A COMPARATIVE STUDY BETWEEN THE PREVENTIVE USE OF TILMICOSIN PHOSPHATE (PULMOTIL PREMIX®) AND MYCOPLASMA HYOPNEUMONIAE VACCINATION IN A PIG HERD WITH CHRONIC RESPIRATORY DISEASE

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ABSTRACT

The objective of the study was to compare the effects of a preventive in-feed medication program using tilmicosin (Pulmotil® 200 premix, Elanco Animal Health) at 200 ppm with those of a Mycoplasma hyopneumoniae (Mh) vaccination program (Stellamune™ Mycoplasma, Pfizer Animal Health) in a pig herd with chronic respiratory disease. In total, 208 piglets were randomly allocated to either the medication (P) or the vaccination (V) group. Pigs of the P group received medicated feed during 3 weeks after weaning, and during 2 weeks at the start of the finishing period. The piglets of the V group were vaccinated twice, at 4 and 22 days of age. Both groups were compared by ADG and FCR (major variables) and by a coughing index, pneumonia lesions and serology for Mh (minor variables). No significant differences (P>0.05) were observed between both groups. In this type of herd, the preventive use of tilmicosin had similar beneficial effects compared to Mh vaccination.

1. INTRODUCTION

Although major efforts have been made to control respiratory disease in modern pig herds, they continue to be an important problem for the pig industry. 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 (10). Control measures against respiratory disease problems usually aim to prevent clinical symptoms and to reduce the associated economic losses. Because of the high pig herd density in some areas in Belgium and the frequent contacts between herds, eradication programs to make and maintain herds free of certain respiratory pathogens like Mh are very seldom implemented in commercial pig farms. In most cases of respiratory problems in pig farms, some management practices and housing conditions should be improved such as the use of all-in/all-out (AIAO) production, compartmentalization, minimizing mixing and moving pigs, optimizing ventilation etc. However, some farms continue to suffer from respiratory problems although no major shortcomings can be detected in the management and housing. In such cases, vaccination or anti-microbial medication programs are frequently used.

Tilmicosin is a semi-synthetic macrolide antibiotic with excellent in vitro activity against Mh and many pathogenic bacteria found in the respiratory tract of pigs (1, 2, 8). The in vivo activity of this antibiotic for the prevention of respiratory disease has also been demonstrated in studies conducted under experimental (9) and field conditions (7). Mh vaccines are widely used for the control of enzootic pneumonia. Different studies have shown that Mh vaccination can significantly improve the performance of grow-finishing pigs and decrease the number of lung lesions at slaughter (5). The objective of the present study was to compare the effects of a preventive in-feed medication program using tilmicosin (Pulmotil® 200 premix, Elanco Animal Health) at 200 ppm with those of a Mh vaccination program (Stellamune™ Mycoplasma, Pfizer Animal Health) in a pig herd with chronic respiratory disease.

2. MATERIALS AND METHODS

2.1. The production system and farm history

The study was conducted in a 400-sow herd that was part of a closed production system. ‘Van Gennip’ hybrid sows were inseminated with semen of Pietrain boars. The sows were vaccinated against Aujeszky disease virus, Parvovirus, Erysipelothrix rhusiopathiae, atrophic rhinitis and E. coli. Piglets received an iron injection at three days of age and male piglets were castrated at about 7 days of age. The pigs were weaned at 22 days and
transferred into a nursery unit in which they were raised until 14 weeks (approximately 40 kg liveweight). 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 herd. During the nursery and finishing period, the pigs received a commercial feed containing 40 ppm salinomycin as a performance enhancer.

According to the farmer and the herd health veterinarian, the nursery-finishing herd had a history of chronic respiratory disease. 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 or pleuritis lesions, respectively. Out of 10 randomly taken blood samples in pigs of 80 kg, 100% showed antibodies against Mh.

2.2. Study population and experimental design

In total, 208 piglets were selected for the trial. They were derived from 21 sows and were born within a timespan of one week. At three days of age, they were ear tagged and randomly allocated to either the medication (P) or vaccination (V) group. An equal number of vaccinated and control pigs per sow were selected (block randomisation per sow). Pigs belonging to the P group received a feed containing 200 ppm tilmicosin phosphate (Pulmotil® 200 premix, Elanco Animal Health) during three weeks, starting approximately one week after weaning, and during two weeks starting at 77 days of age. Pigs of the V group were vaccinated twice against Mh (Stellamune™ Mycoplasma, Pfizer Animal Health) according to label instructions, namely at 4 and 22 days of age. Preventive measures (castration, iron injection, tail docking) and other management practices were identical for both groups. During the nursery-finishing period, the two groups were housed in 2 separated, identical compartments. The 2 compartments consisted of 8 pens (13 pigs/pen) and were located in the same building. The ventilation and feeding system were identical. There was one feeder with two integrated drinking nipples per two pens.

