Copan Italia SpA). Sows were sampled during the first 24 hours post partum (more specifically, 12–24 hours after the expulsion of the last piglet). Piglets were sampled at weaning (before moving them in the nursery units) and seven days after weaning. In herd A, the piglets were vaccinated against *M. hyopneumoniae* on the day of weaning, after the samplings had been completed.

Oral fluid samples were collected from each pen hosting pigs originating from the selected sows at seven days after weaning. Samplings were done with cotton ropes (Swine oral fluids kit, Tego), using a ratio of one rope for each 25 pigs with a 30-minute exposure.<sup>17</sup> The bottom part of each rope was adjusted so as to match with the average height of the pigs' shoulder joint. Oral fluid samples were collected into individual containers.

Blood samples were collected from the selected primiparous sows and their pigs at 12–24 hours after farrowing and at weaning, respectively. For the pigs originating from the selected litters in each farrowing batch, the extent (total area) of macroscopically visible *Mycoplasma*-like lung lesions (lung lesion score (LLS)) and the prevalence of *Mycoplasma*-like pneumonia lesions and fissures were recorded at slaughter.

### Sample processing and testing

On collection, all laryngeal swabs were immediately cooled at 4.0°C and subsequently stored at –20.0°C until analysis. DNA was extracted using a DNeasy Kit manufactured by Qiagen (Blood and Tissue kit, Belgium) according to the protocol used for buccal swabs. Detection of *M. hyopneumoniae* DNA by n-PCR was performed according to Stärk and others.<sup>20</sup> The n-PCR products were analysed by gel electrophoresis on a 1.5 per cent agarose gel in Tris-Borate-EDTA (TBE) buffer and stained with GelRed (Biotium) with visualisation under UV illumination. Extracted DNA from a pure culture of the virulent *M. hyopneumoniae* F7.2C field strain was used as a positive control.<sup>24</sup>

During sampling, the individual containers with the oral fluid samples were cooled at 4.0°C and stored at –20.0°C until analysis. Oral fluid samples from each pen were pooled after thawing and let to stand for one hour at room temperature. Then, the samples were centrifuged for 10 minutes at 80g and the supernatant was collected. Following the aforementioned centrifugation step, all supernatants were centrifuged for 30 minutes at 13,500g. The collected pellets were then used to perform the DNA extractions using the DNeasy Kit manufactured by Qiagen (Blood and Tissue kit, Belgium) according to the protocol used for the blood or body fluids. Detection of *M. hyopneumoniae* DNA by n-PCR was performed as described for the laryngeal swabs.

All blood samples collected were used to measure antibodies against *M. hyopneumoniae* via a blocking ELISA (IDEIA, *M. hyopneumoniae* EIA Kit, Oxoid, UK) as previously described by Feld and others.<sup>25</sup> This ELISA is based on monoclonal antibodies against a 74-kDa protein of *M. hyopneumoniae*.<sup>25</sup> Inhibition percentages (IP) for all sera were calculated considering the optical density value of each serum sample as well as the negative control according to Sibila and others.<sup>26</sup> Classification of individual sera on the basis of IP values was as follows: IP<30.0 per cent, negative; IP>50.0 per cent, positive; IP≥30.0 per cent and ≤50.0 per cent, equivocal.

At slaughter, the LLS was quantified according to the scoring system described by Morrison and others.<sup>27</sup> Briefly, this LLS expresses the total area (percentage) of the lung tissue affected by pneumonia lesions. An average LLS score was calculated for each production batch based on the LLS of the individual animals. *Mycoplasma*-like pneumonia lesions (catarrhal bronchopneumonia) were defined as red to purplish areas of cranioventral consolidation raised above the surface or at the surface of each lobe and with a liver-like consistency. Chronic *Mycoplasma*-like pneumonia lesions (fissures) were grey to purplish cranioventral scars, shrunken below the surface of the lobes.

