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## Research article

# Longitudinal study of respiratory infection patterns of breeding sows in five farrow-to-finish herds

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## ABSTRACT

A longitudinal study was carried out in five French farrow-to-finish herds differently affected by respiratory diseases to describe the carrying and infection patterns of batches of sows to various respiratory pathogens during gestation and lactation. An entire batch of sows was followed during two successive reproduction cycles. Nasal, tonsillar and oropharyngeal swabs and blood samples were taken from each sow 9 and 4 weeks before farrowing and 1 and 4 weeks after farrowing. *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis* and *Streptococcus suis* were detected from swab samples using PCR assays. Blood samples were tested for antibodies against *M. hyopneumoniae*, *A. pleuropneumoniae* serotypes 1–9–11 and 2, Porcine Circovirus type-2 (PCV-2) and Porcine Reproductive and Respiratory Syndrome virus (PRRSV) by ELISA tests. Antibodies against H<sub>1</sub>N<sub>1</sub>, H<sub>1</sub>N<sub>2</sub> and H<sub>3</sub>N<sub>2</sub> Swine Influenza Viruses (SIV) of European lineages were tested by hemagglutination inhibition assay. The results indicated that *S. suis* is widespread among sows (67.1% of PCR-positive sows). *A. pleuropneumoniae*, *P. multocida*, and *H. parasuis* were detected by PCR in 30.9%, 24.6% and 23.4% of the sows, respectively. Antibodies against *M. hyopneumoniae* were recovered from more than 55% of the sows in all herds whereas the micro-organism was detected in 2.4% of the sows. Although PCV-2 and SIV infections were highly prevalent, the PRRSV infection patterns ranged from no infection in farms mildly affected by respiratory diseases to active circulation in more severely affected herds. The sow population thus constitutes a reservoir for a continuous circulation of respiratory pathogens and needs to be properly considered in control strategies.

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## 1. Introduction

Respiratory diseases are some of the most common and costly diseases affecting growing-finishing pigs reared under intensive confined conditions. Several bacterial and viral agents are involved in the pathogenesis of these diseases. *Mycoplasma hyopneumoniae*, most frequently in

combination with other bacteria like *Pasteurella multocida*, *Haemophilus parasuis*, *Streptococcus suis* and *Actinobacillus pleuropneumoniae*, or viruses such as Swine Influenza Virus (SIV), Porcine Reproductive and Respiratory Syndrome virus (PRRSV), Porcine Circovirus type-2 (PCV-2) and Porcine Respiratory Coronavirus (PRCV) are the most important micro-organisms responsible for lung diseases (Sorensen et al., 2006). Together they are involved in the Porcine Respiratory Disease Complex (PRDC).

Some studies have indicated that disease outcome may be influenced by the infection pattern and infectious

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pressure of the pathogens in growing-finishing pigs, particularly for *M. hyopneumoniae* (Sibila et al., 2004; Fano et al., 2007). Indeed, the infection pressure had to be at a very low level throughout the pigs' lifetime in order to reduce disease severity. Particular attention to critical transmission points is required to reduce pathogen transmission between animals. For many of the microorganisms involved in respiratory diseases vertical transmission, relying on sow to piglet contamination, is suspected to occur in endemically infected herds during the suckling phase (Clark et al., 1991; Amass et al., 1996; Vigre et al., 2002; Zimmerman et al., 2006). Sows can thus serve as a reservoir for the continuous circulation of infectious pathogens especially in farrow-to-finish systems, where both populations, (i.e., the breeding herd and the growing-finishing pigs), are reared on the same site (Calsamiglia and Pijoan, 2000; Sorensen et al., 2006). Reducing the infection pressure in the sow herd may help to decrease spreading in the offspring. However, at present, little is known about the shedding and infection dynamics of respiratory pathogens, especially bacteria, in sow populations. Such information would be helpful to (i) properly design epidemiological studies for assessing/understanding infection dynamics within a herd and detecting the risk factors of respiratory diseases, (ii) provide accurate information for modeling studies, i.e. parameterization and validation of epidemiological models, and (iii) implement adequate control strategies. The aim of the present study was therefore to describe the carrying and infection patterns of batches of sows to various respiratory pathogens during gestation and lactation in herds differently affected by respiratory disorders.

## 2. Materials and methods

### 2.1. Herds selection

A longitudinal study was carried out in five single site farrow-to-finish herds located in Brittany (Western France). The farms were included on the basis of the

veterinarian's knowledge of the farm and the results of an initial screening of 12 herds. Five herds were selected for different patterns regarding their respiratory health status in growing-finishing pigs and to tally with the most common herd health patterns, housing and herd management practices encountered in the field at that time. They were also selected for convenience as the farmer had to agree with our protocol. The screening visit took place 2–3 months before the present longitudinal field study was started. Clinical signs of respiratory disorders in the farrowing, post-weaning and finishing sections were recorded. Nasal, tonsillar and oro-pharyngeal swabs and blood samples were obtained from 30 pigs: 10 in the post-weaning section, 10 at the beginning and 10 at the end of the finishing phase. Lungs from at least 30 of the sampled finishing pigs were collected at the slaughterhouse. Macroscopic lung lesions of pneumonia were scored (Maded and Kobisch, 1982) and samples of lung lesions were taken. Swabs and lung samples were examined by PCR in our laboratory to detect *M. hyopneumoniae*, *P. multocida*, *A. pleuropneumoniae*, *H. parasuis* and *S. suis*. All sera were analysed for the detection of antibodies to PRRSV and SIV (H<sub>1</sub>N<sub>1</sub>, H<sub>3</sub>N<sub>2</sub> and H<sub>1</sub>N<sub>2</sub>).

The retained farms differed in terms of clinical severity, infection profile, and the type and extent of lung lesions (Table 1). Disease severity and complexity showed a gradual increase from farms A to E. Herd A was mildly affected by respiratory diseases, according to clinical diagnosis and macroscopic lung lesions examination. The growing-finishing pigs were free from PRRSV and SIV. The lowest level of lung lesions in the five herds, was recorded in herd A. Herds B and C were endemically infected by PRRSV and differed in lung lesion severity, the finishing pigs in farm C being more affected by pneumonia. The last 2 farms (herds D and E) were the most severely affected by lung lesions as pneumonia and pleuritis were detected in more than 90% and 25% of the pigs, respectively. The finishing pigs were PRRSV and SIV positive, whereas the pigs from herd D tested positive for H<sub>1</sub>N<sub>1</sub> antibodies, the growing pigs from farm E had antibodies against both SIV subtypes (H<sub>1</sub>N<sub>1</sub> and H<sub>1</sub>N<sub>2</sub>).

