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Title: Longitudinal study of respiratory infection patterns of breeding sows in 5 farrow-to-finish herds

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32 of European lineages were tested by hemagglutination inhibition assay. The results indicated that *S.*  
33 *suis* is widespread among sows (67.1% of PCR-positive sows). *A. pleuropneumoniae*, *P. multocida*,  
34 and *H. parasuis* were detected by PCR in 30.9%, 24.6% and 23.4% of the sows respectively.  
35 Antibodies against *M. hyopneumoniae* were recovered from more than 55% of the sows in all herds  
36 whereas the micro-organism was detected in 2.4% of the sows. Although PCV-2 and SIV infections  
37 were highly prevalent, the PRRSV infection patterns ranged from no infection in farms mildly affected  
38 by respiratory diseases to active circulation in more severely affected herds. The sow population thus  
39 constitutes a reservoir for a continuous circulation of respiratory pathogens and needs to be properly  
40 considered in control strategies.

41

42 Keywords: respiratory diseases, sows, pathogen detection, infection dynamics

43

## 44 1. Introduction

45

46 Respiratory diseases are some of the most common and costly diseases affecting growing finishing  
47 pigs reared under intensive confined conditions. Several bacterial and viral agents are involved in the  
48 pathogenesis of these diseases. *Mycoplasma hyopneumoniae*, most frequently in combination with  
49 other bacteria like *Pasteurella multocida*, *Haemophilus parasuis*, *Streptococcus suis* and  
50 *Actinobacillus pleuropneumoniae*, or viruses such as Swine Influenza Virus (SIV), Porcine  
51 Reproductive and Respiratory Syndrome virus (PRRSV), Porcine Circovirus type 2 (PCV-2) and  
52 Porcine Respiratory Coronavirus (PRCV) are the most important micro-organisms responsible for lung  
53 diseases (Sorensen et al., 2006). Together they are involved in the Porcine Respiratory Disease  
54 Complex (PRDC).

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

74

## 75 2. Material and methods

### 76 2.1. Herds selection

77 A longitudinal study was carried out in five single site farrow-to-finish herds located in Brittany  
78 (Western France). The farms were included on the basis of the veterinarian's knowledge of the farm  
79 and the results of an initial screening of 12 herds. Five herds were selected for different patterns  
80 regarding their respiratory health status in growing finishing pigs and to tally with the most common  
81 herd health patterns, housing and herd management practices encountered in the field at that time.  
82 They were also selected for convenience as the farmer had to agree with our protocol. The screening  
83 visit took place two to three months before the present longitudinal field study was started. Clinical  
84 signs of respiratory disorders in the farrowing, post-weaning and finishing sections were recorded.  
85 Nasal, tonsillar and oro-pharyngeal swabs and blood samples were obtained from 30 pigs: 10 in the  
86 post-weaning section, 10 at the beginning and 10 at the end of the finishing phase. Lungs from at least  
87 30 of the sampled finishing pigs were collected at the slaughterhouse. Macroscopic lung lesions of  
88 pneumonia were scored (Madec and Kobisch, 1982) and samples of lung lesions were taken. Swabs  
89 and lung samples were examined by PCR in our laboratory to detect *M. hyopneumoniae*, *P.*  
90 *multocida*, *A. pleuropneumoniae*, *H. parasuis* and *S. suis*. All sera were analysed for the detection of  
91 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>).

92 The retained farms differed in terms of clinical severity, infection profile, and the type and extent of  
93 lung lesions (Table 1). Disease severity and complexity showed a gradual increase from farms A to E.  
94 Herd A was mildly affected by respiratory diseases, according to clinical diagnosis and macroscopic  
95 lung lesions examination. The growing-finishing pigs were free from PRRSV and SIV. The lowest level  
96 of lung lesions in the 5 herds, was recorded in herd A. Herds B and C were endemically infected by  
97 PRRSV and differed in lung lesion severity, the finishing pigs in farm C being more affected by  
98 pneumonia. The last 2 farms (herds D and E) were the most severely affected by lung lesions as  
99 pneumonia and pleuritis were detected in more than 90 and 25% of the pigs, respectively. The  
100 finishing pigs were PRRSV and SIV positive. Whereas the pigs from herd D tested positive for H<sub>1</sub>N<sub>1</sub>  
101 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>).

### 102 2.2. Animals and sampling scheme

103 The herds were managed all-in all-out by room in the farrowing, post-weaning and fattening sections  
104 with a 3-week batch interval between farrowing for 3 farms, 4-week interval for herd A and 1-week

105 interval for herd B. The main features of the five herds are detailed in Table 2. After checking for  
106 pregnancy, the sows in each herd were housed in a gestation room and were then moved to farrowing  
107 rooms about one week before the expected farrowing time. The piglets were weaned around 4 weeks  
108 after farrowing except in farms A and C where the piglets were weaned at 3 weeks of age. During the  
109 gestation and lactation periods, the sows were housed in closed facilities with mechanical ventilation  
110 and slatted floor. The sows in the selected herds were not vaccinated against *M. hyopneumoniae*  
111 during the study. Whether the sows were vaccinated earlier in life in the multiplier herd was unknown.  
112 The sows in herd C were vaccinated against *A. pleuropneumoniae*. The breeding sows in herd B were  
113 vaccinated against PRRSV and SIV. The same commercial SIV vaccine (antigens A/New Jersey/8/76  
114 (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  
115 used in this herd during the study. An inactivated PRRSV vaccine was used for the gilts in herd E. All  
116 farms except E vaccinated the sows and gilts against atrophic rhinitis.  
117 All farms were visited eight times. An entire batch of pregnant sows in each herd was followed during  
118 two successive gestation and lactation periods. The sows were sampled twice during gestation, nine  
119 and four weeks before the expected farrowing date, and twice during the farrowing phase, then one  
120 and four weeks after farrowing. Prior to the first visit, the sows were individually identified (ear tag) by  
121 the farmer. Clinical observations and samplings were made by four specially trained operators from  
122 our laboratory throughout the study. To reduce the bias due to the observer, each operator was  
123 specialized in an activity: animal restraining (1), sow's mouth opening (1), swabbing (1) and blood  
124 sampling and clinical observations (1).