### Statistical analysis

With regard to the detection rates of *M. hyopneumoniae* in laryngeal swabs at weaning, the inclusion of a minimum of 55 piglets from the selected primiparous sows per farrowing batch allowed to detect with 95 per cent certainty and 80 per cent statistical power a difference of 10.5 percentage points between pigs of the NV and V sow batches. The inclusion of a minimum of 55 pigs in each farrowing batch allowed to assess a difference of 4.6 points in LLS (sd=12.50) with 95 per cent certainty and 80 per cent statistical power (IBM SPSS Sample Power 3, Illinois, USA). The primary outcome parameters were the detection rates of *M. hyopneumoniae* in the swabs obtained from the selected sows at 12–24 hours post-farrowing as well as the swabs taken from their piglets at weaning and seven days after weaning, and the LLS of those pigs at slaughter. The secondary parameters were the percentage of nursery pens where *M. hyopneumoniae* detection occurred seven days after weaning by oral fluids, and the percentage of sows and piglets showing *M. hyopneumoniae* antibodies at 12–24 hours post-farrowing and weaning, respectively. *M. hyopneumoniae* detection rates in laryngeal swabs were analysed using binary logistic regression with vaccination status (NV or V) and farrowing batch as predictors for the model. Logistic regression was also used to compare between individual farrowing batches the percentage of nursery pens that were positive for *M. hyopneumoniae* by oral fluids as well as the percentage of seropositive sows

**Table 2** Percentage and number (in parentheses) of sows with a *M. hyopneumoniae* nested-PCR-positive laryngeal swab

Herd A								
	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value
12–24 hours post-farrowing	0.0 (0/5)	0.0 (0/5)	20.0 (1/5)	0.0 (0/5)	0.999	10.0 (1/10)	0.0 (0/10)	0.999
Herd B								
	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value
12–24 hours post-farrowing	0.0 (0/5)	40.0 (2/5)	40.0 (2/5)	20.0 (1/5)	0.902	20.0 (2/10)	30.0 (3/10)	0.608

Vaccination status: NV (non-vaccinated sow batches) and V (vaccinated sow batches). The P value refers to the comparisons between the individual sow batches, and the P\* value to the comparisons between the summaries of the NV (a–c) and V (b–d) batches. Differences between batches were not statistically significant (P>0.05).

and piglets. In herd A, the percentage of pigs showing *Mycoplasma*-like pneumonia lesions and fissures between individual farrowing batches were also analysed via logistic regression, including the fattening unit as a predictor for the model.

Fisher's exact test was used to analyse the percentage of nursery pens positive for *M. hyopneumoniae* by oral fluids according to the vaccination status of their piglets. Fisher's exact test was also used to analyse the total percentage of seropositive sows and piglets between the NV and V farrowing batches as well as the total percentage of pigs with *Mycoplasma*-like pneumonia lesions and fissures. *M. hyopneumoniae* detection rates in laryngeal swabs obtained from the piglets at weaning and seven days after weaning as well as the percentage of seropositive piglets at weaning were correlated via Spearman's rank correlation with the LLS of the same pigs at slaughter. Kruskal-Wallis analysis of variance was used to analyse data that did not fulfil the criteria of normality and homogeneity of variances, namely the serology IP and the LLS. For all the above-mentioned analyses, statistical results were considered significant when P values were ≤0.05 (two-sided test). The statistical package SPSS V.23.0 was used to analyse the data.

## Results

### Detection of *M. hyopneumoniae* using n-PCR in laryngeal swabs

In both herds, no statistically significant differences were found in the percentage of the selected n-PCR positive sows at 12–24 hours post-farrowing between the NV and V batches (herd A: P=0.999; herd B: P=0.608, [table 2](#)). The same was observed for the comparisons between individual sow batches (herd A: P=0.999; herd B: P=0.902, [table 2](#)).

Concerning the percentage of n-PCR positive piglets at weaning, in both herds there were no significant differences between the piglets of the selected NV and V sows (herd A: P=0.225; herd B: P=0.948, [table 3](#)). In herd A, significantly more piglets originating from the selected NV sows were found to be n-PCR positive at seven days after weaning compared with those of the selected V sows (P=0.039, [table 3](#)). In herd B, there were no significant differences between the selected NV and V sows in the percentage of n-PCR-positive piglets at seven days after weaning (P=0.738, [table 3](#)).

### Detection of *M. hyopneumoniae* using n-PCR in oral fluids

The percentages of nursery pens that were positive for *M. hyopneumoniae* by oral fluids in herds A and B are presented in [table 4](#). In both herds, no significant differences between the pens hosting piglets of the selected NV and V sows were detected (herd A: P=0.429; herd B: P=1.000).

