Mycoplasma hyopneumoniae prevalence in Belgian and Dutch pig herds using a tracheo-bronchial swab technique

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INTRODUCTION

Mycoplasma hyopneumoniae (M. hyopneumoniae) – one of the main pathogens of the Porcine Respiratory Disease Complex (PRDC) – is still very important in modern intensive swine farming in Europe. Economic losses due to mycoplasmal pneumonia (enzootic pneumonia) are related to coughing, slower growth and higher feed conversion in combination with higher susceptibility of infected pigs for other, mainly secondary pathogens, such as Pasteurella multocida and Actinobacillus pleuropneumoniae (Maes, 1998). Diagnosis of mycoplasmal infections can be performed using different approaches (Strait & Thacker, 2005; Sibila et al., 2009): clinical signs, slaughterhouse checks of affected lungs (Meyns et al., 2011; Fraile et al., 2010), serological examinations of relevant animal groups or direct identification of the pathogen through polymerase chain reaction (PCR)-techniques (Calsamiglia and Pijoan, 2000; Marois et al., 2010). Various sampling sites have been used throughout recent years, such as nasal swabs, tonsil scrapings and broncho-alveolar lavage fluids. Recently, a new sampling technique has been developed and validated for use in pigs, namely the tracheo-bronchial swab (TBS) technique (Fablet et al., 2010). The aim of the present study was to obtain data on distribution of M. hyopneumoniae infection throughout closed pig herds in Belgium and The Netherlands, using this tracheo-bronchial swab technique. Sampling was mainly focused on early diagnosis, since piglets can already be infected during suckling through the sow (Calsamiglia & Pijoan, 2000; Fano et al., 2007; Sibila et al., 2007; Nathues et al., 2010; Villarreal et al., 2010; Segalés et al., 2011) and further spread of infection occurs after weaning (Meyns et al., 2004; 2006).

MATERIALS AND METHODS

Closed pig herds were randomly selected through regular contacts with local veterinary practices. Following inclusion criteria were installed: at least 200 sows, known vaccination status against M. hyopneumoniae, no antibiotic treatment with molecules active against M. hyopneumoniae and no specific clinical problems with M. hyopneumoniae in fattening pigs. In total 50 pig herds were included in the study, among which 20 in Belgium and 30 in The Netherlands. In every pig herd, 30 pigs were sampled in three age groups. Sampling was always performed by the same veterinarian thoroughly trained on the TBS sampling procedure. The standard sampling protocol included 10 pigs around weaning (3-4 weeks of age), 10 pigs at 5-6 weeks of age and 10 pigs at the end of the nursery stage (7-11 weeks of age). Tracheo-bronchial swabs were collected following fixation of the piglets with a nose strap and mouth spreader. The TBS was introduced through the mouth, glottis and passed through the trachea up to the tracheo-bronchial split. Mucus was collected at this location and suspended into 1 mL of buffered saline solution and stored fresh (5°C) until analysis. Nested PCR (nPCR) analysis was performed according to the standard operating procedure of the laboratory (IVD GmbH, Hannover, Germany) and PCR results were reported as negative or
positive for the presence of *M. hyopneumoniae*. The detection limit of the nPCR test was set at 300 DNA copies of *M. hyopneumoniae* per mL of TBS suspension.

**RESULTS**

Prevalence results of the *M. hyopneumoniae* screening using the new TBS technique are given in Table 1.

<table>
<thead>
<tr>
<th>Country</th>
<th>Age group</th>
<th># herd positive</th>
<th>% herd positive</th>
<th># pigs positive</th>
<th>% pigs positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>3-4</td>
<td>4/12</td>
<td>33.3</td>
<td>7/110</td>
<td>6.36</td>
</tr>
<tr>
<td></td>
<td>5-6</td>
<td>5/13</td>
<td>38.5</td>
<td>15/160</td>
<td>9.38</td>
</tr>
<tr>
<td></td>
<td>7-11</td>
<td>6/18</td>
<td>33.3</td>
<td>19/215</td>
<td>8.84</td>
</tr>
<tr>
<td></td>
<td>3-11</td>
<td>10/20</td>
<td>50.0</td>
<td>41/485</td>
<td>8.45</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>3-4</td>
<td>7/27</td>
<td>25.9</td>
<td>19/275</td>
<td>6.91</td>
</tr>
<tr>
<td></td>
<td>5-6</td>
<td>5/24</td>
<td>20.8</td>
<td>22/235</td>
<td>9.36</td>
</tr>
<tr>
<td></td>
<td>7-11</td>
<td>5/29</td>
<td>17.2</td>
<td>22/325</td>
<td>6.77</td>
</tr>
<tr>
<td></td>
<td>3-11</td>
<td>9/30</td>
<td>30.0</td>
<td>63/835</td>
<td>7.54</td>
</tr>
</tbody>
</table>

**DISCUSSION**

When compared to a recently published prevalence study (Villarreal et al., 2010), the percentages of herds with evidence of infections with *M. hyopneumoniae* at 3 to 4 weeks of age were lower in this study. Indeed, in our study, 33.3 and 25.9% of the Belgian and Dutch herds, respectively had at least one piglet positive for *M. hyopneumoniae* at 3 to 4 weeks of age, whereas these percentages were 66.7 and 83.3% in the study of Villarreal and co-workers. On the other hand, the individual animal prevalences at 3 to 4 weeks of age were higher in both Belgium and The Netherlands (6.4 and 6.9%, respectively) when compared to the study of Villarreal and coworkers (3.3 and 7.7%, respectively). This difference can be explained by the use of the more sensitive TBS technique in this study, whereas Villarreal and co-workers used nasal swabs. Indeed, tracheo-brochial swabs have been shown to be 3.5 times more sensitive than nasal swabs for the detection of *M. hyopneumoniae* infections (Fablet et al., 2010). In the study of Villarreal and co-workers, only pig herds with typical clinical signs related to *M. hyopneumoniae* were selected, whereas in our study, inclusion criteria required no specific clinical respiratory problems in the fattening pigs. It is possible that the prevalence rates would be even higher when selecting only herds with characteristic *M. hyopneumoniae* problems. The individual prevalence rates were higher at 5-6 weeks of age, but decreased again at 7-11 weeks of age. The latter is in contrast with experimental infection studies, showing that one piglet infected before weaning will infect on average 1.16 to 3.51 penmates during the nursery phase (Meyns et al., 2004; 2006). This might eventually be due to the transversal sampling protocol used in the present study, sampling different age groups at the same moment. It is known that variation in prevalence of *M. hyopneumoniae* may occur between subsequent batches of pigs within the same pig herd throughout time (Fano et al., 2007). Therefore, to get more reliable data on the spread of *M. hyopneumoniae* under field circumstances, longitudinal screening of the same batch of pigs throughout time should be considered (Villarreal et al., 2011).

**CONCLUSION**

In conclusion, the present study confirms that under Belgian and Dutch field conditions, piglets may already be infected very early in their life (prevalence of 6.4 and 6.9% at 3 to 4
weeks of age, respectively). Further spread of *M. hyopneumoniae* during the early post-weaning period seems to occur, as evidenced by the higher prevalences at 5 to 6 weeks of age.

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REFERENCES