Vaccination of Piglets up to 1 Week of Age with a Single-Dose Mycoplasma hyopneumoniae Vaccine Induces Protective Immunity within 2 Weeks against Virulent Challenge in the Presence of Maternally Derived Antibodies

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Enzootic pneumonia, resulting from infection with Mycoplasma hyopneumoniae, is of considerable economic importance to the pig industry and normally is controlled through active vaccination of piglets. We have demonstrated that administration of an inactivated Mycoplasma hyopneumoniae vaccine to piglets less than 1 week old is efficacious under field conditions and reduces the level of lung lesions observed in comparison to that in control pigs. Here, the results of two separate studies, one in piglets with and the second one in piglets without maternal antibodies, conducted to satisfy the requirements of the European Pharmacopoeia (monograph no. 07/2009:2448), are reported. Piglets received either minimal titer Suvaxyn MH-One or saline at less than 1 week of age and were challenged with Mycoplasma hyopneumoniae 2 weeks later. The number of lung lesions was recorded 4 weeks after challenge, and bronchial swab and lung tissue specimens were analyzed for quantification of Mycoplasma hyopneumoniae DNA. In the presence and absence of maternal antibodies, vaccination of piglets at less than 1 week of age was efficacious, with vaccinated piglets having significantly lower percentages of lung with lesions and lower Mycoplasma hyopneumoniae counts detected in bronchial swab and lung tissue specimens at necropsy. In conclusion, the vaccination of piglets at 1 week of age with Suvaxyn MH-One is efficacious in the presence of high levels of maternal antibodies.

Enzootic pneumonia in pigs is a serious problem for commercial operations. The disease can result from infection with Mycoplasma hyopneumoniae, is characterized mainly by a nonproductive cough (1), and is responsible for considerable economic losses in pig production worldwide (2, 3), resulting from higher feed conversion, decreased body weight gains, and increased medication costs. Studies have shown that infection with M. hyopneumoniae also predisposes pigs to subsequent infection by other bacterial pathogens, such as Actinobacillus (formerly Haemophilus) pleuropneumoniae (4), or Pasteurella multocida (5). Apart from coinfections, factors such as the management of the animals, their environment, and their general health status can also affect the severity of mycoplasmal disease. Therefore, strategies for the control of enzootic pneumonia are extremely important in pig production.

The majority of piglets in commercial systems are routinely vaccinated against M. hyopneumoniae, and numerous studies have evaluated the efficacy and safety of vaccines under field conditions (6–8). Vaccination of pigs has been shown to reduce body weight losses and the prevalence of pigs actually observed with (pneumonic) lung lesions, as well as the severity of lesions in those pigs that are affected. Piglets are often infected by M. hyopneumoniae from their mothers (3), and field prevalence studies have indicated that between 1.5% and 4% of 1- and 3-week-old piglets tested with either nasal swabs or necropsies show positive PCR results for M. hyopneumoniae (9).

We demonstrated previously that vaccination of piglets with an inactivated M. hyopneumoniae vaccine (Suvaxyn MH-One; Zoetis) at less than 1 week of age (4 to 5 days old) under field conditions significantly reduces lung lesions and also reduces body weight losses (10). The efficacy of single-shot vaccines against M. hyopneumoniae at commercial-release titers, when administered to 1-week-old pigs in the presence of maternal antibodies, has been demonstrated previously (11, 12). However, the studies reported here evaluated the efficacy of Suvaxyn MH-One at a minimal release titer, administered to piglets up to 1 week old, in the absence or presence of high maternally derived antibody levels with challenge 2 weeks postvaccination.

MATERIALS AND METHODS

Experimental design. We conducted two studies that were identical in design apart from the serological status of the sows and piglets with respect to Mycoplasma hyopneumoniae; one study used maternally derived antibody (MDA)-negative pigs, while the second study used MDA-positive pigs. Both studies followed a generalized randomized block design, with blocks determined by litter.