### Serological investigation

The serological results for *M. hyopneumoniae* are presented in [table 5](#). The NV batches exhibited a significantly lower percentage of sows that were seropositive than the V batches (herd A: P=0.001; herd B: P=0.023). The same trend was observed regarding the differences in the IP values (herd A: P=0.000; herd B: P=0.017). At weaning, a significantly lower percentage of seropositive piglets originated from the selected NV sows compared with the selected V sows (both herds A and B: P=0.000). Similarly, the piglets of the selected NV sows exhibited lower average IP values than those of their V counterparts (both herds A and B: P=0.000).

In both herds, there was no significant difference between the percentages of seropositive and seronegative piglets that were found to be n-PCR

**Table 3** Percentage and number (in parentheses) of piglets with a *M. hyopneumoniae* nested-PCR-positive laryngeal swab

Herd A									
	Age (weeks)	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value
Weaning	3	25.4 (15/59)	14.9 (10/67)	14.7 (9/61)	13.3 (8/60)	0.277	20.0 (24/120)	14.2 (18/127)	0.225
Nursery period	4	12.5 (7/56)	0.0 (0/66)	1.7 (1/59)	1.8 (1/57)	0.088	7.0 (8/115) <sup>A</sup>	0.8 (1/123) <sup>B</sup>	0.039
Herd B									
	Age (weeks)	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value
Weaning	4	0.0 (0/65)	0.0 (0/57)	1.6 (1/61)	1.7 (1/58)	0.997	0.8 (1/126)	0.9 (1/115)	0.948
Nursery period	5	3.1 (2/64)	1.8 (1/55)	1.7 (1/58)	1.8 (1/55)	0.943	2.5 (3/122)	1.8 (2/110)	0.738

Vaccination status: NV (pigs originating from non-vaccinated sow farrowing batches) and V (pigs originating from vaccinated sow farrowing batches). The P value refers to the comparisons between the individual farrowing batches, and the P\* value to the comparisons between the summaries of the NV (a–c) and V (b–d) batches. Values with different superscripts within a row are significantly different (P<0.05).

**Table 4** Percentage and number (in parentheses) of pen-based oral fluid samples positive by nested-PCR for *M. hyopneumoniae*

Herd A									
	Age (weeks)	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value
Nursery period	4	100.0 (2/2)	0.0 (0/2)	0.0 (0/2)	0.0 (0/2)	N/A†	50.0 (2/4)	0.0 (0/4)	0.429
Herd B									
	Age (weeks)	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value
Nursery period	5	0.0 (0/9)	0.0 (0/7)	25.0 (2/8)	12.5 (1/8)	0.941	11.8 (2/17)	6.7 (1/15)	1.000

Vaccination status: NV (pens hosting pigs from non-vaccinated sow farrowing batches) and V (pens hosting pigs from vaccinated sow farrowing batches). The P value refers to the comparisons between the individual farrowing batches, and the P\* value to the comparisons between the summaries of the NV (a-c) and V (b-d) batches. Differences between batches were not statistically significant (P>0.05).  
†Non-applicable due to insufficient number of observations.

positive at weaning (herd A: P=0.145; herd B: P=1.000). In herd A, a significantly lower percentage of piglets being seropositive at weaning were found to be n-PCR positive at seven days after weaning compared with their seronegative counterparts (P=0.024). In herd B, no such difference was observed (P=1.000).

### Lung lesions

In herd A, lung lesion evaluation at slaughter was performed on 68/120 and 64/127 pigs that originated from the selected NV and V sows (across batches a, b, c and d), respectively (table 6). Lost ear tags was the reason why not all pigs could be evaluated. In herd B, only batches a and b were investigated at slaughter, with the lung lesion evaluation performed on 47/65 and 55/57 pigs originating from the selected sows, respectively (table 6). Pigs that originated from batches c and d were, due to miscommunication, already slaughtered before the pre-arranged time.

In both herds, logistic regression analyses showed that the different fattening units did not have a significant effect on the prevalence of *Mycoplasma*-like pneumonia lesions and fissures. In herd A, significant differences between the different production batches were only obtained for the prevalence and extent of *Mycoplasma*-like pneumonia lesions. More specifically, a significantly higher percentage of pigs originating from the NV sows had *Mycoplasma*-like pneumonia lesions compared with those of the V sows (P=0.045, table 6). The pigs of the NV sows exhibited a significantly higher LLS than those of the V sows (P=0.021, table 6). In herd

B, there were no significant differences between the pigs of the NV and V sows regarding the prevalence and extent of *Mycoplasma*-like pneumonia lesions as well as the prevalence of fissures (table 6).

