

# Evaluation of the effectiveness of a macrolide antibiotic on reduction of respiratory pathogens in 12-day and 21-day weaned pigs

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## Summary

**Objective:** To determine the effect of a new feed-grade antibiotic (tilmicosin) on *Mycoplasma hyopneumoniae*-challenged pigs and to determine whether the *M. hyopneumoniae* status of pigs weaned at 21 days, when tilmicosin is used, would be similar to the *M. hyopneumoniae* status of piglets weaned at 12 days. Additionally, the effect of tilmicosin on other respiratory pathogens in all treated pigs was assessed.

**Methods:** Fifty commercial pigs were randomly allocated to five treatment groups. Three groups were weaned when 12 days old and were either 1) challenged with *M. hyopneumoniae* and treated with tilmicosin (CEW treated), 2) challenged but not treated with tilmicosin (CEW untreated), or 3) left untreated and unchallenged (EW controls). Two groups were weaned when 21 days old, were not challenged with *M. hyopneumoniae*, and were either treated (LW treated) or not treated (LW controls) with tilmicosin. Some of the pigs in all treatment groups developed clinical signs similar to those of *Haemophilus parasuis* and were treated with penicillin for 3 consecutive days immediately before the two groups of the early-weaned pigs were challenged. Weight gain for each pig was measured as the difference in weight between 12 and 56 days of age. The presence of respiratory disease was measured by the number of pigs observed coughing each day and by lung lesion scores at necropsy. Pigs were euthanized when 56 days old and examined for the presence of *M. hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *H. parasuis*, *Pasteurella multocida*, *Streptococcus suis*, and *Bordetella bronchiseptica*. Additionally, sera of all pigs were assessed for the presence of *M. hyopneumoniae* and *A. pleuropneumoniae* when pigs were 12 and 56 days old.

**Results:** Growth rates of the different groups of pigs were unaffected by tilmicosin. Tilmicosin reduced ( $P < .01$ ) the coughing in the CEW treated pigs. Although the lung lesion scores of the CEW treated pigs were not significantly lower than those in the CEW untreated group ( $P > .05$ ), tilmicosin did appear to delay the onset of the infection during the course of the treatment. *Actinobacillus pleuropneumoniae*, *B. bronchiseptica*, and *P. multocida* were not isolated from tissues of any of the pigs. Four of the seven pigs from which *S. suis* was isolated were from the LW control group, whereas *S. suis* was not isolated from any of the LW treated pigs. *Haemophilus parasuis* was isolated at necropsy from 26 of the pigs in this trial: 19 of 20 LW pigs and seven of 30 early-weaned pigs. Within weaning age groups, tilmicosin did not influence the rate of isolation of *H. parasuis*. Pigs in all five groups were seropositive to *A. pleuropneumoniae* when 12 days old, but titers declined during the experiment. Two of the ten LW control pigs seroconverted to *M. hyopneumoniae* during the experiment, whereas none of the LW treated or EW control pigs seroconverted.

**Implications:** Tilmicosin did not reduce lesions of mycoplasmal pneumonia when added to the feed of CEW treated pigs. Tilmicosin delayed onset of coughing and thus probably delayed the development of lung pathology while it was fed to CEW treated pigs. Tilmicosin reduced *S. suis* colonization and *M. hyopneumoniae* seroconversion in LW pigs.

**Keywords:** swine, *Mycoplasma hyopneumoniae*, early weaning, tilmicosin

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**S**egregated early weaning (SEW) has been shown to inhibit the vertical transmission of some respiratory pathogens of pigs.<sup>1,2</sup> Pigs from the herd used in this study, reared using SEW technology, attain market weight about 40 days sooner and these pigs require approximately 0.5 lb (0.23 kg) less feed per lb (kg) of gain compared to the same measures from a large database for pigs reared

in continuous-flow systems.<sup>3,4</sup> Because of the improved health and performance of SEW pigs, many producers are considering using SEW technology. Some producers, however, are reluctant to adopt SEW because of its drawbacks: it is expensive to implement, particularly in smaller herds, and it can have a negative effect on reproductive performance. The objectives of this study were to investigate the effectiveness of a macrolide antibiotic (tilmicosin [Pulmotil], ELANCO, Indianapolis, Indiana) in preventing the development of mycoplasmal pneumonia in 12-day-old challenged pigs and unchallenged-littermate pigs

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**Figure 1**

Group	Birth	Allocate, bleed, and weigh; Wean	Rx, tilmicosin and/or Wean	Rx (Procaine Pen G)						Necropsy: bleed, weigh, score lungs
				Challenge	Stop Rx					
CEW, RX	●	AW	R	●●●●		●				●
CEW	●	AW		●●●●						●
EW, CONTROLS	●	AW		●●●						●
LW, RX	●	A	RW	●●●		●				●
LW, CONTROLS	●	A	W	●●●						●
Day:	1	12	21	27 28 29 30		42				56

Experimental design

weaned at 21 days of age. Additionally, the effect of tilmicosin on vertical transmission of other respiratory pathogens to pigs weaned at these two ages was investigated.