**Table 1**

Clinical, viral and bacteriological profiles and lung lesions of growing and finishing batches in the five selected farrow-to-finish pig herds (nasal, tonsillar, oro-pharyngeal swabs and blood samples of 30 growing-finishing pigs, lung examination of 30 slaughtered pigs, findings of the screening visit performed 2–3 months before the start of the longitudinal study).

Herd	Clinical signs (cough) <sup>a</sup>	Pathogen detection by PCR in swab samples (number of positive pigs)					Viral status <sup>b</sup>		Lung lesions		Pathogen detection by PCR in lung tissues <sup>b</sup>				
		Mhp	Pm	App	Hps	Ssuis	PRRS	SIV	Pneumonia mean score (/28) (σ) <sup>c</sup>	% pleuritis	Mhp	Pm	App	Hps	Ssuis
A	Fin.	1	9	0	28	30	–	–	0.9 (2.5)	5.1	+	–	–	–	+
B	Fin.	0	16	0	30	2	+	–	2.1 (3.7)	15	+	+	–	–	–
C <sup>d</sup>	Fin.	0	20	0	17	17	+	–	9.6 (4.2)	0	+	–	+	+	–
D <sup>e</sup>	Far./Fin.	3	26	0	30	18	+	H <sub>1</sub> N <sub>1</sub>	10.1 (6.3)	38.5	+	+	–	–	–
E	PW/Fin.	0	4	2	29	13	+	H <sub>1</sub> N <sub>1</sub> , H <sub>1</sub> N <sub>2</sub>	6.4 (5.1)	28.6	+	–	–	+	–

<sup>a</sup> Fin.: finishing; Far.: farrowing; PW: post-weaning.

<sup>b</sup> (+) positive; (–) negative.

<sup>c</sup> Madec and Kobisch (1982).

<sup>d</sup> 10 growers and 10 market-aged pigs.

<sup>e</sup> Nasal swabs.

## 2.2. Animals and sampling scheme

The herds were managed all-in all-out by room in the farrowing, post-weaning and fattening sections with a 3-week batch interval between farrowing for 3 farms, 4-week interval for herd A and 1-week interval for herd B. The main features of the five herds are detailed in Table 2. After checking for pregnancy, the sows in each herd were housed in a gestation room and were then moved to farrowing rooms about 1 week before the expected farrowing time. The piglets were weaned around 4 weeks after farrowing except in farms A and C where the piglets were weaned at 3 weeks of age. During the gestation and lactation periods, the sows were housed in closed facilities with mechanical ventilation and slatted floor. The sows in the selected herds were not vaccinated against *M. hyopneumoniae* during the study. Whether the sows were vaccinated earlier in life in the multiplier herd was unknown. The sows in herd C were vaccinated against *A. pleuropneumoniae*. The breeding sows in herd B were vaccinated against PRRSV and SIV. The same commercial SIV vaccine (antigens A/New Jersey/8/76 (H<sub>1</sub>N<sub>1</sub>) and A/Port Chalmers/1/73 (H<sub>3</sub>N<sub>2</sub>)) had previously been used in herd A but no SIV vaccine was used in this herd during the study. An inactivated PRRSV vaccine was used for the gilts in herd E. All farms except E vaccinated the sows and gilts against atrophic rhinitis.

All farms were visited 8 times. An entire batch of pregnant sows in each herd was followed during two successive gestation and lactation periods. The sows were sampled twice during gestation, 9 and 4 weeks before the expected farrowing date, and twice during the farrowing phase, then 1 and 4 weeks after farrowing. Prior to the first visit, the sows were individually identified (ear tag) by the farmer. Clinical observations and samplings were made by four specially trained operators from our laboratory throughout the study. To reduce the bias due to the observer, each operator was specialized in an activity: animal restraining (1), sow's mouth opening (1), swabbing (1) and blood sampling and clinical observations (1).

## 2.3. Sample collection

At each visit, nasal, tonsillar and oro-pharyngeal swabs and blood samples were taken from each sow. The animals were restrained by placing a conventional cable snare over the maxilla. The animal's mouth was held open with a gag to obtain samples from the tonsillar and oral-pharyngeal areas. The surface of the tonsils was swabbed with "CytoBrushes" (VWR International, Fontenay-sous-Bois, France). Oral-pharyngeal samples were obtained by swabbing the surface of the oral-pharyngeal cavity thoroughly but gently with a brush protected by a catheter (Ori Endometrial Brush™, Orifice Medical AB, Ystad, Sweden). For nasal sampling, both nasal cavities were swabbed with "CytoBrushes" (VWR International), inserted into the nostrils and rotated to reach deeply into the turbinates. All samples were placed in 2 ml of Buffered Peptone Water Broth. They were individually identified and delivered to the laboratory for processing on the day of collection (Initial Suspension, IS).

**Table 2**  
Main characteristics of the five farrow-to-finish herds under study.

Herd	Sow herd features		Vaccination scheme/ respiratory diseases	Husbandry		Technical performances					
	Number of sows	Breed		% gilts	Batch interval	Weaning age (weeks)	ADWG <sup>a</sup> (g/day; 7–25 kg)	FCR <sup>b</sup> (7–25 kg)	ADWG <sup>a</sup> (g/day; 25–105 kg)	FCR <sup>b</sup> (25–105 kg)	Age at 105 kg live weight (days)
A	130	Naïma	22	4 weeks	3	492	1.55	777	2.67	164	5.1
B	700	LW × LR	30	1 week	4	458	1.59	789	2.61	165	9.5
C	170	Naïma + LW × LR	31	3 weeks	3	501	1.64	817	2.62	160	7.0
D	220	Naïma	19	3 weeks	4	426	1.60	728	2.93	168	6.5
E	190	LW × LR	22	3 weeks	4	na <sup>c</sup>	na	na	na	na	na

<sup>a</sup> Average daily weight gain.

<sup>b</sup> Feed conversion ratio.

<sup>c</sup> Not available.

Blood samples were collected by jugular vein puncture, using evacuated Tubes (Vacuette, Dutscher SAS, Brumath, France) without additive. The samples were individually identified, and delivered to the laboratory, where they were processed. Serum was obtained by centrifugation for 10 min at  $3500 \times g$  and stored at  $-20^\circ\text{C}$  until subsequent analysis.

#### 2.4. Laboratory analyses

##### 2.4.1. PCRs on swab samples for bacterial pathogen DNA detection

Briefly, 1 ml of each IS was centrifuged ( $12,000 \times g$ , 20 min) and the pellets were resuspended in  $800 \mu\text{l}$  of lysis solution. The remaining IS was stored at  $-70^\circ\text{C}$  with glycerol (20%) for future studies. Lysates were incubated for 1 h at  $60^\circ\text{C}$ , 10 min at  $95^\circ\text{C}$  and then kept at  $-20^\circ\text{C}$  until PCR analysis. All swabs were tested by PCR, as previously described, for DNA detection of *M. hyopneumoniae* (Marois et al., 2007), *A. pleuropneumoniae* (Schaller et al., 2001), *P. multocida* (Marois et al., 2008), *S. suis* (Marois et al., 2004) and *H. parasuis* (Oliveira et al., 2001). When an inhibition of the PCR reaction was observed, DNA was re-extracted from the lysates with phenol/chloroform/isoamyl(alcohol (25/24/1) (Marois et al., 2004). A sample was considered positive to a pathogen when the specific PCR tested positive.