In both herds, there was no significant difference in the prevalence of *Mycoplasma*-like pneumonia lesions at slaughter between the pigs that were n-PCR positive at weaning (herd A: P=0.125; herd B: P=0.238) or seven days after weaning (herd A: P=0.680; herd B: P=1.000) and their n-PCR-negative counterparts. Concerning the LLS, there was no significant difference between the pigs that were n-PCR positive at weaning (herd A:  $r^2=0.156$ , P=0.143; herd B:  $r^2=-0.036$ , P=0.714) or seven days after weaning (herd A:  $r^2=0.123$ , P=0.252; herd B:  $r^2=-0.052$ , P=0.603) and their n-PCR-negative counterparts.

In herd A, a significantly lower prevalence of *Mycoplasma*-like pneumonia lesions (P=0.048) and a lower LLS ( $r^2=-0.267$ , P=0.011) at slaughter was observed in the pigs being seropositive at weaning compared with their seronegative counterparts. In herd B, no such difference was observed for the prevalence of *Mycoplasma*-like pneumonia lesions (P=0.484) and the LLS ( $r^2=0.025$ , P=0.804).

### Discussion

The present study investigated whether *M. hyopneumoniae* vaccination of sows at the end of gestation influenced the *M. hyopneumoniae* colonisation status of their piglets during the peri-weaning period as well as the prevalence and extent of *Mycoplasma*-like

**Table 5** Percentage and number (in parentheses) of seropositive sows and pigs at 12–24 hours post-farrowing and at weaning, respectively, together with the inhibition percentages (IP)

Herd A									
	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value	
Sows seropositive	20.0 (1/5)	80.0 (4/5)	0.0 (0/5)	100.0 (5/5)	0.380	10.0 (1/10) <sup>A</sup>	90.0 (9/10) <sup>B</sup>	0.001	
Sows IP	25.6 <sup>AB</sup>	77.5 <sup>ABC</sup>	26.8 <sup>B</sup>	94.4 <sup>C</sup>	0.005	26.2 <sup>A</sup>	86.0 <sup>B</sup>	0.000	
Pigs seropositive	6.8 (4/59) <sup>A</sup>	74.6 (50/67) <sup>B</sup>	5.1 (3/59) <sup>C</sup>	90.0 (54/60) <sup>D</sup>	0.000	5.9 (7/118) <sup>A</sup>	81.9 (104/127) <sup>B</sup>	0.000	
Pigs IP	15.6 <sup>A</sup>	68.1 <sup>B</sup>	29.0 <sup>A</sup>	79.6 <sup>B</sup>	0.000	22.3 <sup>A</sup>	73.2 <sup>B</sup>	0.000	
Herd B									
	a	b	c	d	P value	a-c (NV)	b-d (V)	P* value	
Sows seropositive	40.0 (2/5)	80.0 (4/5)	0.0 (0/4)	80.0 (4/5)	0.532	22.0 (2/9) <sup>A</sup>	80.0 (8/10) <sup>B</sup>	0.023	
Sows IP	39.0 <sup>AB</sup>	56.4 <sup>AB</sup>	19.9 <sup>A</sup>	68.8 <sup>B</sup>	0.049	30.5 <sup>A</sup>	62.6 <sup>B</sup>	0.017	
Pigs seropositive	42.2 (27/64) <sup>A</sup>	66.7 (38/57) <sup>B</sup>	3.3 (2/60) <sup>C</sup>	77.6 (45/58) <sup>B</sup>	0.000	23.4 (29/124) <sup>A</sup>	72.2 (83/115) <sup>B</sup>	0.000	
Pigs IP	42.4 <sup>A</sup>	79.3 <sup>B</sup>	24.6 <sup>C</sup>	65.2 <sup>D</sup>	0.000	38.5 <sup>A</sup>	72.5 <sup>B</sup>	0.000	

Vaccination status: NV (non-vaccinated sow batches and their pigs) and V (vaccinated sow batches and their pigs). The P value refers to the comparisons between the individual farrowing batches, and the P\* value to the comparisons between the summaries of the NV (a-c) and V (b-d) batches. Values with different superscripts within a row are significantly different (P<0.05 and P\*<0.05).