In the MDA-negative study, 36 piglets were enrolled into one of two treatment groups (28 piglets in each group) receiving either saline as the control product or a minimal-titer batch of Suvaxyn MH-One. In the MDA-positive study, 78 piglets were enrolled into one of two treatment groups (39 piglets in each group), again receiving either saline as the control product or a minimal-titer batch of Suvaxyn MH-One. Two weeks after vaccination, the pigs in both studies were challenged with Mycoplasma hyopneumoniae; 28 days after the challenge, necropsies and lung lesion scoring were performed. The proposed use of animals and conduct of the studies were reviewed and approved by the local Zoetis Institutional Animal Care and Use Committee.
Animals. Piglets were farrowed from sows obtained from commercial suppliers (Genetiporc, Morris, MN) in the United States; sows were pre-screened by enzyme-linked immunosorbent assays (ELISA) for the presence or absence of *Mycoplasma hyopneumoniae* antibodies using the IDEXX *M. hyopneumoniae* antibody test (S/P ratio) per the manufacturer’s instructions (IDEXX Laboratories Inc., Westbrook, MA), and for the absence of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) antibodies. Sows were transported to Zoetis facilities (Charles City, IA [MDA positive], or Kalamazoo, MI [MDA negative]) prior to farrowing and were administered Lulytase (Zoetis) to ensure that the piglets were farrowed within a 4-day period. Piglets from each sow were randomly allocated to treatment groups in each study, blocked on litter. In the MDA-positive study, the parent sows had been vaccinated as gilts, although not with SuvaMyn GH-One, and were confirmed to be free of the *Mycoplasma hyopneumoniae* antigen by culturing and analysis of nasal swabs. All piglets were allowed to suckle from their own mothers prior to the challenge at 3 weeks of age, and no cross-fostering of litters was permitted. Sows and pigs were housed according to the guidelines in *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (24), in concrete pens with slatted flooring. Feed and water were available *ad libitum*. All animals were observed at least once daily by experienced farm personnel and, if required, a veterinarian was notified.

Vaccine. A minimal-titer batch (relative potency, <1.0) of SuvaMyn GH-One was prepared in Zoetis biological development laboratories following commercial production principles; a minimal-titer vaccine was used to demonstrate efficacy in compliance with the European Pharmacopoeia (monograph no. 07/2009:2448). Sterile saline obtained from Zoetis production facilities was used as the control product. On day 0, when piglets were 4 to 7 days old, each animal was administered 2 ml of either sterile saline for injection or SuvaMyn GH-One by intramuscular injection into the left neck muscle per the randomized treatment plan. A new sterile, 1-inch, 20-gauge needle and syringe were used for each animal.

Challenge. The challenge material was *Mycoplasma hyopneumoniae* strain 232 obtained by Zoetis from Iowa State University; this strain is widely used and has been well characterized (13). A total of 7 ml of pig lung homogenate diluted 1:100 in Friis medium, equivalent to 100 color-changing units/ml, was administered to each pig. Pigs in all treatment groups were sedated with Telazol (tiletamine hydrochloride and zolazepam hydrochloride) prior to challenge administration. The challenge culture was administered tracheally with a sterile needle and syringe. Within approximately 1 h postchallenge, no adverse events were observed.

Observations. All piglets were observed daily during the course of the studies to ensure good general health and also to determine if specific clinical symptoms attributable to either vaccination or challenge (for example, coughing or respiratory distress) were observed. Euthanasia and necropsy were performed, the lungs were removed according to standard practices, and the level of lung lesions observed in each lobe (left cranial, left middle, left caudal, right cranial, right middle, right caudal, and accessory) was scored and recorded as a percentage (0% to 100%) (14). Within each individual study, lungs from all the pigs were examined and scored by a single operator. Following necropsy and lung lesion evaluation on day 42, two representative lung samples, one from the right middle lung lobe and one from the left middle lung lobe, were collected into a single container for detection of *Mycoplasma hyopneumoniae* DNA. Blood samples for serology were collected from all pigs on day 0 (prior to vaccine or control product administration) and subsequently on day 13 (prior to challenge) and day 42 (prior to euthanasia). Bronchial swab specimens were collected from all pigs following euthanasia and necropsy. Each pig’s bronchus was swabbed at the bifurcation with a polyester-tipped swab, which was then swirled for 5 s in a tube containing 2 ml sterile phosphate-buffered saline.