### 125 **2.3. Sample collection**

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

135 identified and delivered to the laboratory for processing on the day of collection (Initial Suspension:  
136 IS).

137 Blood samples were collected by jugular vein puncture, using evacuated Tubes (Vacurette, Dutscher  
138 SAS, Brumath, France) without additive. The samples were individually identified, and delivered to the  
139 laboratory, where they were processed. Serum was obtained by centrifugation for 10 min at 3500g  
140 and stored at -20°C until subsequent analysis.

## 141 **2.3. Laboratory analyses**

### 142 **2.3.1 PCRs on swab samples for bacterial pathogen DNA detection**

143 Briefly, 1 ml of each IS was centrifuged (12.000g, 20 min) and the pellets were resuspended in 800 µl  
144 of lysis solution. The remaining IS was stored at -70°C with glycerol (20%) for future studies. Lysates  
145 were incubated for 1 h at 60°C, 10 min at 95°C and then kept at -20°C until PCR analysis. All swabs  
146 were tested by PCR, as previously described, for DNA detection of *M. hyopneumoniae* (Marois et al.,  
147 2007), *A. pleuropneumoniae* (Schaller et al., 2001), *P. multocida* (Marois et al., 2008), *S. suis* (Marois  
148 et al., 2004) and *H. parasuis* (Oliveira et al., 2001). When an inhibition of the PCR reaction was  
149 observed, DNA was re-extracted from the lysates with phenol/chloroform/isoamyl(ic) alcohol (25/24/1)  
150 (Marois et al., 2004). A sample was considered positive to a pathogen when the specific PCR tested  
151 positive.

### 153 **2.3.2 Serological analyses**

154 All sera were tested for *M. hyopneumoniae* antibodies with a blocking enzyme-linked immunosorbent  
155 assay (ELISA), according to the manufacturer's instructions (DAKO ELISA, Kitvia, Labarthe-Inard,  
156 France). The inhibition percentage (IP) for each serum was calculated with the following formula:  
157  $IP=100-[100 \times (\text{sample mean OD} / \text{buffer control mean OD})]$ . A sample was classified as positive if the IP  
158 value was >50%. The sensitivity and specificity of the ELISA were previously reported to be 100% and  
159 100% respectively, in experimental trials (Sørensen et al., 1997). Sera were also analysed to detect *A.*  
160 *pleuropneumoniae* antibodies serotype 2 and serogroup 1-9-11 using 2 ELISA commercial kits  
161 (Swinecheck App2 and Swinecheck App1911, Biovet, AES Laboratoire, Combourg, France). The  
162 positive threshold was fixed at an optical density (OD) of 0.5 and 0.55 for serotype 2 and serogroup 1-  
163 9-11 respectively. The sensitivity and specificity of the two ELISA tests were 77.8% and 97.4% and  
164 89.5% and 95.1%, respectively, according to the manufacturer.



165 Sera of all herds, except herd A, were tested for PCV-2 antibodies using an ORF-2 protein based  
166 ELISA test (Blanchard et al., 2003) at 4 sampling dates: one and four weeks post partum at the first  
167 reproduction cycle and nine and four weeks before farrowing in the second cycle. An OD ratio of 1.5  
168 served as positivity cut-off. The sensitivity and specificity of the PCV-2 ELISA were 98.2% and 94.5%  
169 respectively. PRRS virus antibodies were tested (ELISA test, IDDEX laboratory, Eragny, sur Oise,  
170 France) in sera of every herd at 3 sampling dates: nine weeks before farrowing in the first reproduction  
171 cycle and four weeks after farrowing in both cycles. The sensitivity and specificity of the PRRS ELISA  
172 were 97.4% and 99.6% respectively. During the first reproduction cycle of each herd, random samples  
173 of sera obtained one week post partum from 12 sows were tested with an hemagglutination inhibition  
174 (HI) test (Van Reeth et al., 2008) for antibodies against European SIVs, using strains  
175 A/Sw/Finistere/2899/82 (H<sub>1</sub>N<sub>1</sub>), A/Sw/Gent/1/84 (H<sub>3</sub>N<sub>2</sub>) and A/Sw/Scotland/410440/94 (H<sub>1</sub>N<sub>2</sub>) as antigens.  
176 An animal was considered positive against a given SIV subtype when the HI antibody titre was 20 or  
177 more (Van Reeth et al., 2008).

178

#### 179 **2.4. Statistical analysis**

180 The contamination and infection status was considered at the sow level. At each sampling date, an  
181 animal was considered contaminated by a bacterial pathogen when at least one out of the three  
182 samples tested positive by specific PCR assay. Generalized linear models using the Generalized  
183 Estimation Equations (GEE) method were used to assess the influence of parity, farm, physiological  
184 stage and cycle on the sow contamination status determined by PCR assays and the infection status  
185 determined by serological assays (*M. hyopneumoniae* and *A. pleuropneumoniae*) (proc genmod, SAS  
186 9.1, SAS Inst Inc.). The influence of the sampling site on the sow contamination status was also  
187 assessed using GEE models. Since the data consisted in repeated measurements on the same  
188 animals over time and space, observations could not be considered as independent. Sow was taken  
189 as a repeated statement to assess the relationships between the dependent variable and parity, farm  
190 and sampling site whereas a farm cluster effect was considered in models assessing the influence of  
191 the physiological stage and cycle on the dependent variable. The score statistics for type-3 GEE  
192 analysis was used to test the significance of main effects ( $p < 0.05$ ). GEE models were also used to  
193 estimate the frequency of positive animals by PCR and serological assays as regards bacterial  
194 pathogens throughout the survey period. Within-herd seroprevalences were adjusted according to  
195 sensitivity and specificity of ELISA assays.