2.3. Major variables of comparison

Average Live Weight (ALW) and Average Daily weight Gain (ADG)
The live weight of each pig was determined at five different ages: at 4 days (first vaccination), 22 days (at second vaccination), 70 days and 107 days of age and at slaughter (212 days of age). The ADG (g per pig per day) during the different production stages was computed as the difference between starting and final weight divided by the duration of that production stage.

Average daily Feed Consumption (AFC) and Feed Conversion Rate (FCR)
The AFC (g per pig per day) was estimated per 2 pens during the nursery and the finishing period. The FCR of each pen was estimated as the ratio of AFC to ADG.

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

2.4. Minor variables of comparison

Serological testing
Thirty pigs of each group were randomly selected at pen level, and they were successively bled at the following ages: 70 days, 107 days, 167 days and at 212 days (slaughter). The blood samples were analyzed for 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 or negative, respectively. Intermediate OD-values were considered doubtful. The sera from the 30 blood samples taken at slaughter were additionally tested for presence of antibodies against Influenza H1N1 and H3N2 viruses and Actinobacillus pleuropneumoniae (App) biotype 1 serotype 2 and 9. Fifteen out of the 30 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), the Herd Check® PRRS ELISA (Idexx Laboratories, Westbrook, ME, USA) to detect PRRSV antibodies, and a complement fixation test to detect antibodies against the App serotypes. HI-titers ≥ 4 and ≥ 20 were considered positive for H1N1 and H3N2 viruses, respectively. For PRRSV sera with S/P-values > 0.4 and < 0.3 were considered positive or negative, respectively. CBR-titers ≥ 40 for the App serotypes were considered positive.
Coughing index
A coughing index was performed weekly by the investigator and compared for both groups. The pigs in each pen were observed for a period of ten minutes after they had been moved about for two minutes. The number of pigs that coughed was recorded and divided by the total number of pigs present in these pens.

Macroscopic lung lesions
The presence of pneumonia, interlobular fissures, abscesses, App-lesions and pleuritis was recorded at slaughter. The lungs were thoroughly palpated and sliced for inspection if necessary. The percentage of pigs with lesions was compared for the two treatment groups.

2.5. Statistical analysis
Variables were considered to be significant at the 0.05 level (two-sided). ALW, ADG, AFC and FCR were summarized across the pens for each treatment group and compared using two-sample t-tests. Mortality rate was analyzed using chi-square tests (with correction for continuity). Serological results and prevalences of pneumonia, interlobular fissures, abscesses, App-lesions and pleuritis were compared using chi-square tests. Fisher’s exact tests were applied when small numbers were involved. Statistical analyses were performed using SAS.

3. RESULTS
At the start of the study, the ALW of the pigs in the P and V group were 2.66kg and 2.75kg, respectively (P = 0.81). Throughout the study, ADG and FCR were generally similar for both groups as shown in table 1.

Table 1. Results of average daily gain (ADG) (g/day) and feed conversion rate (FCR) in the medicated (P) and vaccinated (V) groups during different periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period</th>
<th>P group</th>
<th>V group</th>
<th>Difference (P-V)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG</td>
<td>D7 - D22</td>
<td>225</td>
<td>214</td>
<td>11</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>D23 - D70</td>
<td>370</td>
<td>354</td>
<td>16</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>D71 - D107</td>
<td>648</td>
<td>696</td>
<td>-48</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>D108 - D212</td>
<td>626</td>
<td>591</td>
<td>35</td>
<td>0.18</td>
</tr>
<tr>
<td>FCR</td>
<td>D22 - D97</td>
<td>1.30</td>
<td>1.32</td>
<td>-0.02</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>D98 - D212</td>
<td>3.31</td>
<td>3.28</td>
<td>0.03</td>
<td>0.89</td>
</tr>
</tbody>
</table>

More pigs died in the P group (10%) than in the vaccination group (5%), but this difference was not statistically significant (P = 0.24).

All death pigs were necropsied. This led to diverse results concerning the causes of death. Bacteriological culture of lung tissue was performed for 2 pigs of the P group. This revealed the presence of *Streptococcus suis* spp.