The serum samples obtained were analyzed by Zoetis Laboratory Sciences (Kalamazoo, MI) using an *M. hyopneumoniae* antibody test (same-to-positive [S/P] ratio) according to the manufacturer’s instructions (IDEXX Laboratories Inc.). Bronchial swab and lung tissue specimens were tested by Zoetis Laboratory Sciences (Kalamazoo, MI) to quantify *Mycoplasma hyopneumoniae* DNA by quantitative PCR (12) using standard laboratory procedures. Briefly, DNA was extracted from each of the samples using a QIAcube robot and a QIAamp DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer’s 200-µl 1- to 11-sample protocol. DNA was then analyzed using a CFX96 real-time PCR detection system and C1000 thermal cycler (Bio-Rad, Hercules, CA), PCR primers for the *Mycoplasma hyopneumoniae* 16S rRNA region, and a *Mycoplasma hyopneumoniae*-specific probe. DNA counts were expressed in cells/ml. Validation studies performed by Zoetis Laboratory Sciences staff confirmed the specificity of the assay for detecting only *Mycoplasma hyopneumoniae*.

Statistical analysis. A company biometrics representative was responsible for data summaries and analyses of data (SAS/STAT version 9.2; SAS Institute, Cary, NC). The percentage of total lung with lesions was calculated using the formula 

\[
\frac{(0.10 \times \text{left cranial}) + (0.10 \times \text{left middle}) + (0.25 \times \text{left caudal}) + (0.10 \times \text{right cranial}) + (0.10 \times \text{right middle}) + (0.25 \times \text{right caudal}) + (0.10 \times \text{accessory})}{100}\%
\]

Transformed lung lesions were analyzed with a general linear mixed model. Pairwise comparisons were made between treatment groups if the treatment effect was significant. Back-transformed least-squares means of the percentages of total lung with lesions, their standard errors, and their 95% confidence limits, as well as the minima and maxima, were calculated. Frequency distributions were calculated for each treatment for the following categories: 0% to <5%, 5% to <10%, 10% to <20%, 20% to <30%, and ≥30%.

Serology data were transformed using an appropriate logarithmic transformation and were analyzed with a general linear repeated-measures mixed model. Treatment least-squares means (back-transformed for serology) were calculated for each time point, and the values were plotted for each treatment over time.

The frequency distribution for whether an animal ever had mycoplasma present postchallenge was calculated for each treatment. Bronchial swab or lung tissue data were transformed with an appropriate logarithmic transformation and were analyzed using a general linear mixed model. Linear combinations of the parameter estimates were used in a *priori* contrasts after testing for a significant 

\[P \leq 0.05\]

Treatment least-squares means and 95% confidence limits were back-transformed for presentation.

RESULTS

Animals. In the MDA-negative study, 27 control (T01) and 26 vaccinated (T02) pigs completed the study. In the MDA-positive study, 34 control (T03) and 37 vaccinated (T04) pigs completed the trial. No abnormal health or clinical symptoms resulting from vaccination or challenge were observed in any treatment group in either of the two studies. A number of pigs enrolled had to be euthanized; diagnoses included umbilical hernias, weakness and lethargy, and lameness or joint damage as a result of being stepped on by their parent sow.

Lung lesions. A summary of the percentages of total lung with lesions in the two studies is shown in Table 1. In the MDA-negative study, 54% of the vaccinated pigs were shown to have low levels of lung lesions (between 0 and 5%), with 4% of pigs having more than 20% lung lesions, compared with the controls, which had 26% in the lower category and 15% with more than 20% lung lesions. In the MDA-positive study, 87% of the vaccinated pigs were shown to have low levels of lung lesions (between 0 and 5%), with 5% of pigs having more than 20% lung lesions, compared with the controls, which had 47% in the lower category (data not shown) and none in the higher category. This result is skewed by the vaccines having two pigs having high overall lung lesion...
scores and the rest being essentially clear of mycoplasma-induced lesions, compared with the controls, nearly all of which had low lesion scores. In the MDA-negative study, there were highly significant differences (P = 0.003) between vaccinated and control pigs, with a mean percentages of lungs with lesions of 4.0% and 10.7%, respectively. In the MDA-positive study, there were also highly significant differences (P = 0.0002) in mean percentage of lungs with lesions, albeit at a lower level than the MDA-negative results, between vaccinated and control pigs, with values of 1.0% and 4.8%, respectively (data not shown).