196 **3. Results**197 **3.1. PCR analyses for bacterial pathogens detection**

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

206 DNA of *M. hyopneumoniae*, *H. parasuis*, *P. multocida*, *A. pleuropneumoniae* and *S. suis* were  
207 detected from at least one out of the three swab samples in 2.4% (Confidence Interval at 95% (C.I.<sub>95%</sub>)  
208 (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  
209 67.1% (C.I.<sub>95%</sub>: (63.8;70.2)) of the sows respectively. Whatever the micro organism, the model-based  
210 estimated frequency of positive animals varied from farm to farm (Table 4). In all five herds, all the  
211 pathogens, except *M. hyopneumoniae* were detected at least once in the upper respiratory tract of the  
212 sows. *S. suis* was the pathogen the most frequently recovered in all herds, with more than 40% of  
213 positive sows. To a lesser extent *A. pleuropneumoniae*, *P. multocida* and *H. parasuis* were also  
214 frequently detected in almost all herds with herd levels ranging from 11.6 to 48%, 8.4 to 45% and 4.7  
215 to 50% of positive sows, respectively.

216 In the sub sample of 109 sows which were swabbed during all eight visits, for all the sows found  
217 positive to *M. hyopneumoniae*, the DNA of the organism was detected at one sampling occasion,  
218 whereas genomes of *P. multocida*, *H. parasuis*, *A. pleuropneumoniae* and *S. suis* were detected on 2  
219 sampling occasions or more for 60.4%, 66.3%, 75.5% and 98.1% of the positive sows respectively.  
220 Whatever the micro-organism, the frequency of positive animals in the herd varied over time, without  
221 any significant effect of the physiological stage (Figure 1 and Table 5) whereas a significant effect of  
222 the reproduction cycle was observed for *S. suis* and *H. parasuis*. A significant effect of the farm on the  
223 sow contamination status of the upper respiratory tract was identified for each micro-organism  
224 ( $p<0.05$ , Table 5), except for *M. hyopneumoniae* which could not be estimated due to the absence of  
225 detection in some farms. The ability to detect contaminated sows was significantly influenced by the  
226 sampling site for four out of the five bacteria (Table 5). Swabbing the tonsils significantly increased the

227 probability to detect sows contaminated by *A. pleuropneumoniae* and *H. parasuis* than sampling the  
228 nasal cavities or the oro-pharyngeal area ( $p < 0.05$ , Table 6). The detection of *P. multocida* PCR  
229 positive sows was significantly improved when nasal swabs were used ( $p < 0.05$ , Table 6). The odds for  
230 a sow being PCR-positive to *S. suis* by oro-pharyngeal swabbing was significantly lower than by nasal  
231 or tonsillar sampling ( $p < 0.05$ , Table 6).

232

### 233 3.2. Serological analyses

#### 234 3.2.1. Bacterial pathogens

235 Sows seropositive to *M. hyopneumoniae*, *A. pleuropneumoniae* serogroups 1-9-11 and 2 were  
236 detected in all five herds at different frequencies depending on the farm (Table 7). The odds of sows  
237 from herd E being *M. hyopneumoniae* seropositive were significantly lower than those of herds A, C  
238 and D ( $p < 0.05$ ). The odds of being seropositive to *A. pleuropneumoniae* serogroup 1-9-11 was  
239 significantly higher in herds C and E than in the three other herds whereas for serotype 2 it was higher  
240 in herds A and C. The lowest proportions of seropositive sows to *A. pleuropneumoniae* serogroup 1-9-  
241 11 and serotype 2 were found in herds A, B, D and D respectively.

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

#### 248 3.2.2. Viral pathogens

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

255 All herds tested positive for SIV by HI assays (Figure 4). The highest frequencies of SIV-seropositive  
256 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  
257 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

258 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  
259 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  
260 those of the most prevalent subtype were detected in herd A in which the sows used to be vaccinated  
261 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>  
262 subtype observed in herds D and E were considered to be due to cross-reactions in the HI test.  
263 PCV-2 antibodies were detected in more than 75% of sows in the four tested herds at one and four  
264 weeks after farrowing for the first reproduction cycle and at nine and four weeks before farrowing for  
265 the second cycle.

266

#### 267 **4. Discussion**

268 The aim of the present study was to describe the carrying and infection patterns of batches of sows for  
269 various respiratory pathogens during two successive gestation and lactation periods. Three sampling  
270 sites in the upper respiratory tract, *i.e.*, nasal cavities, tonsils, oro-pharyngeal area, were chosen to  
271 describe the carrier state of the animals regarding five bacteria involved in PRDC. The sampling areas  
272 were selected according to the site where each bacteria was likely to be recovered (Møller et al.,  
273 1993; Pijoan, 1999; Rapp-Gabrielson, 1999; Marois et al., 2004; Fablet et al., 2010). On the other  
274 hand, as no test is perfect, sensitivity should be increased by sampling several sites. Therefore, for  
275 each bacterium, samples from all three sites were tested by PCR to enhance the likelihood of  
276 detecting positive animals. The carrier state was hence studied at the animal level.