As a result of vaccination, there was a significant difference (P < 0.001) in seroprevalence for Mh at 70 days of age. However, at 107, 167 and 212 days, there was no significant difference in seroprevalence for Mh. Likewise, there was no significant difference for Influenza H1N1 and H3N2, for PRRSV and for App biotype 1 serotype 2 and 9 at slaughter age (Table 2).
Table 2. Seroprevalence of *Mycoplasma hyopneumoniae* (Mh) throughout the study period, and of Influenza H1N1 and H3N2, PRRSV and App serotype 2 and 9 at 212 days of age (at slaughter) in the medicated (P) and vaccinated (V) group

<table>
<thead>
<tr>
<th>% of pigs with positive serum antibodies</th>
<th>P-group (n=30)</th>
<th>V-group (n=30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mh - 70 days</td>
<td>0</td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mh - 107 days</td>
<td>0</td>
<td>7</td>
<td>0.49</td>
</tr>
<tr>
<td>Mh - 167 days</td>
<td>25</td>
<td>52</td>
<td>0.07</td>
</tr>
<tr>
<td>Mh - 212 days</td>
<td>89</td>
<td>96</td>
<td>0.56</td>
</tr>
<tr>
<td>Influenza H1N1</td>
<td>91</td>
<td>82</td>
<td>0.67</td>
</tr>
<tr>
<td>Influenza H3N2</td>
<td>78</td>
<td>74</td>
<td>1.00</td>
</tr>
<tr>
<td>PRRSV</td>
<td>100</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>App serotype 2, 9</td>
<td>0</td>
<td>15</td>
<td>0.11</td>
</tr>
</tbody>
</table>

No significant differences were found between both groups in terms of prevalence of pneumonia, interlobular fissures, abscesses, App-lesions and pleuritis (Table 3).

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>Prevalence of lesion (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-group (n=82)</td>
<td>V-group (n=96)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td>Fissures</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Abscesses</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>App-lesions</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Pleuritis</td>
<td>18</td>
<td>19</td>
</tr>
</tbody>
</table>

The mean coughing index was generally similar for both groups throughout the study (figure 1, see appendix).

4. DISCUSSION

This study compared the effects of a preventive in-feed medication program using tilmicosin at 200 ppm with those of a Mh vaccination program in a pig herd with chronic respiratory disease. The results showed that in this herd infections with Mh were highly prevalent, mainly occurring during the second half of the finishing period (Table 2). The seroprevalence increased from 25% to almost 90% at slaughter age. The results of the necropsies and the additional serological results at slaughter indicated that infections with other bacterial and viral respiratory pathogens were also common in this herd. Approximately 80% of the pigs was seropositive for both Influenza viruses and all pigs were seropositive for PRRSV. These high percentages of seropositives at slaughter age are also frequently found in other pig farms in Flanders (4, 6).

The preventive medication with tilmicosin during 3 weeks after weaning and during 2 weeks at approximately 10 weeks conferred similar beneficial effects compared to a Mh vaccination program. Because of practical reasons, there was no negative control group. The improvement of performance parameters ADG and FCR
associated with Mh vaccination in chronically infected herds usually amounts to approximately 5% (3, 5). These 2 medication periods were selected because in closed pig herds, respiratory disease outbreaks usually occur at these times. During the first period, the pigs are weaned and usually mixed with other pigs to form homogeneous pens. In addition, a lot of other stress factors take place such as transferring pigs to the nursery unit, change of feed etc. The medication program was not initiated immediately after weaning because the feed intake might have been insufficient to obtain the pursued body levels in these pigs. Therefore, the medication was postponed to the second week after weaning. The second medication period was initiated when the pigs were approximately 11 weeks old. At that time, pigs are usually placed in the finishing unit. Respiratory problems during the first month of the finishing period are very commonly encountered in pig farms.

The Mh vaccine was applied according to label directions namely at 4 and 22 days of age. Since the first seroconversion to Mh was observed at the earliest at day 167, vaccination at a later age would also have been successful in this herd. Moreover, since the sows were seropositive to Mh, the effect of vaccination would probably have been better when the pigs were vaccinated later. Although it is not yet fully understood, it is assumed that maternal antibodies may interfere to some degree with the efficacy of early Mh vaccination.

In conclusion, the study documented that in this chronically infected farm with its specific production system and infection pattern, the preventive use of tilmicosin during 3 weeks in the nursery unit and during 2 weeks from 11 weeks of age has similar beneficial effects compared to Mh vaccination. Further studies preferably including a larger number of pigs and pig herds should be conducted to confirm the results.

5. ACKNOWLEDGEMENTS

This work was partially supported by a grant of Eli Lilly Benelux, Elanco Animal Health. The authors thank the personnel from the Regional Investigation Centre of East Flanders (Drongen) for the laboratory work and the herd owner for participation in this study.

6. REFERENCES

7. APPENDIX

Figure 1. Results of the coughing index in the medicated (P) and vaccinated (V) group throughout the entire study period.