A Comparative Study of the Preventive Use of Tilmicosin Phosphate (Pulmotil premix®) and Mycoplasma hyopneumoniae Vaccination in a Pig Herd with Chronic Respiratory Disease

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With 1 figure and 3 tables

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Summary

This study was conducted to compare the effects of a preventive in-feed medication programme using tilmicosin (Pulmotil® 200 premix, Elanco Animal Health) at 200 p.p.m. with those of a Mycoplasma hyopneumoniae (Mh) vaccination programme (StellamuneTM Mycoplasma, Pfizer Animal Health). A pig herd with chronic respiratory disease in which infection with Mh played an important role was selected, and a total of 204 piglets were randomly allocated to either the medication (P) or the vaccination (V) group. Pigs in the P group received medicated feed for 3 weeks after weaning (days 34–55), and for 2 weeks late in the nursery period (days 77–98). The piglets in the V group were vaccinated twice intramuscularly, at 4 and 22 days of age. The two groups were compared on the basis of average daily gain (ADG), feed conversion rate (FCR), additional curative medication days (CMD), overall mortality (major variables), a coughing index, pneumonia lesions, and serology against Mh, influenza H1N1 and influenza H3N2 viruses, Actinobacillus pleuropneumoniae (App) and porcine reproductive and respiratory syndrome virus (PRRSV) (minor variables). No significant differences (P > 0.05) were observed for ADG (555 g/day in P group; 567 g/day in V group), FCR (2.64 in P group; 2.41 in V group) and mortality rate (11% in P group; 7% in V group). The average number of additional curative medication days (CMD) per pig was significantly higher (P < 0.01) in the P group (1.5) than in the V group (0.58). At slaughter age, the serological results and the prevalence of macroscopic lung lesions were comparable in the two groups (P > 0.05). With the exception of CMD, the preventive use of tilmicosin at this swine farm was found to confer similar beneficial effects to Mh vaccination.

Introduction

Within the chronic respiratory disease complex, infections with Mycoplasma hyopneumoniae (Mh) are generally considered to play a key role, since they can render pigs more susceptible to infections with other respiratory pathogens (Ross, 1999). Because of the high pig herd density in some areas in Belgium and the frequent contacts between herds, eradication programmes to render and maintain commercial herds free of certain respiratory pathogens such as Mh are seldom implemented. In most instances, control measures are used such as all-in/all-out (AIAO) production, compartmentalization, minimization of mixing and moving pigs, optimization of ventilation and
improvement of housing conditions (Straw, 1992). However, some farms continue to suffer from respiratory problems although no major shortcomings can be detected in management or housing. In such cases, antimicrobial medication or vaccination programmes are frequently used.

By adopting strategic medication programmes, as in this field trial, antimicrobials are provided during high-risk periods, before an outbreak is expected. Strategic medication can limit the clinical and economical consequences of infections, and the pathogen proliferation within the herd (Henry, 1986). Tilmicosin, a semisynthetic macrolide antibiotic (Ose, 1987), has excellent in vitro activity against gram-positive bacteria and mycoplasma, but also against certain gram-negative bacteria (Ose, 1987; Debono et al., 1989; Blackball et al., 1995). Under in vivo experimental conditions, tilmicosin has been effective in controlling and preventing Actinobacillus pleuropneumoniae (App) outbreaks (Moore et al., 1996b; Paradis et al., 1998; Wallgren et al., 1999). The efficacy of this antibiotic for controlling App infections (Binder et al., 1993; Moore et al., 1996b) or respiratory disease not primarily due to App infections (Olsen and Bäckström, 2000) under field conditions has also been demonstrated (Moore et al., 1996a; Olsen and Bäckström, 2000).

Commercial Mh vaccines are widely used for the control of enzootic pneumonia and related respiratory diseases in pig herds. A number of field studies have shown that Mh vaccination can significantly improve the average daily weight gain (ADG) of grow-finishing pigs and decrease the number of lung lesions at slaughter (Doohoo and Montgomery, 1996; Schatzmann et al., 1996; Maes et al., 1998, 1999). In addition, some studies showed that Mh vaccination significantly improved the feed conversion rate by 1–4% (Maes et al., 1999; Baum, 2000; Bouwkamp et al., 2000; Van Nes et al., 2000).