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

288 To the best of our knowledge, this is the first time that a comprehensive description of the microbial  
289 flora of the airways of naturally infected sows has been obtained under field conditions. We showed  
290 that the upper part of the airways of clinically healthy sows harboured a variety of respiratory  
291 pathogens such as *S. suis*, *A. pleuropneumoniae*, *P. multocida* and *H. parasuis*. This is in agreement  
292 with results of previous studies indicating that these bacteria are common inhabitants of the upper  
293 respiratory tract of asymptomatic pigs raised under commercial conditions (Dritz et al., 1996; Baele et  
294 al., 2001; MacInnes et al., 2008). Even if for *H. parasuis*, *A. pleuropneumoniae*, *S. suis* and *P.*  
295 *multocida* only a limited number of serotypes are known to be highly pathogenic, PCR assays  
296 detecting species of pathogens were used as general descriptors of the infectious status towards the  
297 main bacteria species involved in respiratory diseases. An overall low pathogen load in a herd being  
298 recommended, the aim was to assess, at the pathogen species level, whether different infection  
299 pressure may be identified and were related to the overall respiratory health status of the farm. The  
300 results of our study in sows indicated that whatever the pathogen, its prevalence varied between farms  
301 and over time within herds. As only a small percentage of sows (<10%) was consistently detected  
302 positive to a given pathogen at all eight sampling times, it can be hypothesized that the pathogen  
303 might transiently be present in small quantities, *i.e.*, under the detection threshold of the PCR assay in  
304 the upper respiratory tract of the sows, and that shedding was intermittent in most of the animals.  
305 While *S. suis*, *A. pleuropneumoniae*, *P. multocida* and *H. parasuis* were the most frequent pathogens  
306 identified in the upper part of the airways of the sows, these pathogens were seldom detected in lung  
307 tissues of slaughtered pigs during the recruitment step. *M. hyopneumoniae* was indeed the only  
308 pathogen detected at slaughter from lungs of finishing pigs in all five herds. Even if the slaughtered  
309 sampled pigs were not the offspring of the sows followed in the present study, we can assume that  
310 other factors than the bacterial carrier state of the upper part of the respiratory tract of the sows may  
311 influence the infectious status of the lung in growing finishing pigs.

312 *M. hyopneumoniae* plays a central role in PRDC and is widespread in pig herds (Thacker, 2006).  
313 Interestingly, even if the *M. hyopneumoniae* genome was detected in four out of the five selected  
314 herds, the micro-organism was relatively seldom identified at the sow level. The detection threshold of  
315 the simple PCR was estimated to be ten to one hundred fold higher than that of nested-PCR (Marois  
316 et al., 2007). Therefore this could have limited our capacity to detect sows carrying low numbers of *M.*  
317 *hyopneumoniae* (<1000 mycoplasma). The true prevalence of positive animals may in turn have been  
318 underestimated. On the other hand, when studying infection dynamics, the bacterial load carried by

319 the animals is more important than a positive shedding status. Further studies using quantitative tests  
320 are needed to improve our knowledge on the course of *M. hyopneumoniae* infection under field  
321 conditions. Although *M. hyopneumoniae* DNA was not consistently detected, antibodies against the  
322 micro-organism were recovered in sows from all herds. Positive reactions in serum from sows may  
323 reflect previous exposure to *M. hyopneumoniae*, as vaccination was not carried out in the investigated  
324 herds. Interestingly, in the herd that showed the lowest number of seropositive sows, *M.*  
325 *hyopneumoniae* carriage was not detected at any sampling time. It can be speculated that *M.*  
326 *hyopneumoniae* infection dynamics within the sow population remained limited in this farm. The fact  
327 that *M. hyopneumoniae* was detected in the upper respiratory tract of seropositive sows, indicated, as  
328 already reported (Sibila et al., 2007), that circulating antibodies against *M. hyopneumoniae* did not  
329 prevent colonization of the airways. *M. hyopneumoniae* DNA was detected in sows from the first until  
330 the tenth parity suggesting that even the oldest sows may carry the organism and have the potential to  
331 spread the bacteria to susceptible animals, especially their offspring. In previous studies, relationships  
332 between sow parity and *M. hyopneumoniae* infection were inconsistent (Calsamiglia and Pijoan, 2000;  
333 Ruiz et al., 2003; grosse Beilage et al., 2009). Our study, being primarily descriptive, may lack  
334 sufficient power to find any significant association. Thus, further research seems to be required to  
335 conclude about the influence of sow age on *M. hyopneumoniae* infection, but, due to the expected low  
336 prevalence of carrier sows, altogether, these results emphasize the need to sample a large number of  
337 sows of different parity groups when assessing *M. hyopneumoniae* infection dynamics.

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

348 As expected, the cluster herd effect had a significant influence on infection status of the sows for all  
349 the examined pathogens which demonstrated variation between farms as a result of differences in

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

364 The seroprevalence of PCV-2 infection was high in the four herds we tested, hence confirming the  
365 widespread occurrence of PCV-2 in French sow herds as described earlier (Rose et al., 2003).  
366 Serological investigations also showed that SIV antibodies were highly prevalent in the sows which is  
367 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  
368 populations in France (Kuntz-Simon and Madec, 2009). HI tests revealed infections with H<sub>1</sub>N<sub>1</sub> and/or  
369 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-  
370 reactions in the HI test as already reported (Van Reeth et al., 2006). The infection pattern of the sow  
371 batches towards PRRSV differed between the five herds in which the growing pigs were experiencing  
372 different levels of severity of respiratory disorders. While in herd A, being the less affected by  
373 respiratory diseases, PRRSV infection was not detected in the sows during the study, an increase in  
374 the frequency of PRRSV-seropositive sows was found in the herd the most severely affected by lung  
375 diseases in finishing pigs (herd E). This increase in seropositivity frequency indicated that PRRSV was  
376 actively circulating in the sow herd. A higher and stable frequency of PRRSV infection was recovered  
377 in the second herd with growing pigs severely affected by respiratory diseases. Conversely a lower  
378 and decreasing rate of infected sows was described in herd C, suggesting that the virus was no longer  
379 actively spreading in the sow population. In the latter herd, the growing-finishing pigs which were  
380 tested remained sero-negative throughout the course of the study (data not shown). In herd B, despite

381 the use of a commercial inactivated vaccine against PRRSV, from 50 to 64% of the sows tested  
382 positive by serology. Under experimental and field conditions, some variation in seropositivity rates  
383 following vaccination with a killed vaccine have been described (Reynaud et al., 1999). In the same  
384 line, Papatsiros et al., (2006) found that the humoral response of sows following two vaccinations was  
385 short, lasting 40 days after the booster vaccination. In the present study, the antibodies detected 72  
386 days after the vaccination were probably related to active infection. In addition, the PRRSV antibodies  
387 detected in growing pigs confirmed that this herd was not virus-free.