Serology. In both studies, no significant differences in mean serology titers were seen between the vaccinated and the control pigs in samples taken prior to vaccination or prior to the challenge. By day 42, 28 days after the challenge with *Mycoplasma hyopneumoniae*, the vaccinated pigs had significantly (P = 0.05) higher serology titers for *Mycoplasma hyopneumoniae* than did the control pigs. Figures 1 and 2 show the mean serology titers at each of the sampling time points for the MDA-negative (Fig. 1) and MDA-positive (Fig. 2) studies; S/P ratio values of >0.4 were considered positive. As can be seen in Fig. 1, the mean serology titers were below the defined cutoff/negative value prevaccination and prechallenge, and while the vaccinated and control groups had increases in antibody titers following challenge, the vaccinated group had higher mean values. In Fig. 2, the mean serology titers show all pigs to be seropositive prior to vaccination; the control group showed a decline in antibody titers with time and a negative mean titer by day 42. The vaccinated group was able to respond to the challenge on day 14, with an increase in antibody titers seen by day 42.

Quantification of *Mycoplasma hyopneumoniae* DNA from bronchial swab specimens. A summary of the quantifications of *Mycoplasma hyopneumoniae* DNA in bronchial swab fluid specimens for the two studies is shown in Table 2. In both the MDA-negative and MDA-positive studies, there were highly significant differences between the vaccinated and the control pigs (P = 0.0001), with the vaccinated pigs having lower mean amounts of mycoplasma detected in both cases.

Quantification of *Mycoplasma hyopneumoniae* DNA from lung tissue specimens. A summary of the quantifications of *Mycoplasma hyopneumoniae* DNA in lung tissue samples for the two studies is shown in Table 3. In both the MDA-negative and MDA-positive studies, there were highly significant differences between the vaccinated and the control pigs (MDA negative, P = 0.0009; MDA positive, P = 0.0001), with the vaccinated pigs having lower mean amounts of mycoplasma detected in both cases.

**DISCUSSION**

The present study investigated whether the administration of a minimal-titer batch of an inactivated *Mycoplasma hyopneumoniae* vaccine, Suvaxyn MH-One, to piglets less than 1 week of age would be effective in reducing the levels of lung lesions in the presence and absence of maternally derived antibodies. In both cases, vaccination resulted in highly significant reductions in lung lesions compared to those in the control pigs. In addition, the

**TABLE 1 Frequency distribution of lung lesion categories by treatment group**

| Treatment group | 0% < 5% | 5% < 10% | 10% < 20% | 20% < 30% | ≥ 30% | Total no. of observations |
|-----------------|---------|---------|-----------|-----------|-------|---------------------------|
| T01             | 7       | 25.9    | 6         | 22.2      | 10    | 37.0                      | 27 |
| T02             | 14      | 53.8    | 6         | 23.1      | 5     | 19.2                      | 26 |
| T03             | 16      | 47.1    | 13        | 38.2      | 5     | 14.7                      | 34 |
| T04             | 32      | 86.5    | 1         | 2.7       | 2     | 5.4                       | 37 |

* At slaughter, lungs were scored by lobe for the percentage of lung with lesions; the total number of lesions (n) per lung was then categorized.

* T01, MDA-negative control pigs; T02, MDA-negative vaccinated pigs; T03, MDA-positive control pigs; T04, MDA-positive vaccinated pigs.