Farmers often ask veterinarians for professional advice as to whether they should medicate or vaccinate their pigs to control respiratory disease caused by Mh infections. Although strategic medication and Mh vaccination are both commonly practised, we are not aware of any study that has compared the two control strategies within the same farm. Most of the decisions are not evidence-based but are the result of practical and economic considerations and the clinical experience of the veterinarian. The objective of the present study was to compare the effects of a preventive in-feed medication programme using tilmicosin at 200 p.p.m. with those of a Mh vaccination programme in a Mh-infected pig herd with chronic respiratory disease. The strategies were compared based on clinical, performance and pathological parameters.

### Materials and Methods

#### The production system and farm history

The study was conducted in a 400-sow herd that was part of a closed production system. The herd was free from Aujeszky disease virus. Van Gennep hybrid sows were inseminated with semen of Piétrain boars. The sows were vaccinated against Aujeszky disease virus, parvovirus, Erysipelothrix rhusiopathiae, atrophic rhinitis and Escherichia coli. Piglets received an iron injection at 3 days of age and male piglets were castrated when they were about 7 days old. The pigs were weaned at 22 days and transferred into a nursery unit in which they were raised until about 15 weeks (45 kg live weight). Thereafter, they were moved into the finishing unit in which they were housed until slaughter age (6–7 months). The nursery and finishing unit were located at the same site approximately 4 km from the sow unit. During the nursery and finishing periods, the pigs received a commercial feed containing 40 p.p.m. salinomycin as a performance enhancer.

According to the farmer and the herd health veterinarian, there was a history of chronic respiratory disease, mainly in the nursery unit. This was confirmed by clinical, pathological and serological investigations prior to the start of the study. From a sample of 195 slaughter pigs, 52 and 28% had pneumonia and pleuritis lesions, respectively. Out of 10 randomly taken blood samples in pigs of 80 kg, 100% showed antibodies against Mh.
Study population and experimental design

In total, 204 piglets derived from 21 sows were selected for the trial. All of them were born within a time span of 1 week. At 3 days of age, they were ear-tagged and randomly allocated to either the medication (P) or vaccination (V) group. An equal number of control and vaccinated pigs per sow were selected (block randomization per sow) (Kleinbaum et al., 1982). Pigs belonging to the P group received a feed containing 200 p.p.m. tilmicosin phosphate (Pulmotil® 200 premix, Elanco Animal Health, Indianapolis, USA) for 3 weeks starting approximately 1 week after weaning, and for 2 weeks starting at 77 days of age. Pigs of the V group were vaccinated twice against Mh (Stellamune™ Mycoplasma, Pfizer Animal Health, New York, USA) according to label instructions, namely at 4 and 22 days of age. Preventive measures (castration, iron injection and tail docking) and other management practices were identical for the two groups. During the nursery-finishing period, the two groups were housed in two separated, identical compartments. The two compartments consisted of eight pens (13 pigs/pen) divided by solid partitions and they were located within the same building. Each compartment had identical ventilation and feeding systems. There was one feeder with two integrated drinking nipples per two pens. The pigs were not regrouped when they were transferred from the nursery to the finishing unit.

Major variables of comparison

Average daily weight gain (ADG). The live weight of each pig (kg) was determined at four different ages: at 4 days (first vaccination), 22 days (second vaccination), 107 days and 212 days (slaughter) of age. The ADG (g/pig/day) during the different production stages was computed as the difference between starting and final weights divided by the duration of that production stage. Data for dead or removed pigs were included in the calculations. The live weights of the pigs at slaughter were computed by multiplying the carcass weights by 1.3.

Feed conversion rate (FCR). The average daily feed consumption (AFC) (g/pig/day) was calculated for the entire study period using the two-pen feed consumption, the duration of the period and the average number of pigs in the pen during this period. The FCR of each pen was estimated as the ratio of AFC to ADG.

Additional curative medication days (CMD) per pig. CMD denotes the number of days that a pig was treated individually against respiratory disease. Other medications were not included in the analysis because they were the same for both groups and because they were not relevant for the purpose of this study. Treatments were applied by the farmer and recorded daily on medication sheets. The farmer was unaware of the allocation of the pigs to either the P or the V group.