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

396

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1 **Table 1: Clinical, viral and bacteriological profiles and lung lesions of growing and finishing batches in the five selected farrow-to-finish pig herds**  
 2 (nasal, tonsillar, oropharyngeal swabs and blood samples of 30 growing finishing pigs, lung examination of 30 slaughtered pigs, findings of the screening visit  
 3 performed two to three months before the start of the longitudinal study)  
 4

Herd	Clinical signs (cough) <sup>1</sup>	Pathogen detection by PCR in swab samples (number of positive pigs)					Viral status <sup>2</sup>		Lung lesions		Pathogen detection by PCR in lung tissues <sup>2</sup>				
		Mhp	Pm	App	Hps	Ssuis	PRRS	SIV	Pneumonia mean score (/28) ( $\sigma$ ) <sup>***</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*	Fin.	0	20	0	17	17	+	-	9.6 (4.2)	0	+	-	+	+	-
D**	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	+	-	-	+	-

5 1: Fin.: Finishing, Far.: Farrowing, PW: Post-Weaning; 2: +: positive, -: negative; \* (10 growers and 10 market-aged pigs) \*\* nasal swabs \*\*\* Madec and  
 6 Kobisch, 1982

7  
 8

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

Herd	Sow herd features			Husbandry			Technical performances					
	Number of sows	Breed	% gilts	Vaccination scheme/ respiratory diseases	Batch interval	Weaning age (weeks)	ADWG <sup>1</sup> (g/day; 7-25 kg)	FCR <sup>2</sup> (7-25kg)	ADWG <sup>1</sup> (g/day; 25-105 kg)	FCR <sup>2</sup> (25-105 kg)	Age at 105 kg live weight (days)	% mortality from weaning to slaughter
<b>A</b>	130	Naïma	22	Atrophic Rhinitis	4-week	3	492	1.55	777	2.67	164	5.1
<b>B</b>	700	LW*LR	30	Atrophic Rhinitis, PRRSV, SIV	1-week	4	458	1.59	789	2.61	165	9.5
<b>C</b>	170	Naïma + LW*LR	31	Atrophic Rhinitis, App	3-week	3	501	1.64	817	2.62	160	7.0
<b>D</b>	220	Naima	19	Atrophic Rhinitis	3-week	4	426	1.60	728	2.93	168	6.5
<b>E</b>	190	LW*LR	22	PRRSV (gilts)	3-week	4	na <sup>3</sup>	na	na	na	na	na

10 <sup>1</sup>ADWG: Average Daily Weight Gain11 <sup>2</sup>FCR: Feed Conversion Ratio12 <sup>3</sup>na : not available

13

14 **Table 3 : Parity mean distribution during two successive reproduction cycles of five batches of sows**  
15 **from farrow-to-finish herds**

16

17

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

18

19 **Table 4: Estimated frequencies (with 95% confidence interval) of *M. hyopneumoniae*, *A.***  
 20 ***pleuropneumoniae*, *P. multocida*, *H. parasuis* and *S. suis* detection in breeding sows followed in five**  
 21 **farrow-to-finish herds (184 sows, 8 sampling times, nasal, tonsillar and oro-pharyngeal swabs, PCR assays,**  
 22 **Generalised Estimation Equations models with an autoregressive correlation matrix)**

23

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

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

25

26



27 **Table 5: Results of the score statistics for type-3 Generalized Estimation Equations analysis to test the**  
 28 **significance of herd, parity, physiological stage, cycle effects and sampling site on the sow**  
 29 **contamination status determined by PCR assays and the infection status determined by serological**  
 30 **assays**

31

Dependant variable	Independant variable				
	Herd	Parity number	Physiological stage	Cycle	Sampling site
· Mhp PCR-status	n.e.*	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	-
· App 1-9-11 serological status	<0.001	0.005	0.19	0.11	-
· App 2 serological status	<0.001	0.002	0.29	0.41	-

32 \*n.e. : not estimable

33

34

35 Table 6: Results of the sampling site comparison to detect PCR positive sows to *M. hyopneumoniae*, *A. pleuropneumoniae*, *P. multocida*, *H.*  
 36 *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% (CI  
 37 95%))  
 38

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

39 **Table 7: Adjusted frequencies (with 95% confidence interval) of *M. hyopneumoniae* (Mhp), *A.***  
 40 ***pleuropneumoniae* serogroup 1-9-11 (App 1-9-11) and serotype 2 (App 2) detection in breeding sows**  
 41 **followed in five farrow-to-finish herds (184 sows, 8 sampling times, ELISA assays, Generalised Estimation**  
 42 **Equations models with an autoregressive correlation matrix)**