**Table 6** Percentage of pigs with *Mycoplasma hyopneumoniae*-like lung lesions (*Mhyo*) and fissures at slaughter, and lung lesion score (LLS; average  $\pm$ SD)

Herd A								
	a (n=33)	b (n=33)	c (n=35)	d (n=31)	P value	a-c (NV; n=68)	b-d (V; n=64)	P* value
Prevalence of <i>Mhyo</i> -like lesions	95.7 <sup>A</sup>	65.2 <sup>B</sup>	60.0 <sup>B</sup>	47.6 <sup>B</sup>	0.038	77.1 <sup>A</sup>	56.8 <sup>B</sup>	0.045
Prevalence of fissures	33.3	17.4	0.0	0.0	0.676	16.3	9.1	0.360
LLS	29.4 $\pm$ 19.1 <sup>A</sup>	14.9 $\pm$ 16.7 <sup>B</sup>	23.6 $\pm$ 28.6 <sup>AB</sup>	16.1 $\pm$ 21.8 <sup>B</sup>	0.032	26.4 $\pm$ 24.4 <sup>A</sup>	15.5 $\pm$ 19.1 <sup>B</sup>	0.021
Herd B								
	a (n=47)	b (n=55)	c	d	P value	a-c (NV; n=47)	b-d (V; n=55)	P* value
Prevalence of <i>Mhyo</i> -like lesions	78.7	74.1	N/At	N/At	N/At	78.7	74.1	0.649
Prevalence of fissures	46.8	37.9	N/At	N/At	N/At	46.8	37.9	0.428
LLS	8.4 $\pm$ 8.5	9.7 $\pm$ 11.6	N/At	N/At	N/At	8.4 $\pm$ 8.5	9.7 $\pm$ 11.6	0.541

Vaccination status: NV (pigs originating from non-vaccinated sow farrowing batches) and V (pigs originating from vaccinated sow farrowing batches). The P value refers to the comparisons between the individual farrowing batches, and the P\* value to the comparisons between the summaries of the NV (a-c) and V (b-d) batches. Values with different superscripts within a row are significantly different (P<0.05 and P\* $\leq$ 0.05).

†Non-applicable as pigs of batches c and d were slaughtered before the pre-arranged time and thus, no lung lesion evaluation was performed.

pneumonia lesions at slaughter. Results showed that in herd A pigs originating from the selected V primiparous sows had a significantly lower percentage of colonised pigs at seven days post-weaning compared with their counterparts from the selected NV primiparous sows. Additionally, at slaughter, the pigs of the selected V primiparous sows had a significantly lower prevalence of *Mycoplasma*-like pneumonia lesions and a significantly lower LLS than those of the selected NV primiparous sows. In herd B, there were no significant differences detected for any of the aforementioned parameters and a possible explanation might be the lower colonisation pressure observed.<sup>7</sup>

The reason why sow vaccination was performed twice in the present study (in sow batches b and d) was that the levels of *M. hyopneumoniae* IgG antibodies in the colostrum are comparable to the levels of *M. hyopneumoniae* IgG antibodies in the serum of the sows four weeks ante partum.<sup>28 29</sup> Then, until the time of partus the serum IgG antibody levels decrease, whereas the colostrum IgG antibody levels increase.<sup>30 31</sup> Hence, it was desired to achieve a high amount of maternal immunity transferred to the colostrum by boosting the first vaccination with a second one.

Concerning the results obtained in herd A, it should be taken into account that double sow vaccination during late gestation was applied in addition to piglet vaccination at weaning. A different study design was used by Díaz and others,<sup>32</sup> where the prevalence of *Mycoplasma*-like pneumonia lesions at slaughter was compared between one herd that vaccinated the sows in addition to piglet vaccination (herd 1) and one herd that vaccinated only the piglets (herd 2). At the beginning of the study, the prevalence of pneumonia lesions across herds 1 and 2 was 22 per cent and 21 per cent, respectively. After an eight-month implementation period, the prevalence of pneumonia lesions across herds 1 and 2 was 7 per cent and 18 per cent, respectively. Nevertheless, the study of Díaz and others<sup>32</sup> did not include parameters such as the detection rates of *M. hyopneumoniae* in the piglets and the LLS at slaughter. Additionally, sows were vaccinated only once at three weeks before farrowing.