Mortality rate. The percentage of pigs that died during the nursery and finishing periods was compared for the two groups. The weight and age of the dead pigs were recorded. All pigs that died during the trial were necropsied by the investigator to assess the possible cause of death. Where appropriate, dead pigs were processed for further laboratory examination.

Minor variables of comparison

Serology. Thirty pigs of each group (29%) were randomly selected at pen level, and were successively bled at the following ages: 70 days, 107 days (first week of the finishing period), 167 days and 212 days (slaughter). The blood samples were analysed for the presence of antibodies against Mh using the DAKO® Mh ELISA (DAKO, Glostrup, Denmark). Sera with optical density (OD) values < 50% and ≥ 65% of the OD buffer control were considered positive and negative, respectively. Intermediate OD values (3%) were considered positive in the statistical analysis. The sera from the blood samples taken at slaughter were additionally tested for presence of antibodies against influenza H1N1 and H3N2 viruses and Actinobacillus pleuropneumoniae (App) biotype 1 serovars 2 and 9. Fifteen blood samples taken at slaughter from each group were tested for the presence of antibodies against porcine reproductive and respiratory syndrome virus (PRRSV). A standard haemagglutination-inhibition test was used to detect antibodies against the influenza viruses (Palmer et al., 1975), a complement fixation test was used to detect antibodies against the App serovars (Lombin et al., 1985) and the Herd Check® PRRS ELISA (Idexx Laboratories, Westbrook, ME)
was used to detect PRRSV antibodies. HI titres $\geq 4$ and $\geq 20$ were considered positive for H1N1 and H3N2 viruses, respectively. Complement Fixation (CF) titres $\geq 40$ for the App serovars were considered positive. PRRSV sera with sample-to-positive (S/P) values $> 0.4$ and $< 0.3$ were considered positive and negative, respectively. There were no intermediate S/P values.

*Coughing index.* A coughing index based on all pigs in both groups was recorded every 2 weeks by the principal investigator. The pigs in each pen were observed for a period of 10 min after they had been moved for about 2 min. The number of pigs that coughed was recorded and divided by the total number of pigs present in these pens. This number was multiplied by 100 to obtain the coughing index.

*Macroscopic lung lesions.* At slaughter, the presence of pneumonia, interlobular fissures, abscesses, App lesions and pleuritis was recorded blind for all pigs in the P and V groups. In addition, an average lung severity score per group was calculated. The lungs were thoroughly palpated and sliced for inspection if necessary. The percentage of pigs with lesions was compared for the two treatment groups. An average lung severity score per group was calculated based on the percentage area affected by pneumonia in each lobe multiplied by the relative weight of each lobe, and the lung pneumatic score was the sum of all individual lung scores. The apical lobes were each considered to represent 10% of the lung; the cardial lobes 7% each, the accessory lobe 6%, and both diaphragmatic lobes 30% each (Morrison et al., 1985). Potential values for the average lung severity score ranged from 0 to 100%.

*Economic evaluation.* A preliminary economic evaluation in Euros was performed based on the inherent costs of the two control strategies and the costs of the statistically different production parameters.

### Statistical analysis

ADG and FCR were compared using two-sample $t$-tests. The unit of analysis was the individual pig in the case of ADG and two pens for FCR. CMD was analysed using Poisson regression analysis, and mortality rate was analysed using a chi-square test. Serological results and prevalence of lung lesions in the two groups were compared using chi-square tests. Fisher’s exact tests were applied when small numbers were involved. The average lung severity score per group was analysed using one-way ANOVA. Variables were considered to be significant at the 0.05 level (two-sided). Statistical analyses were performed using SPSS (SPSS 10, SPSS Inc., Chicago, IL, USA).