##### 2.4.2. Serological analyses

All sera were tested for *M. hyopneumoniae* antibodies with a blocking enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (DAKO ELISA, Kitvia, Labarthe-Inard, France). The inhibition percentage (IP) for each serum was calculated with the following formula:  $\text{IP} = 100 - [100 \times (\text{sample mean OD} / \text{buffer control mean OD})]$ . A sample was classified as positive if the IP value was  $>50\%$ . The sensitivity and specificity of the ELISA were previously reported to be 100% and 100%, respectively, in experimental trials (Sørensen et al., 1997). Sera were also analysed to detect *A. pleuropneumoniae* antibodies serotype 2 and serogroup 1-9-11 using 2 ELISA commercial kits (Swinecheck App2 and Swinecheck App1911, Biovet, AES Laboratoire, Combourg, France). The positive threshold was fixed at an optical density (OD) of 0.5 and 0.55 for serotype 2 and serogroup 1-9-11, respectively. The sensitivity and specificity of the two ELISA tests were 77.8% and 97.4% and 89.5% and 95.1%, respectively, according to the manufacturer.

Sera of all herds, except herd A, were tested for PCV-2 antibodies using an ORF-2 protein based ELISA test (Blanchard et al., 2003) at 4 sampling dates: 1 and 4 weeks post-partum at the first reproduction cycle and 9 and 4 weeks before farrowing in the second cycle. An OD ratio of 1.5 served as positivity cut-off. The sensitivity and specificity of the PCV-2 ELISA were 98.2% and 94.5%, respectively. PRRS virus antibodies were tested (ELISA test, IDDEX Laboratory, Eragny, sur Oise, France) in sera of every herd at 3 sampling dates: 9 weeks before farrowing in the first reproduction cycle and 4 weeks after farrowing in both cycles. The sensitivity and specificity of the PRRS

ELISA were 97.4% and 99.6%, respectively. During the first reproduction cycle of each herd, random samples of sera obtained 1-week post-partum from 12 sows were tested with an hemagglutination inhibition (HI) test (Van Reeth et al., 2008) for antibodies against European SIVs, using strains A/Sw/Finistere/2899/82 ( $\text{H}_1\text{N}_1$ ), A/Sw/Gent/1/84 ( $\text{H}_3\text{N}_2$ ) and A/Sw/Scotland/410440/94 ( $\text{H}_1\text{N}_2$ ) as antigens. An animal was considered positive against a given SIV subtype when the HI antibody titre was 20 or more (Van Reeth et al., 2008).

#### 2.5. Statistical analysis

The contamination and infection status was considered at the sow level. At each sampling date, an animal was considered contaminated by a bacterial pathogen when at least one out of the three samples tested positive by specific PCR assay. Generalized linear models using the Generalized Estimation Equations (GEE) method were used to assess the influence of parity, farm, physiological stage and cycle on the sow contamination status determined by PCR assays and the infection status determined by serological assays (*M. hyopneumoniae* and *A. pleuropneumoniae*) (proc genmod, SAS 9.1, SAS Inst Inc.). The influence of the sampling site on the sow contamination status was also assessed using GEE models. Since the data consisted in repeated measurements on the same animals over time and space, observations could not be considered as independent. Sow was taken as a repeated statement to assess the relationships between the dependent variable and parity, farm and sampling site whereas a farm cluster effect was considered in models assessing the influence of the physiological stage and cycle on the dependent variable. The score statistics for type-3 GEE analysis was used to test the significance of main effects ( $p < 0.05$ ). GEE models were also used to estimate the frequency of positive animals by PCR and serological assays as regards bacterial pathogens throughout the survey period. Within-herd seroprevalences were adjusted according to sensitivity and specificity of ELISA assays.

### 3. Results

#### 3.1. PCR analyses for bacterial pathogen detection

In total, 3453 swabs from 184 sows were tested by PCR assays aimed to detect bacterial pathogen genomes. 109 of these 184 sows were followed throughout the entire sampling period due to the culling/replacement process in-between cycles. On average, 29 sows ( $\sigma=6.7$ ) were sampled per batch at each sampling time and sows were sampled 6.9, 6.0, 6.6, 6.5, and 5.4 times in herds A, B, C, D and E, respectively. Parity distributions at each reproduction cycle are given for each herd in Table 3. Sows from herd C were on average younger than those from the other herds since new breeding stock was introduced when moving from a 3-week to a 4-week batch interval. The high average parity in herd D was related to the breeding sows management policy (long lasting time of the sows).

DNA of *M. hyopneumoniae*, *H. parasuis*, *P. multocida*, *A. pleuropneumoniae* and *S. suis* were detected from at least



**Table 3**

Parity mean distribution during two successive reproduction cycles of five batches of sows from farrow-to-finish herds.

Herd	Cycle 1		Cycle 2	
	Mean ( $\sigma$ )	n	Mean ( $\sigma$ )	n
A	4.2 (2.4)	23	3.8 (2.5)	25
B	4.0 (2.6)	35	3.0 (2.1)	36
C	2.7 (1.7)	39	2.3 (1.3)	41
D	5.6 (3.1)	27	4.7 (3.1)	26
E	3.6 (2.0)	25	2.9 (1.9)	25

one out of the three swab samples in 2.4% (confidence interval at 95% (C.I.<sub>95%</sub>) (1.7; 3.3)), 23.4% (C.I.<sub>95%</sub>: (20.6;26.4)), 24.6% (C.I.<sub>95%</sub>: (21.0;27.5)), 30.9% (C.I.<sub>95%</sub>: (28.3;33.7)), and 67.1% (C.I.<sub>95%</sub>: (63.8;70.2)) of the sows, respectively. Whatever the micro-organism, the model-based estimated frequency of positive animals varied from farm to farm (Table 4). In all five herds, all the pathogens, except *M. hyopneumoniae* were detected at least once in the upper respiratory tract of the sows. *S. suis* was the pathogen the most frequently recovered in all herds, with more than 40% of positive sows. To a lesser extent *A. pleuropneumoniae*, *P. multocida* and *H. parasuis* were also frequently detected in almost all herds with herd levels ranging from 11.6% to 48%, 8.4% to 45% and 4.7% to 50% of positive sows, respectively.