The studies of Sibila and others<sup>7</sup> and Ruiz and others<sup>18</sup> were conducted in herds that did not additionally vaccinate their piglets and used farrowing batches where half of the sows were NV and half V. In both studies, the V sows received vaccination at five and three weeks before farrowing. The commercial bacterins used in these studies<sup>7 18</sup> were Mypravac Suis (Hipra) and RespiSure (Zoetis), respectively. The studies reported on the effect of sow vaccination on piglet colonisation using a single sampling point either at weaning<sup>18</sup> or during the post-weaning period,<sup>7</sup> and additionally on the extent of *Mycoplasma*-like pneumonia lesions at slaughter.<sup>7</sup> It was shown that vaccination of the sows reduced the number of pigs colonised with *M. hyopneumoniae*<sup>7 18</sup> and the extent of pneumonia lesions at slaughter.<sup>7</sup> Nevertheless, in the study of Sibila and others<sup>7</sup> the differences in colonisation across the single batch investigated were non-significant, while from the two consecutive production batches included in the study of Ruiz and others<sup>18</sup> significant differences in the colonisation rates between the pigs originating from the NV and V sows were only obtained in the first batch.

All the aforementioned differences between the present study and the studies of Díaz and others,<sup>32</sup> Sibila and others<sup>7</sup> and Ruiz and others<sup>18</sup> show that still further investigations are needed in order to conclude which vaccination programme (sow vaccination alone or in addition to piglet vaccination) is more efficient in reducing the number of piglets colonised with *M. hyopneumoniae* during the peri-weaning period and the lung lesions of these pigs at slaughter.

An additional difference of the present study with the aforementioned studies<sup>7 18 32</sup> is that only litters originating from primiparous sows were used in order to increase the likelihood of detecting piglets colonised with *M. hyopneumoniae*.<sup>10 11</sup> Nevertheless, there was no correlation between the colonisation of the sows and their piglets. The predictability of the piglet colonisation status based on the sow parity number alone may be misleading and still remains to be elucidated.<sup>8 33</sup> In contrast to Fano and others,<sup>11</sup> Calsamiglia and Pijoan<sup>8</sup>

indicated that sows of parity three to seven had a higher potential of shedding *M. hyopneumoniae* to their piglets than younger parity sows. Sibila and others<sup>33</sup> found no relationship between sow parity number and piglet colonisation during the suckling period. Thus, it is not known to what extent the parity of the sows might have influenced the colonisation rates of their piglets in the present study, as apart from primiparous sows there was no selection of older parity sows. Hence, in future studies it would be interesting to sample also piglets from sows of different parities.

An additional fact that should be considered regarding the lack of correlation between the colonisation of the selected sows and the colonisation of their piglets in the present study is that *M. hyopneumoniae* is not only transmitted vertically from the sows to their piglets, but also horizontally between piglets sharing the same environment and belonging to different litters (during the suckling period) or pens (during the nursery period).<sup>34</sup> In the present study, there are numerous factors that might have contributed to enhancing horizontal transmission. These can be the colonisation rates detected across the different sows present in the farrowing unit,<sup>34</sup> biosecurity procedures<sup>2 15</sup> and the single injectable iron dextran provision during the first days of age.<sup>33</sup> Additionally, the climate conditions in both the farrowing and the nursery units might have played a role.<sup>33 35</sup> Thus, horizontal transmission might have played an important role in the colonisation rates detected among the piglets of the primiparous sows.

Last but not least, the present study, together with other studies that focused at the breeding sow level, used n-PCR for detecting the presence of the pathogen in the selected sows and their piglets,<sup>7 8 13 18 32</sup> and not quantitative real-time PCR techniques. The latter techniques have been used though only in studies that focused at the fattening pig level and have shown that vaccinated pigs had a significantly reduced number of *M. hyopneumoniae* organisms in their lungs compared with the non-vaccinated pigs.<sup>36 37</sup> For that reason, future studies investigating the effect of sow vaccination in piglet colonisation, apart from including sows of different parities, they should also employ quantitative real-time PCR techniques for detecting the presence of *M. hyopneumoniae* in both the samples obtained from the sows and their piglets. By that way, it will be possible to further elucidate (a) if the number of *M. hyopneumoniae* organisms in the lungs of the selected sows is linked to the colonisation status of their piglets, and (b) if the vaccinated sows exhibit reduced shedding of the pathogen to their piglets during the suckling period compared with the non-vaccinated sows.