### Results

At the start of the study, the ALW of the pigs in the P and V groups were 2.66 and 2.75 kg, respectively ($P > 0.05$). Due to an unexpected, acute clinical respiratory outbreak in the two compartments, all pigs of both groups were medicated through water supplies with 200 p.p.m. doxycycline (Doxyveto 50% Pulvis®, VMD) for 4 consecutive days, starting from 132 days of age. Several pigs showed high fever ($> 40^\circ$C), coughing and dyspnea but no pig died during or shortly after the outbreak. Although no aetiologic diagnosis could be established, the clinical signs, the high titres for influenza H1N1 (256) and H3N2 (320) and the high S/P values for PRRSV (3.64) in some pigs at slaughter age suggested that these viruses were probably involved in this respiratory outbreak.

Thirteen fast-growing pigs (eight pigs in the V group and five pigs in the P group) were slaughtered 13 days prior to the slaughter of the other animals. Except for lung lesions, all parameters were recorded for these pigs.

Throughout the study, ADG and FCR were not significantly ($P > 0.05$) different between the two groups (Table 1). In the first period (days 22–107), ADG was slightly better in the V group whereas the converse was true in the second period (days 108–212). The CMD per pig was significantly ($P < 0.01$) different between the P and the V groups.
for the different periods of the study (Table 1). The percentage of pigs that died in the P and V groups was 11 and 7%, respectively ($P > 0.05$).

Only one pig, belonging to the P group, died of respiratory disease. This piglet died because of severe pneumonic lesions during the first week after weaning, before the medication programme was initiated. Other causes of deaths were meningitis ($n = 4$), wasting ($n = 4$), complications of hernia inguinalis ($n = 3$), stress ($n = 2$), $Staphylococcus hyicus$ infection ($n = 1$) and unspecified reasons ($n = 3$). Further laboratory investigations performed on lung tissue of two pigs in the P group revealed the presence of $Streptococcus suis$ spp. in the herd. An antibiogram showed that the $Streptococcus suis$ strain was resistant to tetracyclines and sulphonamides.

The seroprevalence for Mh at 70 days of age for the P and V groups was 0 and 33%, respectively ($P < 0.001$). However, at 107, 167 and 212 days, there were no significant differences in seroprevalence for Mh (Table 2). Similarly, no significant differences ($P > 0.05$) between the two groups were observed at slaughter age regarding the seroprevalence of influenza H1N1 and H3N2, App biotype 1 serovars 2 and 9 and PRRSV.

The mean coughing index showed large variation throughout the study but was generally similar for the two groups (Fig. 1). The overall mean coughing index for the P and V groups was 8.8 (SD = 7.8) and 7.6 (SD = 6.5), respectively.

No significant differences were found between the two groups in terms of prevalence of pneumonia, interlobular fissures, abscesses, App lesions and pleuritis

### Table 1. Average daily gain (ADG) (g/day), feed conversion rate (FCR), additional curative medication days per pig (CMD), and mortality (%) in the medicated (P) and vaccinated (V) groups during the study

<table>
<thead>
<tr>
<th>Period</th>
<th>ADG</th>
<th>FCR</th>
<th>CMD</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>V</td>
<td>P</td>
<td>V</td>
</tr>
<tr>
<td>days 22–107</td>
<td>451$^a$</td>
<td>478$^a$</td>
<td>NI$^c$</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>0.69$^a$</td>
<td>0.30$^b$</td>
<td>10$^a$</td>
<td>5$^a$</td>
</tr>
<tr>
<td>days 108–212</td>
<td>707$^a$</td>
<td>673$^a$</td>
<td>NI$^c$</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>0.84$^a$</td>
<td>0.28$^b$</td>
<td>1$^a$</td>
<td>2$^a$</td>
</tr>
<tr>
<td>days 22–212</td>
<td>555$^a$</td>
<td>567$^a$</td>
<td>2.64$^a$</td>
<td>2.41$^a$</td>
</tr>
<tr>
<td></td>
<td>1.5$^a$</td>
<td>0.58$^b$</td>
<td>11$^a$</td>
<td>7$^a$</td>
</tr>
</tbody>
</table>

$^a,b$Values for ADG, FCR, CMD and mortality rate with a different superscript differ significantly ($P < 0.05$) in the two groups.

$^c$NI: not investigated.