In the subsample of 109 sows which were swabbed during all eight visits, for all the sows found positive to *M. hyopneumoniae*, the DNA of the organism was detected at one sampling occasion, whereas genomes of *P. multocida*, *H. parasuis*, *A. pleuropneumoniae* and *S. suis* were detected on 2 sampling occasions or more for 60.4%, 66.3%, 75.5% and 98.1% of the positive sows, respectively. Whatever the

micro-organism, the frequency of positive animals in the herd varied over time, without any significant effect of the physiological stage (Fig. 1 and Table 5) whereas a significant effect of the reproduction cycle was observed for *S. suis* and *H. parasuis*. A significant effect of the farm on the sow contamination status of the upper respiratory tract was identified for each micro-organism ( $p < 0.05$ , Table 5), except for *M. hyopneumoniae* which could not be estimated due to the absence of detection in some farms. The ability to detect contaminated sows was significantly influenced by the sampling site for four out of the five bacteria (Table 5). Swabbing the tonsils significantly increased the probability to detect sows contaminated by *A. pleuropneumoniae* and *H. parasuis* than sampling the nasal cavities or the oro-pharyngeal area ( $p < 0.05$ , Table 6). The detection of *P. multocida* PCR-positive sows was significantly improved when nasal swabs were used ( $p < 0.05$ , Table 6). The odds for a sow being PCR-positive to *S. suis* by oro-pharyngeal swabbing was significantly lower than by nasal or tonsillar sampling ( $p < 0.05$ , Table 6).

### 3.2. Serological analyses

#### 3.2.1. Bacterial pathogens

Sows seropositive to *M. hyopneumoniae*, *A. pleuropneumoniae* serogroups 1–9–11 and 2 were detected in all five herds at different frequencies depending on the farm (Table 7). The odds of sows from herd E being *M. hyopneumoniae* seropositive were significantly lower than those of herds A, C and D ( $p < 0.05$ ). The odds of being seropositive to *A. pleuropneumoniae* serogroup 1–9–11 were significantly higher in herds C and E than in the three other herds whereas for serotype 2 it was higher in herds A

**Table 4**

Estimated frequencies (with 95% confidence interval) of *M. hyopneumoniae*, *A. pleuropneumoniae*, *P. multocida*, *H. parasuis* and *S. suis* detection in breeding sows followed in five farrow-to-finish herds (184 sows, 8 sampling times, nasal, tonsillar and oro-pharyngeal swabs, PCR assays, Generalized Estimation Equations models with an autoregressive correlation matrix).

Herd	<i>Mycoplasma hyopneumoniae</i>	<i>Actinobacillus pleuropneumoniae</i>	<i>Pasteurella multocida</i>	<i>Haemophilus parasuis</i>	<i>Streptococcus suis</i>
A	0.6 <sub>b</sub> (0.0;37.3)	48.0 <sub>c</sub> (34.6;61.7)	8.4 <sub>a</sub> (3.6;18.5)	21.6 <sub>b</sub> (13.6;32.3)	45.9 <sub>a</sub> (30.3;62.3)
B	0.4 <sub>b</sub> (0.1;2.6)	11.6 <sub>a</sub> (9.3;14.4)	20.1 <sub>c</sub> (16.2;24.6)	19.1 <sub>b</sub> (15.7;23.1)	71.2 <sub>b,c</sub> (65.7;76.1)
C	4.1 <sub>a</sub> (0.1;68.2)	29.6 <sub>b</sub> (19.2;42.7)	22.5 <sub>c</sub> (13.4;35.3)	4.7 <sub>a</sub> (2.2;9.6)	76.4 <sub>b</sub> (63.4;85.8)
D	5.7 <sub>a</sub> (0.1;75.5)	34.2 <sub>b</sub> (23.6;46.7)	45.0 <sub>b</sub> (30.9;60.0)	50.2 <sub>c</sub> (37.0;63.3)	54.5 <sub>a</sub> (39.3;68.9)
E	0 <sub>b</sub> (0.0;0.0)	41.2 <sub>b,c</sub> (27.7;56.3)	26.9 <sub>c</sub> (15.8;41.9)	30.1 <sub>d</sub> (19.1;44.0)	79.1 <sub>c</sub> (66.2;88.0)

Herds with different subscripts (a, b, c and d) have a significantly different PCR-prevalence ( $p < 0.05$ , Generalized Estimation Equations model).

**Table 5**

Results of the score statistics for type-3 Generalized Estimation Equations analysis to test the significance of herd, parity, physiological stage, cycle effects and sampling site on the sow contamination status determined by PCR assays and the infection status determined by serological assays.

Dependent variable	Independent variable				
	Herd	Parity number	Physiological stage	Cycle	Sampling site
Mhp PCR-status	n.e. <sup>a</sup>	n.e.	n.e.	n.e.	0.63
App PCR-status	<0.001	0.77	n.e.	n.e.	<0.001
Pm PCR-status	<0.001	0.07	0.55	0.61	0.002
Hps PCR-status	<0.001	0.35	0.31	0.04	0.002
Ssuis PCR-status	<0.001	0.12	0.99	0.05	<0.001
Mhp serological status	0.03	0.79	0.18	0.26	–
App1–9–11 serological status	<0.001	0.005	0.19	0.11	–
App2 serological status	<0.001	0.002	0.29	0.41	–

Shaded values are significant at  $p < 0.05$ .

<sup>a</sup> Not estimable.