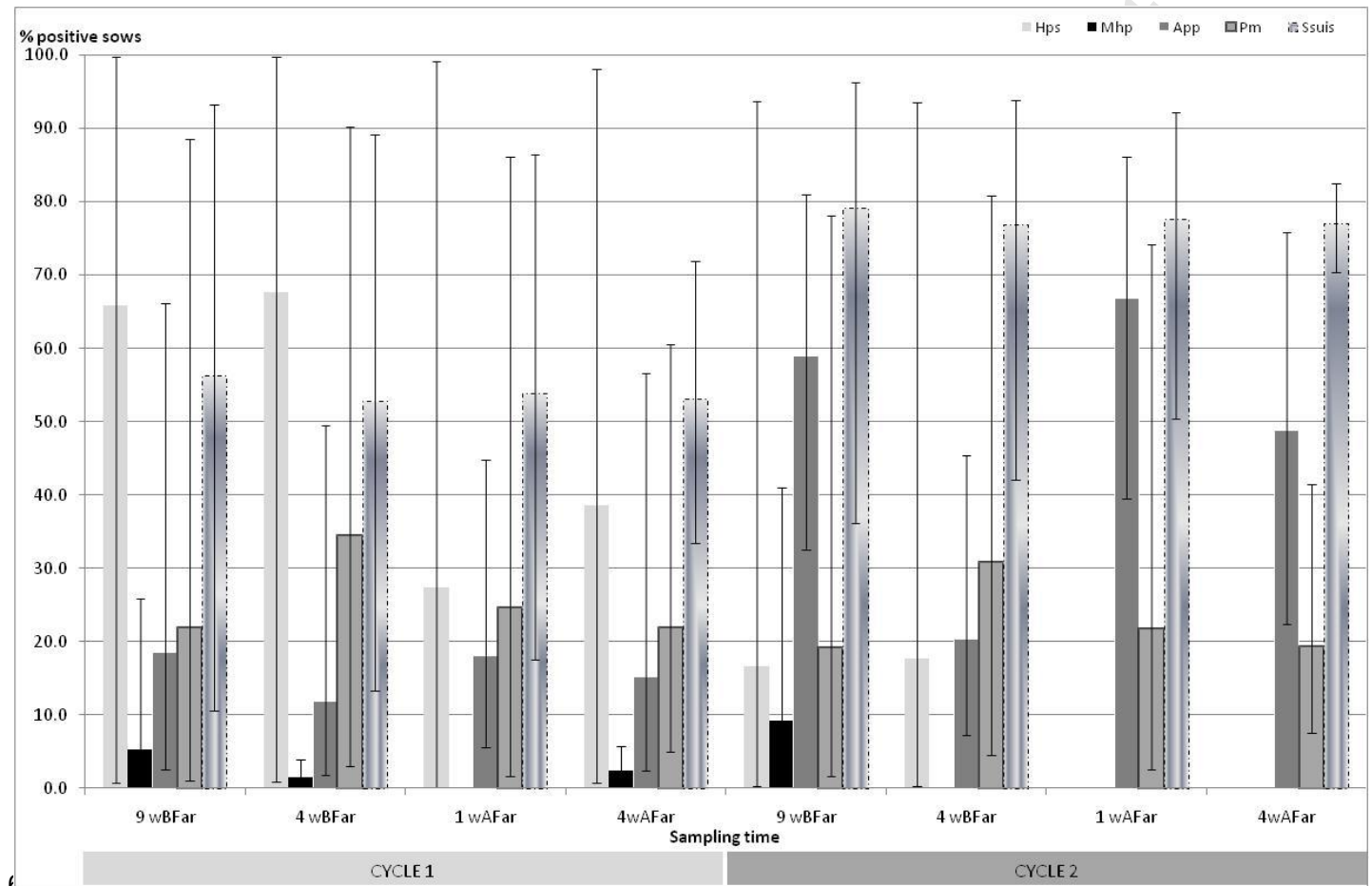
43

Herd	<i>Mhp</i>	<i>App 1-9-11</i>	<i>App 2</i>
<b>A</b>	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>B</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)
<b>C</b>	83.5 <sub>a</sub> (57.1; 95.1)	100 <sub>b</sub> ( 77.1;100)	73.0 <sub>a</sub> (33.6 ;100)
<b>D</b>	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)
<b>E</b>	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)

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

45

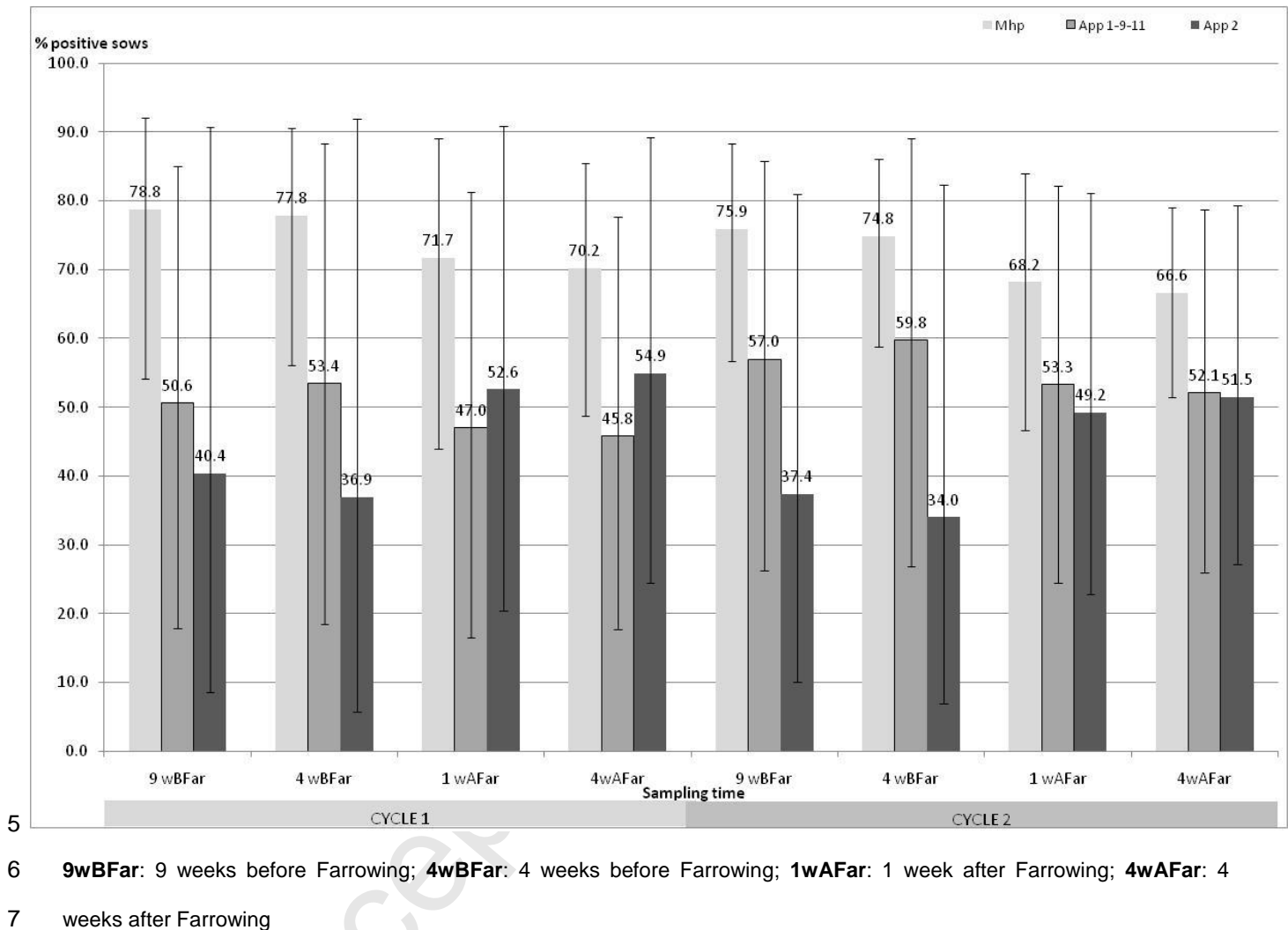
1 **Figure 1: Estimated within herd pathogen detection frequency (and 95% confidence interval) of *M.***  
 2 ***hypopneumoniae* (Mhp), *A. pleuropneumoniae* (App), *P. multocida* (Pm), *H. parasuis* (Hps) and *S. suis***  
 3 **(Ssuis) during two successive gestation and lactation periods (cycles 1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