### Table 2. Seroprevalence of $Mycoplasma hyopneumoniae$ (Mh) at different ages, and of influenza H1N1 and H3N2, $Actinobacillus pleuropneumoniae$ (App) serovars 2 and 9, and porcine reproductive and respiratory syndrome virus (PRRSV) at 212 days of age (at slaughter) in the medicated (P) and vaccinated (V) groups

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Age at blood sampling (days)</th>
<th>% (ratio) of pigs with serum antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Mh</td>
<td>70</td>
<td>0 (0/30)</td>
</tr>
<tr>
<td>Mh</td>
<td>107</td>
<td>0 (0/30)</td>
</tr>
<tr>
<td>Mh</td>
<td>167</td>
<td>25 (7/28)</td>
</tr>
<tr>
<td>Mh</td>
<td>212 (slaughter)</td>
<td>89 (25/28)</td>
</tr>
<tr>
<td>Influenza H1N1</td>
<td>212 (slaughter)</td>
<td>91 (21/23)</td>
</tr>
<tr>
<td>Influenza H3N2</td>
<td>212 (slaughter)</td>
<td>78 (18/23)</td>
</tr>
<tr>
<td>App serovar 2, 9</td>
<td>212 (slaughter)</td>
<td>0 (0/23)</td>
</tr>
<tr>
<td>PRRSV$^a$</td>
<td>212 (slaughter)</td>
<td>100 (15/15)</td>
</tr>
</tbody>
</table>

$^a$15 blood samples per group were analysed for detection of PRRSV antibodies.
The percentage of pigs with pneumonic lesions typical of Mh infections was 28 and 35% in the P and V groups, respectively. The average lung severity scores of the P (2.5%) and V (4.2%) groups were not statistically different ($P > 0.05$).

Based on the finding that only the parameter CMD per pig was statistically different between the two groups, a preliminary assessment of the costs associated with the two control programmes was made. The costs of additional curative medications were 0.34 € per pig in the P group and 0.18 € per pig in the V group. The inherent costs of the in-feed medication strategy with tilmicosin amounted to 1.37 € per pig. This was based on the amount of medicated feed consumed by the pigs, including the costs for manufacture and storage of the medicated feed. The inherent costs of the vaccination strategy were 1.74 € per pig. They were calculated based on the cost of the vaccine (two injections per pig), including costs for labour and storage of the vaccine. The economic difference between the two strategies (1.71 and 1.92 € per pig in the P and V groups, respectively) was 0.21 € per pig in favour of the P group.

**Discussion**

The preventive medication with tilmicosin for 3 weeks after weaning (days 34–55) and for 2 weeks starting at 11 weeks (days 77–98) conferred similar beneficial effects to a Mh vaccination programme at this farm. Production parameters were on average slightly better ($P > 0.05$) in the V group, whereas lung lesions were slightly less prevalent ($P > 0.05$) in the P group. The two medication periods were selected because, in closed

Table 3. Prevalence of the different lung lesions at slaughter in the medicated (P) and vaccinated (V) groups

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>P ($n = 85$)</th>
<th>V ($n = 87$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>28</td>
<td>35</td>
<td>0.32</td>
</tr>
<tr>
<td>Fissures</td>
<td>24</td>
<td>28</td>
<td>0.69</td>
</tr>
<tr>
<td>Abscesses</td>
<td>2</td>
<td>3</td>
<td>1.00</td>
</tr>
<tr>
<td>App lesions</td>
<td>10</td>
<td>9</td>
<td>1.00</td>
</tr>
<tr>
<td>Pleuritis</td>
<td>18</td>
<td>19</td>
<td>1.00</td>
</tr>
</tbody>
</table>
pig herds or in closed production systems, respiratory disease outbreaks at these times are frequently encountered (Christensen and Sørensen, 1999; Stærk 2000), and because the respiratory problems on this farm usually occurred in the nursery unit. The first medication period was not initiated immediately after weaning because the feed intake of recently weaned pigs is not reliable and may vary greatly among pigs (Muirhead and Alexander, 1998). Because medication was postponed to the second week, the low feed intake in the first few days after weaning could not have affected the intended body levels of tilmicosin (10 mg/kg body weight). Early Mh vaccination of the piglets, in the first week of life and at weaning, was chosen because this vaccination scheme is most frequently adopted on Belgian swine farms at this time and because many field trials have demonstrated its beneficial effects on infected swine farms (Doohoo and Montgomery, 1996; Schatzmann et al., 1996; Maes et al., 1998). Since Mh-seropositive pigs in the P group were first detected at day 167, it can be assumed that vaccination at an older age would also have been successful on this farm. Recent studies showed that double vaccination in the nursery unit (Wallgren et al., 2000) or even single vaccination at 10 weeks of age (Baum, 2000; Pommier et al., 2000) conferred beneficial effects on swine farms with late Mh infection. The exact time of infection on this farm could not be assessed because there was no negative control group, i.e. a group that was not medicated or vaccinated. The strategic medication programme may have decreased the Mh infection pressure on this farm and thus may have delayed the time of seroconversion.