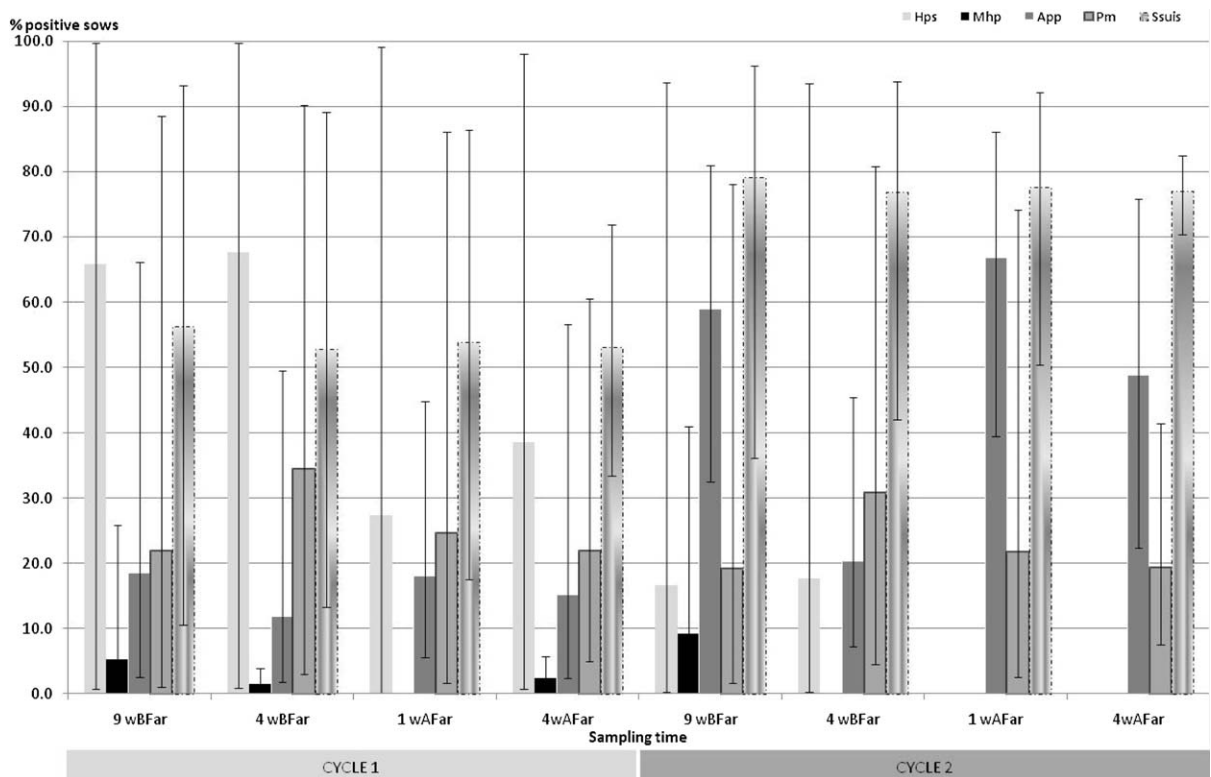


Fig. 1. Estimated within herd pathogen detection frequency (and 95% confidence interval) of *M. hyopneumoniae* (Mhp), *A. pleuropneumoniae* (App), *P. multocida* (Pm), *H. parasuis* (Hps) and *S. suis* (Ssuis) during two successive gestation and lactation periods (cycles 1 and 2) (5 farrow-to-finish herds, 184 sows, 8 sampling times, nasal, tonsillar and oro-pharyngeal swabs, PCR assays, Generalized Estimation Equations with an exchangeable correlation matrix). 9wBFar: 9 weeks before farrowing; 4wBFar: 4 weeks before farrowing; 1wAFar: 1 week after farrowing; 4wAFar: 4 weeks after farrowing.

and C. The lowest proportions of seropositive sows to *A. pleuropneumoniae* serogroup 1-9-11 and serotype 2 were found in herds A, B, D and D, respectively.

Neither physiological stage nor cycle influenced the sow serological status to *M. hyopneumoniae*, *A. pleuropneumoniae* serogroup 1-9-11 or serotype 2 ( $p > 0.05$ , Fig. 2 and Table 5). In herds C and E, an increase of the percentage of *A. pleuropneumoniae* serotype 2-seropositive sows was detected between gestation and lactation in the first reproduction cycle (data not shown). The probability of young sows (parity number  $\leq 2$ ) having antibodies against *A. pleuropneumoniae* 1-9-11 and 2 ( $p < 0.05$ ) was significantly lower. No parity effect was identified for *M. hyopneumoniae* (Table 5).

### 3.2.2. Viral pathogens

The viral infection patterns varied between farms. Sows from herd A were seronegative to PRRSV during the first gestation and lactation periods. Blood samples from the second reproduction cycle were not tested. However, no clinical signs of PRRSV infection were recorded in this farm during the second cycle. The percentage of PRRS-seropositive sows increased between the first gestation and second lactation periods in herd E whereas it decreased in herd C and remained high in herd D as shown in Fig. 3.

All herds tested positive for SIV by HI assays (Fig. 4). The highest frequencies of SIV-seropositive sows were found

for subtype H<sub>1</sub>N<sub>2</sub> in all herds, except in the SIV-vaccinated herd B, where 100% and 91.7% of the sows tested positive for subtypes H<sub>3</sub>N<sub>2</sub> and H<sub>1</sub>N<sub>1</sub>, respectively. At the animal level, three herds (A, B and C) had sows with subtype H<sub>1</sub>N<sub>1</sub>-antibodies titres that were higher than those of the first more frequent subtype and were considered positive for H<sub>1</sub>N<sub>1</sub>. HI titres for subtype H<sub>3</sub>N<sub>2</sub> equal to those of the most prevalent subtype were detected in herd A in which the sows used to be vaccinated before the study. Both the low frequency of positive sows and the low antibodies titres for the H<sub>3</sub>N<sub>2</sub> subtype observed in herds D and E were considered to be due to cross-reactions in the HI test.

PCV-2 antibodies were detected in more than 75% of sows in the four tested herds at 1 and 4 weeks after farrowing for the first reproduction cycle and at 9 and 4 weeks before farrowing for the second cycle.

## 4. Discussion

The aim of the present study was to describe the carrying and infection patterns of batches of sows for various respiratory pathogens during two successive gestation and lactation periods. Three sampling sites in the upper respiratory tract, i.e., nasal cavities, tonsils, and oro-pharyngeal area, were chosen to describe the carrier state of the animals regarding five bacteria involved in PRDC. The sampling areas were selected according to the

**Table 6**  
Results of the sampling site comparison to detect PCR-positive sows to *M. hyopneumonitiae*, *A. pleuropneumonitiae*, *P. multocida*, *H. parasuis* and *S. suis* (5 herds, 184 sows, nasal (NS), tonsillar (TS) and oro-pharyngeal (OPS) swabs, odds ratio (OR) with confidence interval at 95% (C.I.<sub>95%</sub>)).

Sampling site comparison	Site	<i>M. hyopneumonitiae</i>			<i>A. pleuropneumonitiae</i>			<i>P. multocida</i>			<i>H. parasuis</i>			<i>S. suis</i>		
		p	OR	C.I. <sub>95%</sub>	p	OR	C.I. <sub>95%</sub>	p	OR	C.I. <sub>95%</sub>	p	OR	C.I. <sub>95%</sub>	p	OR	C.I. <sub>95%</sub>
NS vs. OPS	NS	0.74	1.11	0.59;2.11	0.17	0.87	0.71;1.06	<b>0.002</b>	1.39	1.13;1.71	<b>0.049</b>	0.81	0.67;0.99	< <b>0.001</b>	1.40	1.23;1.60
	OPS		1	–		1	–		1	–		1	–		1	–
TS vs. NS	TS	0.34	0.72	0.37;1.4	< <b>0.001</b>	1.65	1.35;2.02	< <b>0.001</b>	0.70	0.58;0.86	< <b>0.001</b>	1.43	1.16;1.77	0.48	1.05	0.92;1.18
	NS		1	–		1	–		1	–		1	–		1	–
TS vs. OPS	TS	0.58	0.8	0.37;1.73	< <b>0.001</b>	1.43	1.24;1.66	0.87	0.98	0.79;1.21	<b>0.046</b>	1.17	1.00;1.37	< <b>0.001</b>	1.47	1.29;1.67
	OPS		1	–		1	–		1	–		1	–		1	–

Bold values are values with a p < 0.05.