7 **9wBFar: 9 weeks before Farrowing; 4wBFar: 4 weeks before Farrowing; 1wAFar: 1 week after Farrowing; 4wAFar: 4**  
 8 **weeks after Farrowing**

9

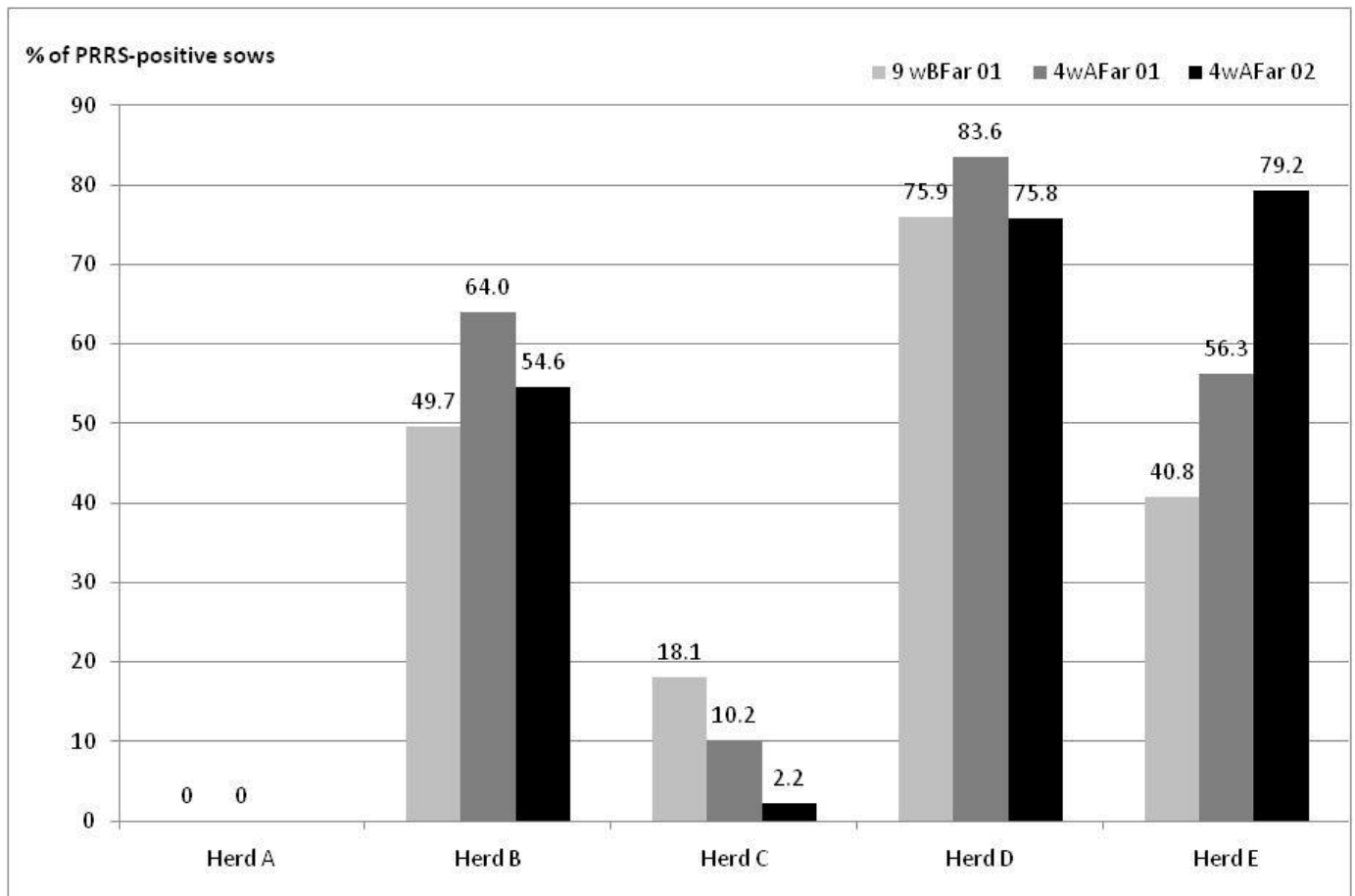
1 **Figure 2: Adjusted within herd antibody detection frequency (with 95% confidence interval) of *M.***  
 2 ***hypopneumoniae* (Mhp), *A. pleuropneumoniae* serogroup 1-9-11 (App1-9-11) and serotype 2 (App 2)**  
 3 **during two successive gestation and lactation periods (cycles 1 and 2) (5 farrow-to-finish herds, 184 sows,**  
 4 **8 sampling times, ELISA assays, Generalised Estimation Equations with an exchangeable correlation matrix)**