In the first period (days 22–107), ADG was slightly better in the V group, whereas the converse was true in the second period (days 108–212). We had expected the opposite outcome because the medication was provided during the nursery period and not in the finishing unit where most Mh infections occurred. The differences, however, were not statistically significant, and ADG during the entire study period was very similar in the two groups. In addition, no significant differences were observed for FCR and mortality. CMD was the only parameter that was significantly different between the two groups, with significantly fewer antimicrobial treatments against respiratory disease being applied in the vaccinated pigs. Although the costs for additional curative medications were lower in vaccinated pigs, the preliminary economic analysis showed that the medication programme was still less expensive than Mh vaccination on this farm.

The Mh seroprevalence in both groups significantly increased when pigs were between 107 and 167 days old. Since the time-span between infection and seroconversion is about 4–6 weeks, Mh infection probably started in the last weeks of the nursery. The high Mh seroprevalences at slaughter age (> 85%) and the fact that more than 25% of the pigs showed pneumonia lesions indicated that Mh infections were indeed highly prevalent on the selected farm. The higher Mh seroprevalence in the V group than in the P group, especially at the first blood samplings, is probably due to the vaccination itself or to the priming effect of the vaccine. Vaccinated pigs may seroconvert earlier following Mh infection compared to non-vaccinated pigs. The results of the necropsies and the additional serological results at slaughter indicated that infections with other bacterial and viral respiratory pathogens were also highly prevalent in this herd. More than 70% of the tested pigs were seropositive for both influenza viruses and all of the pigs were seropositive for PRRSV. Similar numbers of seropositive slaughter pigs have been found on other pig farms in Belgium (Maes et al., 1999, 2000).

The overall coughing index over the entire study period was slightly higher in the P group than in the V group. There was, however, a large variation over time. The acute respiratory outbreak in both groups during the second half of the finishing period had little effect on the mean coughing index at that time. This may be explained by the fact that the outbreak lasted only about 5 days and occurred between two observation times. The pigs were treated during the outbreak for animal welfare reasons. The same antimicrobial, namely doxycycline, was used in both groups to avoid bias in the results.

A strategic medication programme has the advantage that it is not only active against Mh infections, but can also decrease the number of infections with other (respiratory)
pathogens. Mh vaccination is more specific since it is mostly used against Mh infections. However, it has been shown that Mh vaccination can also reduce the number of secondary bacterial infections that accompany Mh infections under field conditions, such as *Pasteurella multocida* (Pm) (Sørensen et al., 1997) and App (Kobisch et al., 1993). A strategic medication programme can be used in a rather flexible way according to the risk periods on the farm. This may be important when the risk of outbreaks changes over time or when there are respiratory problems only during particular months of the year. Initiating Mh vaccination on a farm should be considered in the long run because the main beneficial effects appear after about 6 months, and it is not warranted to interrupt vaccination during periods of low risk for disease outbreaks or during periods of poor market conditions. Vaccination compared to antimicrobial medication has the advantage that no problems will arise with antimicrobial resistance, especially when used for a long time, and that there is no risk of antimicrobial residues in slaughter pigs.

In conclusion, on this swine farm where pigs were suffering from chronic respiratory disease, for its specific production system and infection pattern, the preventive use of tilmicosin for 3 weeks after weaning and for 2 weeks when pigs were 11 weeks old conferred similar beneficial effects to Mh vaccination. Further studies, preferably including a larger number of pigs and pig herds, should be conducted to confirm the results.

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