In the current study, it was decided to use laryngeal swabs as they were considered to be the most suitable method to conduct samplings in both sows and piglets,<sup>6 15</sup> over the whole duration of this

longitudinal study that employed a higher number of animals. The colonisation rates observed in the piglets of herd A decreased between weaning and seven days post weaning. The reasons for this are not clear. Sibila and others<sup>26</sup> and Nathues and others<sup>34</sup> have observed a similar colonisation pattern where the proportion of colonised piglets decreased with age after weaning. This scenario fits with previous suggestions that shedding of *M. hyopneumoniae* is an intermittent and of variable intensity process that still needs to be fully elucidated.<sup>4 5 12 35 38</sup>

Laryngeal swabs were collected from the piglets of this study twice. Samplings were chronologically close to each other (ie, at weaning and seven days post weaning). This was due to the fact that the study focused on the percentage of peri-weaned piglets colonised with *M. hyopneumoniae* between the selected NV and V sows. The aim was to investigate a vaccination protocol that could be applied in herds facing *M. hyopneumoniae* circulation during the early production stages. However, more samplings during the later stages of the nursery and the fattening period might have been beneficial to elucidate how the dynamics of *M. hyopneumoniae* colonisation unfolded between the different production batches, and to which extent under field conditions the shedding of *M. hyopneumoniae* can cease or vary in intensity when comparing pigs from the same production batch.

In the current study, it was attempted to optimise the detection of *M. hyopneumoniae* in oral fluid samples obtained from post-weaned piglets. Oral fluid samples were collected from each pen hosting piglets from the selected NV and V primiparous sows; nevertheless in both herds, these piglets were housed in the same nursery pens with the rest of the piglets originating from the same farrowing batch. Thus, the percentages of pen-based oral fluid samples positive for *M. hyopneumoniae* reflected the colonisation status of a broader population of pigs, rather than the colonisation status of the selected litters. Comparing to the field study of Hernandez-Garcia and others,<sup>17</sup> the oral fluid samples obtained from each pen were pooled in order to increase the sensitivity of detection by n-PCR. Additionally, comparing to both studies of Pieters and others<sup>15</sup> and Hernandez-Garcia and others,<sup>17</sup> n-PCR was used for the detection of the pathogen instead real-time PCR.

In both herds participating in this study, there were no significant differences in the percentage of nursery pens being positive for *M. hyopneumoniae* by oral fluids between the NV and the V production batches. Moreover, pens hosting batches c and d in herd A as well as pens hosting batches a and b in herd B, which included laryngeal swab-positive piglets (as general evidence that colonised pigs were present in these pens), were oral-fluid negative. Hence, the results of this study agree with the studies of Pieters and others<sup>15</sup>



and Hernandez-Garcia and others<sup>17</sup> that the detection of *M. hyopneumoniae* in oral fluid samples obtained from post-weaned pigs needs further optimisation and thus, samplings from multiple pens and on multiple occasions may be needed.

In both herds, the percentage of selected sows that were seropositive at 12–24 hours post-farrowing was influenced by their vaccination status, since 0–40 per cent of the NV sows were seropositive at 12–24 hours post-farrowing versus 80–100 per cent of the V sows. Those differences in the percentage of seropositive sows across the NV and V farrowing batches are in agreement with the studies of Sibila and others<sup>7</sup> and Ruiz and others.<sup>18</sup> It was evident that an improved passive transfer of maternal antibodies via the colostrum was achieved in the piglets of the V sows compared with their counterparts from the NV sows. In the present study, a significantly lower percentage of seropositive piglets at weaning came from NV sows (ranging between 3 per cent and 42 per cent) compared with the V sows (ranging between 67 per cent and 90 per cent). Those differences are also in agreement with previous studies of Ruiz and others<sup>18</sup> and Martelli and others<sup>39</sup> who used similar experimental designs and sampled piglets at the same age.