1 **Figure 3: Frequency of PRRS-seropositive sows during gestation and lactation phases in 5 farrow-to-**  
2 **finish herds (184 sows, 3 sampling dates)**

3

4



5

6 9wBFar01: 9 weeks before Farrowing, replicate 01

7 4wAFar01: 4 weeks after Farrowing, replicate 01

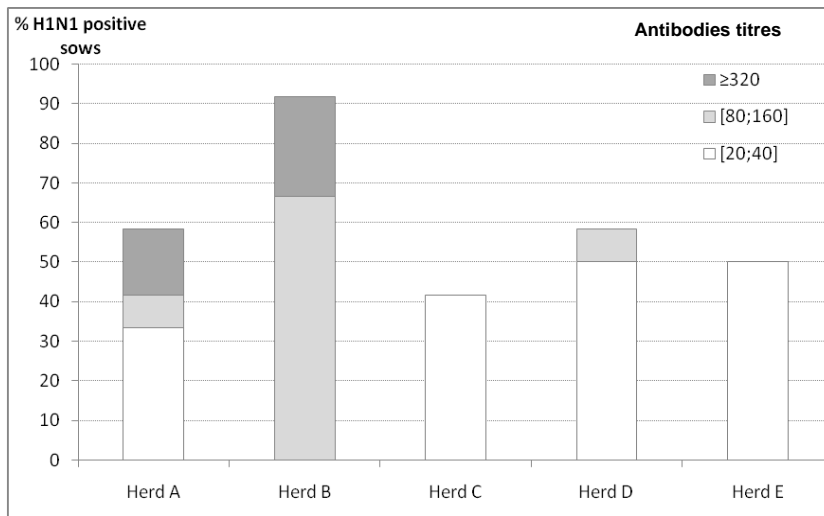
8 4wAFar02: 4 weeks before Farrowing, replicate 02

9

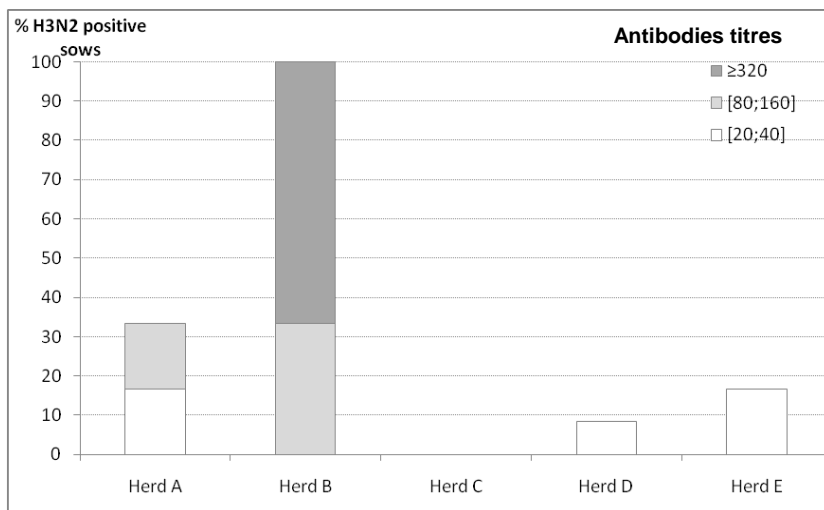
10

1 **Figure 4: Distribution of the frequency of sows with SIV antibodies and antibodies titres for subtypes**  
 2 **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> four weeks after farrowing in five farrow-to-finish pig herds (12 sows/herd)**

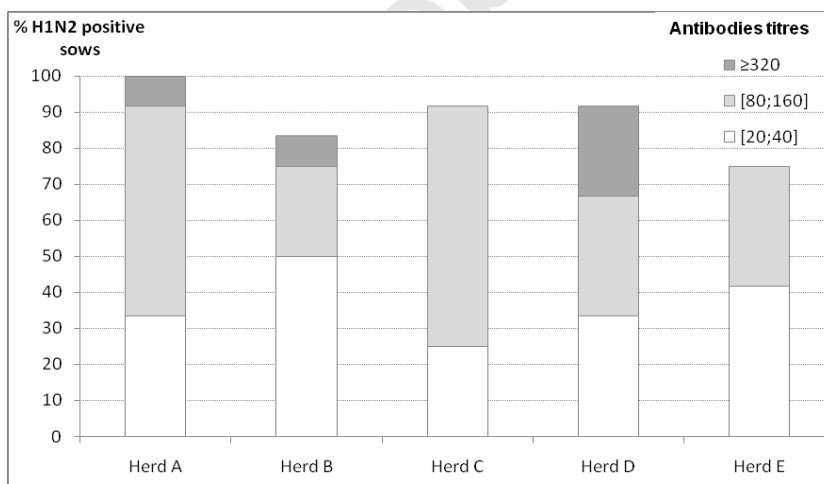
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