![FIG 1 MDA-negative mean serology titers by treatment group and sampling point.](image1)

![FIG 2 MDA-positive mean serology titers by treatment group and sampling point.](image2)
The presence of maternal antibodies has been demonstrated to inhibit the development of the neonatal humoral immune system, especially in humans (17, 18) but also in other species such as cattle (19) and pigs (20). The latter study showed effects of both the age at vaccination and the presence or absence of maternally derived antibodies on subsequent neonatal immune responses to a Mycoplasma hyopneumoniae vaccine. In the absence of maternal antibodies, pigs vaccinated at either 2, 3, or 4 weeks of age had higher antibody responses than did controls 3 weeks after vaccination. The presence of maternal antibodies resulted in pigs vaccinated at 2, 3, or 4 weeks of age having immune responses comparable to those of control pigs at all time points. In both cases, however, high prevaccination titers were associated with lower responses to vaccination, and rapid declines in maternal antibody levels were observed for all pigs (in the vaccinated pigs prevaccination and the controls). In our study, we were able to show rapid increases in antibody titers in vaccinated pigs at day 42 following challenge in both the presence and absence of maternal antibodies, thus demonstrating that the vaccine was able to induce an immune response in 1-week-old neonatal piglets, which then could mount an anamnestic antibody response (15) to the mycoplasma challenge. The lack of responses in control pigs from the MDA-positive study may seem unusual as antibody responses would typically increase following challenge (21), but the individual animal responses we observed were highly variable, with some pigs responding to the challenge and others not responding. The overall mean value trend is consistent with other studies, which showed either a slow increase in antibody titers following challenge (12) in MDA-positive control pigs or a flattening or decrease in titers following challenge, with a slight increase 4 to 5 weeks later (22). Previous work with a similar single-dose vaccine but with a vaccination schedule for older piglets (23) also demonstrated no significant interaction between serological status and vaccination with respect to subsequent lung lesion scores, thus

### TABLE 3 Lung tissue specimen quantitative PCR results

| Treatment group | Geometric mean (cells/ml) | 95% confidence limits | Treatment contrast P value |
|-----------------|--------------------------|-----------------------|---------------------------|
|                 |                          | Lower                | Upper   |                     |
| T01             | 2.40E+06                 | 1.43E+06             | 4.03E+06 | 0.00011c           |
| T02             | 2.37E+05                 | 8.83E+04             | 6.36E+05 |                    |
| T03             | 1.61E+06                 | 8.34E+05             | 3.10E+06 |                    |
| T04             | 1.01E+05                 | 4.55E+04             | 2.25E+05 | 0.0001d            |

a Comparison of T02 with T01.

b Comparison of T04 with T03.

c Shown are back-transformed least-squares means, standard errors, ranges, and confidence limits and the treatment contrast significance (P) values.

d Comparison of T04 with T03.

### TABLE 2 Bronchial swab specimen quantitative PCR results

| Treatment group | Geometric mean (cells/ml) | 95% confidence limits | Treatment contrast P value |
|-----------------|--------------------------|-----------------------|---------------------------|
|                 |                          | Lower                | Upper   |                     |
| T01             | 2.40E+06                 | 1.43E+06             | 4.03E+06 | 0.00011c           |
| T02             | 2.37E+05                 | 8.83E+04             | 6.36E+05 |                    |
| T03             | 1.61E+06                 | 8.34E+05             | 3.10E+06 |                    |
| T04             | 1.01E+05                 | 4.55E+04             | 2.25E+05 | 0.0001d            |

a Comparison of T02 with T01.

b Comparison of T04 with T03.

c Shown are back-transformed least-squares means, standard errors, ranges, and confidence limits and the treatment contrast significance (P) values.

d Comparison of T04 with T03.
confirming that the effect of vaccination was not influenced by the presence of antibody. In addition, two other studies (11, 12) used a single-shot vaccine administered at approximately 7 days of age and demonstrated no impact on onset or duration of immunity according to sow serological status. However, in both cases, those studies used a higher-titer commercial-release batch of vaccine, while the current study used a minimal-release batch to demonstrate efficacy in compliance with the European Pharmacopeia monograph on porcine enzootic pneumonia vaccines.

In conclusion, we have successfully demonstrated that vaccination of piglets at 1 week of age with an inactivated Mycoplasma hyopneumoniae vaccine in the presence or absence of maternally derived antibodies is efficacious. Vaccination significantly reduced the level of lung lesions observed and also the amount of mycoplasma detected in bronchial swab and lung tissue samples.

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