In herd A, a significantly higher number of piglets being seronegative at weaning were found to be n-PCR positive at seven days post-weaning compared with their seropositive counterparts. Additionally, the seronegative piglets had a significantly higher prevalence and extent of *Mycoplasma*-like pneumonia lesions at slaughter than the seropositive ones. In herd B, no such differences were detected and an explanation might be that across all production batches investigated a low colonisation pressure, together with low a LLS, was observed. Also, batches c and d were not evaluated for the prevalence and severity of *Mycoplasma*-like pneumonia lesions at slaughter (due to a miscommunication between the investigators and the slaughtermen), which had reduced the number of animals included in the statistical analysis.

Pieters and others<sup>40</sup> found no relationship between piglet sero-status at weaning and colonisation, and this was attributed to the variable degree of immunity in the sows. In that study, all piglets originated from NV sows.<sup>40</sup> The vaccination of sows against *M. hyopneumoniae* could have possibly further stabilised sow herd immunity, resulting in a uniformly enhanced maternally derived immunity supplied via the colostrum, thus allowing the establishment of a significant relationship between piglet sero-status at weaning and colonisation.

The aforementioned associations observed in herd A between the sero-status of the piglets at weaning and their n-PCR positivity at seven days post-weaning could be attributed to the lower passive transfer of maternal immunity to the piglets of the NV sows compared with their counterparts from the V sows. In fact, 8/9 piglets

found to be n-PCR positive at seven days post-weaning were seronegative at weaning and originated from NV sows. No significant differences were obtained when the data from pigs originating from the NV and V sow batches were analysed separately, likely because of reduced statistical power of the analyses. On a similar way in the same herd, this lower passive transfer of maternal antibodies to the piglets of the NV sows might have played an important role in the differences observed between the seronegative and seropositive pigs in the development of *M. hyopneumoniae*-like pneumonia lesions. In fact, 51/89 pigs with *Mycoplasma*-like pneumonia lesions at slaughter were seronegative at weaning and originated from NV sows versus 22/89 that were seropositive at weaning and originated from V sows.

Colostrum antibodies provide partial protection against both experimentally induced and natural infections.<sup>41</sup> In the present study, the passive transfer of maternal immunity was indirectly measured through an ELISA detecting the levels of serum IgG. Djordjevic and others<sup>42</sup> showed that IgG concentrations (in serum and respiratory tract washings) were not correlated with protection against *M. hyopneumoniae* infection. Thus, there must be additional immunological components, other than the IgG antibodies measured in the current and previous studies, present in the milk of the V sows, whose enhanced presence (due to vaccination) conferred improved resiliency to their pigs against colonisation and *M. hyopneumoniae*-like pneumonia lesion development compared with the pigs originating from the NV sows. These might include antibodies against *M. hyopneumoniae* antigens not measured in the ELISA used here, as well as immune cells. Indeed, Bandrick and others<sup>43</sup> conducted an experimental study and showed that lymphocytes that were passively transferred from V sows to their piglets via the colostrum were able to proliferate and participate in a functional response against *M. hyopneumoniae*.

Overall, in the present study vaccination of the primiparous sows in herd A (which had a sufficient number of colonised piglets at weaning compared with herd B) reduced the number of colonised pigs at seven days post-weaning as well as the prevalence and extent of *Mycoplasma*-like pneumonia lesions at slaughter. Thus, sow vaccination could be a useful tool to control *M. hyopneumoniae* infections in herds that maintain a high proportion of primiparous sows and where colonised piglets are detected during the early production stages. It should be mentioned though that the vaccine used in the present study (Ingelvac MycoFLEX) is currently licensed for use in sows in the USA, but not yet in sows in the European Union. Thus, in countries such as those belonging to the European Union, the veterinarians using the vaccine in sows should comply with the extra-label usage guidelines set by the local authorities. Sow colonisation at 12–24 hours post-farrowing, as

measured with the method used here, was not correlated with piglet colonisation at weaning or post weaning. In this context, the fact that the sero-status of the piglets had an effect on the colonisation rates detected at seven days post weaning, and the prevalence and extent of *Mycoplasma*-like pneumonia lesions at slaughter implies that immunological components passively transferred via the colostrum may play an important role. More studies are needed to elucidate the aforementioned immunological mechanisms of resiliency to colonisation and infection as such is achieved by gestating sow vaccination.

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**Ethics approval** The study was approved by the ethical committee for animal experiments of the Faculty of Veterinary Medicine, Ghent University (EC2014/156).

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