## Materials and methods

### Experimental design

Fifty pigs from a herd known to have *S. suis*, *H. parasuis*, *B. bronchi-septica*, *P. multocida* serotypes A and D, pseudorabies (Aujeszky's disease) virus, porcine reproductive and respiratory syndrome virus (PRRSV), *M. hyopneumoniae*, and *A. pleuropneumoniae* were randomly allotted by litter, sex, and weight to one of five treatment groups (Figure 1):

- CEW treated pigs (n = 10) were weaned at 12 days of age and moved to isolation. These pigs were intratracheally challenged with *M. hyopneumoniae* organisms (3 mL of 10<sup>7</sup> CCU) at 28 days of age. From day 21 to day 42, CEW treated pigs received feed containing 363.6 g tilmicosin per ton. From day 43 to day 56, these pigs received nonmedicated feed.
- CEW untreated pigs (n = 10) were weaned when 12 days old and moved to isolation. At 28 days of age, they were intratracheally challenged with *M. hyopneumoniae* organisms. These pigs did not receive tilmicosin in their feed at any time.
- EW control pigs (n = 10) were weaned when 12 days old and moved to isolation. They received neither challenge with *M. hyopneumoniae* nor treatment with tilmicosin.
- LW treated pigs (n = 10) were weaned at 21 days of age, moved to isolation, and reared to 56 days of age. At weaning, they received feed containing 363.6 g tilmicosin per ton for 21 days and then received untreated feed until they were 56 days old. These pigs were not intratracheally challenged with *M. hyopneumoniae* organisms.
- LW control pigs (n = 10) were weaned at 21 days of age, moved to isolation, and received neither challenge nor treatment during the trial.

All pigs of all groups were euthanized and submitted for necropsy at 56 days of age.

### Facilities

The isolation rooms used in this experiment were at Purdue University, 50 miles (83 km) from the herd of origin. Each room contained two 8 × 4-foot (2.4 × 1.2 m) pens on raised decks. The room air was HEPA filtered with at least 12 air changes per hour. Each of the five groups of 10 pigs was placed in one of the rooms in one of the two pens. Thus, each pig had a space allowance of 4 sq feet (0.4 m<sup>2</sup>) during the trial.

### Feed

Early-weaned groups of pigs received an SEW diet as their initial diet until 21 days of age. All additional diets were formulated as complete diets by ELANCO and did not contain antibiotics other than tilmicosin, as described above.

### Clinical health

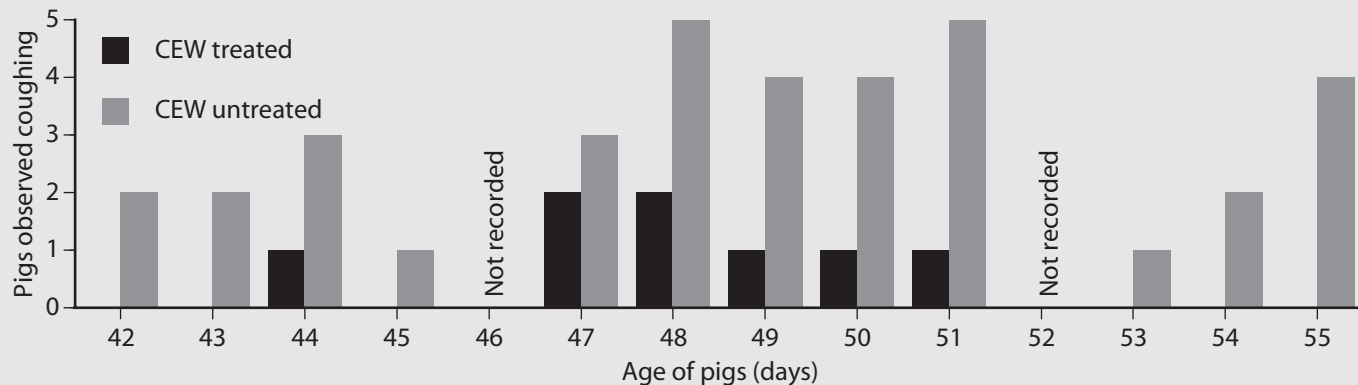
All pigs were observed daily for clinical signs of disease and all clinical signs were recorded. Because some pigs were observed to be clinically ill at 27 days (i.e., febrile, huddling, and anorexic), all pigs in all groups were treated with 1 mL (300,000 IU per mL) of penicillin G for that day and the next 2 days.