**Table 7**

Adjusted frequencies (with 95% confidence interval) of *M. hyopneumonitiae* (Mhp), *A. pleuropneumonitiae* serogroup 1-9-11 (App1-9-11) and serotype 2 (App2) detection in breeding sows followed in five farrow-to-finish herds (184 sows, 8 sampling times, ELISA assays, Generalized Estimation Equations models with an autoregressive correlation matrix).

Herd	Mhp	App1-9-11	App2
A	82.5 <sub>a</sub> (51.6;95.4)	36.9 <sub>a</sub> (8.9;76.1)	65.2 <sub>a</sub> (22.9;100)
B	69.3 <sub>a,b</sub> (57.6;78.9)	24.3 <sub>a</sub> (13.9;37.6)	31.6 <sub>b,c</sub> (19.2;47.6)
C	83.5 <sub>a</sub> (57.1;95.1)	100 <sub>b</sub> (77.1;100)	73.0 <sub>a</sub> (33.6;100)
D	79.9 <sub>a</sub> (51.2;93.8)	29.6 <sub>a</sub> (5.4;69.2)	14.4 <sub>b</sub> (0.2;58.2)
E	55.4 <sub>b</sub> (24.8;82.4)	65.7 <sub>c</sub> (27.8;95.3)	35.1 <sub>c</sub> (10.2;75.5)

Herds with different subscripts (a, b and c) have a significantly different PCR-prevalence (p < 0.05, Generalized Estimation Equations model).

site where each bacteria was likely to be recovered (Møller et al., 1993; Pijoan, 1999; Rapp-Gabrielson, 1999; Marois et al., 2004; Fablet et al., 2010). On the other hand, as no test is perfect, sensitivity should be increased by sampling several sites. Therefore, for each bacterium, samples from all three sites were tested by PCR to enhance the likelihood of detecting positive animals. The carrier state was hence studied at the animal level.

The herds included in this study were not selected at random and may not be representative of the population of French farrow-to finish pig herds. However, they were selected to tally with the different respiratory patterns in growing-finishing pigs most frequently encountered by veterinarians in the field. Their inclusion was based on the results of a preliminary screening step involving 12 herds which were clinically examined and tested for the different agents under investigation. Hence, they were supposed to represent the different categories of herds identified in this screening step as suffering from different levels of severity and complexity of respiratory diseases. As all sows from a batch, selected at random, were included in the study, the cohorts should be appropriate representatives of the herds under study and thereby provide a valid picture of the infection patterns and even their variability between herds. However, one limitation of the study relies on the kind of production system sampled which may not be representative of farming types in use in all other pig producing countries.

To the best of our knowledge, this is the first time that a comprehensive description of the microbial flora of the airways of naturally infected sows has been obtained under field conditions. We showed that the upper part of the airways of clinically healthy sows harboured a variety of respiratory pathogens such as *S. suis*, *A. pleuropneumonitiae*, *P. multocida* and *H. parasuis*. This is in agreement with results of previous studies indicating that these bacteria are common inhabitants of the upper respiratory tract of asymptomatic pigs raised under commercial conditions (Dritz et al., 1996; Baele et al., 2001; MacInnes et al., 2008). Even if for *H. parasuis*, *A. pleuropneumonitiae*, *S. suis* and *P. multocida* only a limited number of serotypes are known to be highly pathogenic, PCR assays detecting species of pathogens were used as general descriptors of the infectious status towards the main bacteria species involved in respiratory diseases. An overall low pathogen load in a herd being recommended, the aim was to assess, at the pathogen species level, whether different infection



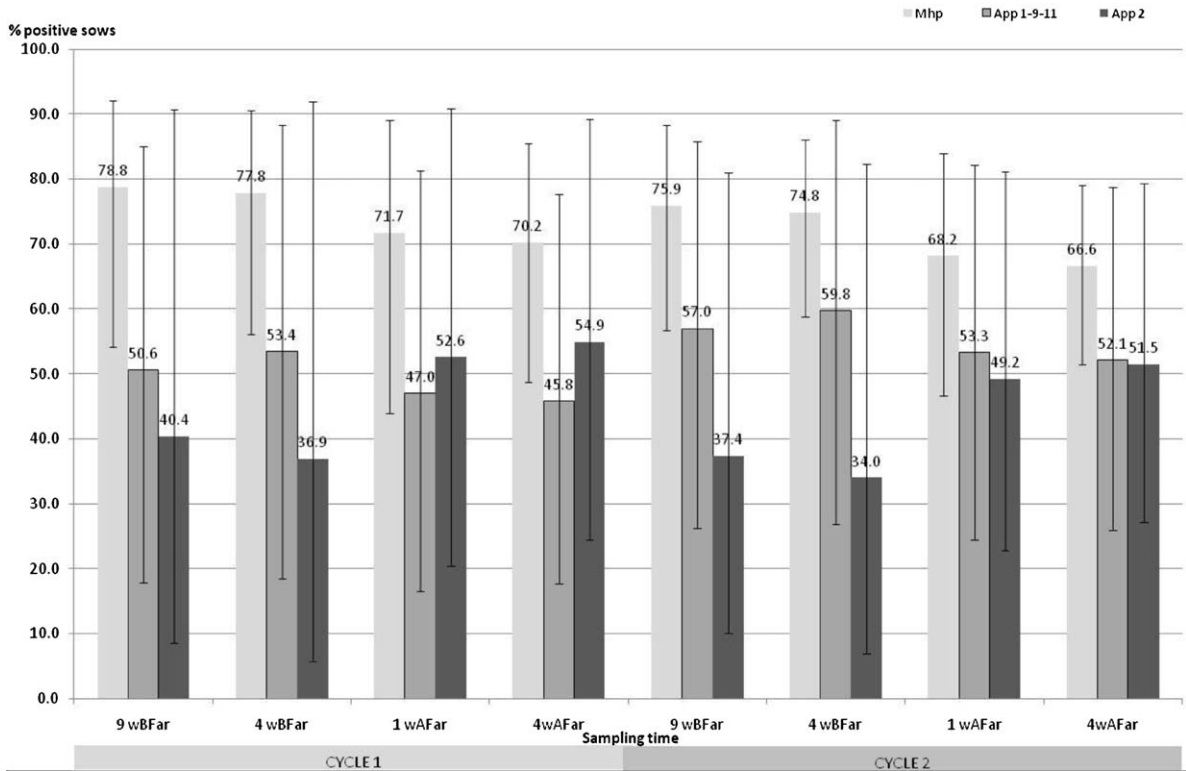


Fig. 2. Adjusted within herd antibody detection frequency (with 95% confidence interval) of *M. hyopneumoniae* (Mhp), *A. pleuropneumoniae* serogroup 1-9-11 (App1-9-11) and serotype 2 (App2) during two successive gestation and lactation periods (cycles 1 and 2) (5 farrow-to-finish herds, 184 sows, 8 sampling times, ELISA assays, Generalized Estimation Equations with an exchangeable correlation matrix). 9wBFar: 9 weeks before farrowing; 4wBFar: 4 weeks before farrowing; 1wAFar: 1 week after farrowing; 4wAFar: 4 weeks after farrowing.