### Growth

At allocation to treatment group when pigs were 12 days old, individual pig weights were recorded. Weights of individual pigs were again recorded just after euthanasia on day 56. Weight gain for each pig was then calculated and used for analysis of differences of growth among groups.

### Coughing

Evidence of coughing was recorded as the number of pigs observed coughing in each treatment group each day. A pig was recorded to have coughed if it was observed coughing during the 15 minutes the animal caretaker cleaned each room and fed the pigs each day.

**Figure 2**

Number of pigs observed coughing per day per treatment group

## Lung lesions, bacterial culture, and fluorescent antibody test

On day 56, all pigs were euthanized by electrocution and a complete necropsy was performed. Pigs were weighed and blood samples were collected. Samples of nasal mucus, tonsils, and tracheal mucus were collected and culturally examined for *S. suis*, *H. parasuis*, *B. bronchiseptica*, and *P. multocida* type D. Lungs were removed from the thoracic cavity and pneumonic lesions were scored by standard methods used at Purdue University.<sup>5,6</sup> Samples of lung lesions (if present) or the ventral margin of the middle lobe of each pig were collected for fluorescent-antibody (FA) tests for *M. hyopneumoniae* and for cultural examination for *P. multocida*.

## Serologic analysis

All pigs in all groups were bled at 12 and 56 days of age. Sera were collected and tested for the presence of antibody titers to *A. pleuropneumoniae* by a hemolysin neutralization assay (HNA).<sup>7</sup> These sera were also tested by an ELISA<sup>8</sup> to determine the presence of *M. hyopneumoniae* titers.

## Statistical analysis

We expected 50% prevalence of the cultured bacterial organisms in the five groups of pigs. Thus, with 10 pigs in a group, we expected to isolate a pathogen 95% of the time in at least one pig in each group if the pathogen was present in the group. We tested the difference in prevalence of clinical illness and of the bacterial pathogens of all possible pairs of treatments using Fisher's Exact test. However, for the purposes of this experiment, the isolation of a pathogen in any pig in a group indicated that the treatment was not effective in eliminating that pathogen. We used a  $\chi^2$  test to determine differences in prevalence of coughing among the groups of pigs, and ANOVA F testing to determine differences in mean optical densities (ELISA) of serological samples, weight gains, and lung lesion scores.

## Results

### Health

More pigs tended to be clinically ill from the LW control group ( $P < .06$ ) than from the LW treated group. However, all pigs in all groups recovered after the second day penicillin was injected.

### Growth

Weights of pigs at the beginning and at the end of the trial did not differ significantly among the treatment groups.

### Coughing

Twelve days after the CEW treated and CEW untreated groups were challenged with *M. hyopneumoniae* (trial day 42), some of the pigs in the CEW untreated group began to cough (Figure 2). An increase in incidence of coughing pigs from 20% to 50% was observed before they were euthanized 2 weeks later. Only one pig in the CEW treated group was observed coughing for the first 17 days after challenge, and only two pigs were observed to cough from days 18–22 postchallenge. No coughing was observed after day 22 postchallenge.

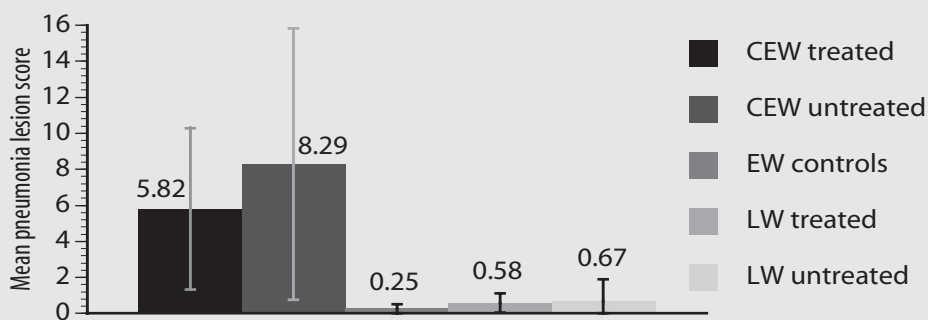
### Lung lesions

Only the CEW treated and untreated groups developed pneumonic lesions typical of mycoplasmal pneumonia. The mean lesion scores of pigs from these two groups were greater ( $P < .05$ ) than the scores of the EW controls. The mean pneumonic lesion score of CEW treated pigs (5.8%), did not differ significantly ( $P > .05$ ) from that of the CEW untreated pigs (8.3%) (Figure 3). The small pneumonic lesions observed in lungs of pigs in the other groups (mean lesion scores less than 1%) were not judged to be typical of mycoplasmal pneumonia.