pressure may be identified and were related to the overall respiratory health status of the farm. The results of our study in sows indicated that whatever the pathogen, its prevalence varied between farms and over time within

herds. As only a small percentage of sows (<10%) was consistently detected positive to a given pathogen at all 8 sampling times, it can be hypothesized that the pathogen might transiently be present in small quantities, i.e., under

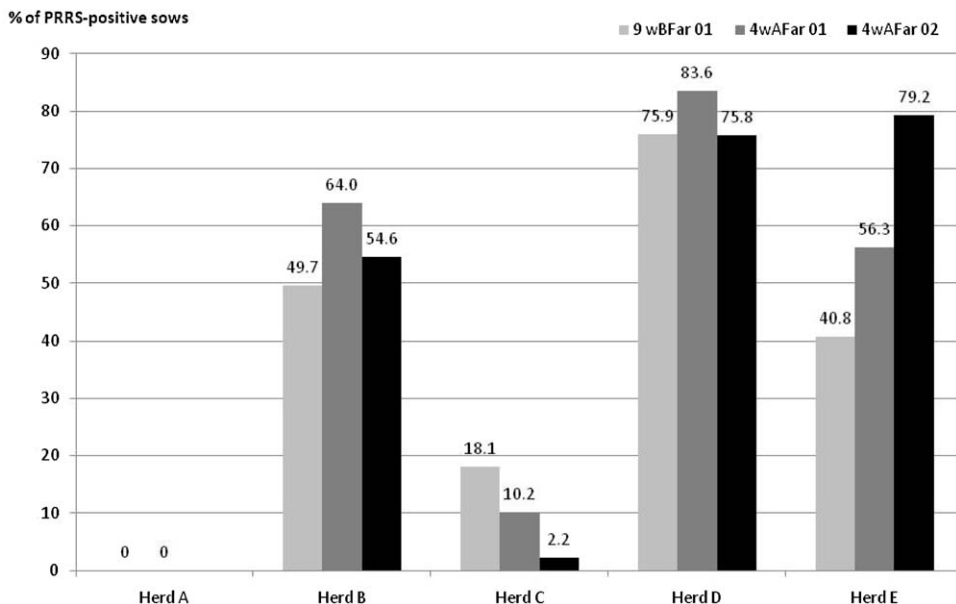


Fig. 3. Frequency of PRRS-seropositive sows during gestation and lactation phases in five farrow-to-finish herds (184 sows, 3 sampling dates). wBFar01: 9 weeks before farrowing, replicate 01; 4wAFar01: 4 weeks after farrowing, replicate 01; 4wAFar02: 4 weeks before farrowing, replicate 02.

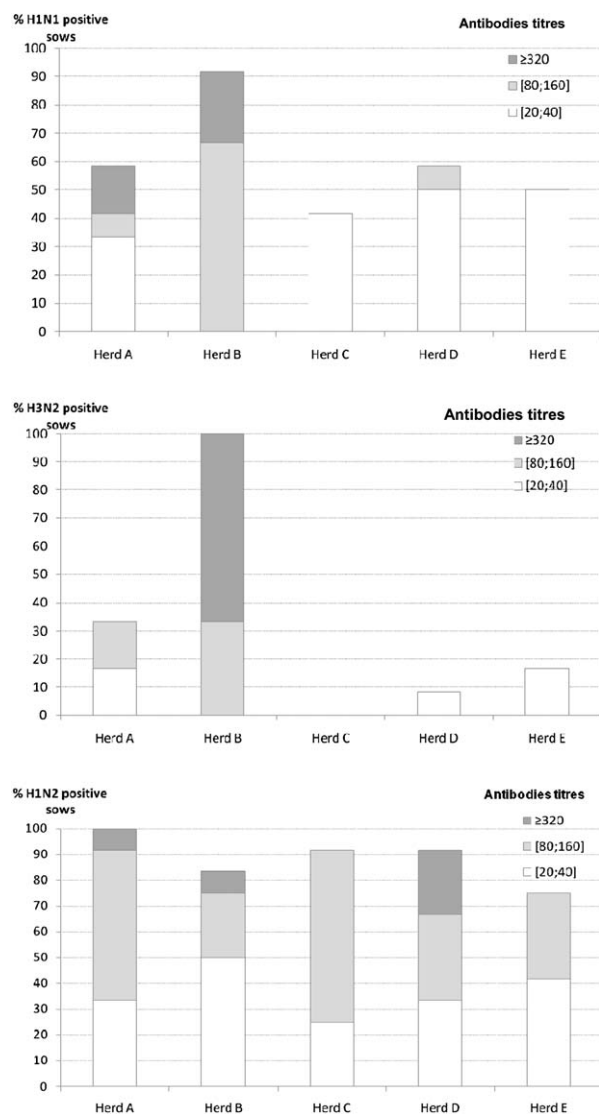


Fig. 4. Distribution of the frequency of sows with SIV antibodies and antibodies titres for subtypes H<sub>1</sub>N<sub>1</sub>, H<sub>1</sub>N<sub>2</sub> and H<sub>3</sub>N<sub>2</sub> 4 weeks after farrowing in five farrow-to-finish pig herds (12 sows/herd).

the detection threshold of the PCR assay in the upper respiratory tract of the sows, and that shedding was intermittent in most of the animals. While *S. suis*, *A. pleuropneumoniae*, *P. multocida* and *H. parasuis* were the most frequent pathogens identified in the upper part of the airways of the sows, these pathogens were seldom detected in lung tissues of slaughtered pigs during the recruitment step. *M. hyopneumoniae* was indeed the only pathogen detected at slaughter from lungs of finishing pigs in all five herds. Even if the slaughtered sampled pigs were not the offspring of the sows followed in the present study, we can assume that other factors than the bacterial carrier state of the upper part of the respiratory tract of the sows may influence the infectious status of the lung in growing-finishing pigs.

*M. hyopneumoniae* plays a central role in PRDC and is widespread in pig herds (Thacker, 2006). Interestingly, even

if the *M. hyopneumoniae* genome was detected in four out of the five selected herds, the micro-organism was relatively seldom identified at the sow level. The detection threshold of the simple PCR was estimated to be 10–100-fold higher than that of nested-PCR (Marois et al., 2007). Therefore, this could have limited our capacity to detect sows carrying low numbers of *M. hyopneumoniae* (<1000 mycoplasma). The true prevalence of positive animals may in turn have been underestimated. On the other hand, when studying infection dynamics, the bacterial load carried by the animals is more important than a positive shedding status. Further studies using quantitative tests are needed to improve our knowledge on the course of *M. hyopneumoniae* infection under field conditions. Although *M. hyopneumoniae* DNA was not consistently detected, antibodies against the micro-organism were recovered in sows from all herds. Positive reactions in serum from sows may reflect previous exposure to *M. hyopneumoniae*, as vaccination was not carried out in the investigated herds. Interestingly, in the herd that showed the lowest number of seropositive sows, *M. hyopneumoniae* carriage was not detected at any sampling time. It can be speculated that *M. hyopneumoniae* infection dynamics within the sow population remained limited in this farm. The fact that *M. hyopneumoniae* was detected in the upper respiratory tract of seropositive sows, indicated, as already reported (Sibila et al., 2007), that circulating antibodies against *M. hyopneumoniae* did not prevent colonization of the airways. *M. hyopneumoniae* DNA was detected in sows from the first until the tenth parity suggesting that even the oldest sows may carry the organism and have the potential to spread the bacteria to susceptible animals, especially their offspring. In previous studies, relationships between sow parity and *M. hyopneumoniae* infection were inconsistent (Calsamiglia and Pijoan, 2000; Ruiz et al., 2003; grosse Beilage et al., 2009). Our study, being primarily descriptive, may lack sufficient power to find any significant association. Thus, further research seems to be required to conclude about the influence of sow age on *M. hyopneumoniae* infection, but, due to the expected low prevalence of carrier sows, altogether, these results emphasize the need to sample a large number of sows of different parity groups when assessing *M. hyopneumoniae* infection dynamics.

The odds for a sow being seropositive for *A. pleuropneumoniae* serogroup 1-9-11 and serotype 2 were significantly lower in first and second parity than in third or more parity sows. These findings indicate that seroconversion is more likely to occur during the first two reproduction cycles and suggest that young sows are exposed to the micro-organism following their introduction in the breeding herd. Antibodies to the two considered serotypes were found in all herds. While the high seropositivity levels observed in herd C could be related to the vaccination, the antibodies detected in the four non-vaccinated herds indicated that sows were naturally infected. This also suggested that the infection is widespread among clinically healthy sow herds as reported for *A. pleuropneumoniae* serotype 2 (Levonen et al., 1994). It should be noted that the infection pressure differed between farms, as revealed by the varying levels of seroprevalence between herds.

As expected, the cluster herd effect had a significant influence on infection status of the sows for all the examined pathogens which demonstrated variation between farms as a result of differences in farm layout and management practices. This is in line with a cross-sectional study showing that *M. hyopneumoniae* seropositivity in sows is influenced by various management factors (grosse Beilage et al., 2009). According to the results of the present study, the carrier state or serological status of the sows did not seem to be affected by whether they were in gestation or lactation. A decrease in the level of *M. hyopneumoniae* serum antibodies during the last month of pregnancy to the farrowing day was previously described (Wallgren et al., 1998). Since the last sampling time during pregnancy was performed 4 weeks before farrowing, our sampling scheme may have limited the ability to detect such a reduction. Whatever the micro-organism, a specific physiological stage with a maximum prevalence of carrier sows was not identified. Therefore, sampling sows at different gestation and lactation stages should be recommended, especially in cross-sectional studies looking for prevalence. On the other hand, the carrying patterns of the same batches of sows, especially for *H. parasuis* and *S. suis*, were different from the first to the second reproduction cycle suggesting a time effect on the level of carriage for a given batch. This tended to indicate that a batch effect is important to take into account in epidemiological studies as well as in diagnostic.

The seroprevalence of PCV-2 infection was high in the four herds we tested, hence confirming the widespread occurrence of PCV-2 in French sow herds as described earlier (Rose et al., 2003). Serological investigations also showed that SIV antibodies were highly prevalent in the sows which is consistent with the fact that viruses of H<sub>1</sub>N<sub>1</sub> and H<sub>1</sub>N<sub>2</sub> European subtypes are widespread in pig populations in France (Kuntz-Simon and Madec, 2009). HI tests revealed infections with H<sub>1</sub>N<sub>1</sub> and/or H<sub>1</sub>N<sub>2</sub> viruses, while H<sub>3</sub>N<sub>2</sub> antibodies could only be detected in vaccinated sows or as a result of cross-reactions in the HI test as already reported (Van Reeth et al., 2006). The infection pattern of the sow batches towards PRRSV differed between the five herds in which the growing pigs were experiencing different levels of severity of respiratory disorders. While in herd A, being the less affected by respiratory diseases, PRRSV infection was not detected in the sows during the study, an increase in the frequency of PRRSV-seropositive sows was found in the herd the most severely affected by lung diseases in finishing pigs (herd E). This increase in seropositivity frequency indicated that PRRSV was actively circulating in the sow herd. A higher and stable frequency of PRRSV infection was recovered in the second herd with growing pigs severely affected by respiratory diseases. Conversely a lower and decreasing rate of infected sows was described in herd C, suggesting that the virus was no longer actively spreading in the sow population. In the latter herd, the growing-finishing pigs which were tested remained sero-negative throughout the course of the study (data not shown). In herd B, despite the use of a commercial inactivated vaccine against PRRSV, from 50% to 64% of the sows tested positive by serology. Under experimental and field conditions, some variations in seropositivity rates following vaccination with a killed

vaccine have been described (Reynaud et al., 1999). In the same line, Papatsiros et al. (2006) found that the humoral response of sows following two vaccinations was short, lasting 40 days after the booster vaccination. In the present study, the antibodies detected 72 days after the vaccination were probably related to active infection. In addition, the PRRSV antibodies detected in growing pigs confirmed that this herd was not virus-free.

In conclusion, the results of the present study indicate that in conventional commercial farrow-to-finish herds, the relevant infectious agents involved in PRDC are present in the sow herd, with limited consequences on the health status of the sows, and thus confirms that the reproductive herd constitutes a reservoir for the continuous circulation of respiratory pathogens. Therefore, these results suggest that the sow population needs to be properly considered in the measures taken to control respiratory diseases. However, further studies may help to know more about the factors that influence the infection process in sow herds and to evaluate the consequences on the infection dynamics in their offspring.

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