### Bacterial cultural and FA

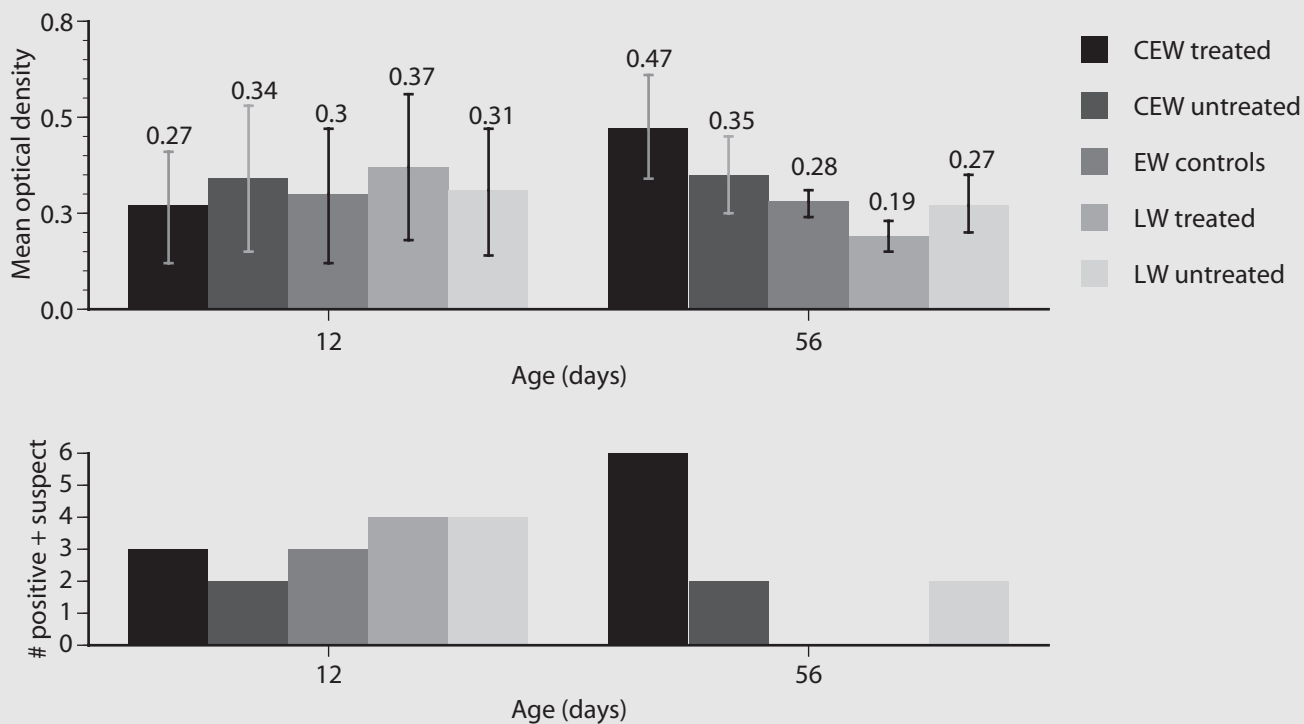
*Actinobacillus pleuropneumoniae*, *B. bronchiseptica*, and *P. multocida* were not isolated from any of the samples of any of the pigs (Table 1). *Streptococcus suis* was isolated from only seven of the pigs; most commonly from the tonsils. Four of the isolations were from LW

**Figure 3**



Lung lesions scores on day 56

**Figure 4**



*M. hyopneumoniae* serostatus of pigs on days 12 and 56

untreated pigs, whereas *S. suis* could not be isolated from LW treated pigs. *Haemophilus parasuis* was isolated from 26 of 30 samples from early-weaned pigs, most commonly from the nasal and tracheal swabs. The prevalence of *H. parasuis* did not differ significantly among the early-weaned or late-weaned groups of pigs.

*Mycoplasma hyopneumoniae* could not be detected by FA tests of the lungs of pigs in treatment groups that did not receive a challenge. Eight of the 20 CEW treated and untreated pigs' lungs were FA positive for *M. hyopneumoniae*; the prevalence of this pathogen did not differ significantly between CEW treated and untreated pigs.

## Serology

At 12 days of age, all pigs in all treatment groups were seropositive to *A. pleuropneumoniae*. When pigs were euthanized at day 56, the anti-

body titers had decayed such that the following percentages of pigs' titers were classified as suspect:

- 60% of CEW treated pigs,
- 70% of CEW untreated pigs,
- 40% of EW control pigs,
- 60% of LW treated pigs, and
- 30% LW untreated pigs.

However, mean titers among treatment groups did not differ significantly at either day 12 or 56 of the study. The mean *A. pleuropneumoniae* titers were significantly higher ( $P < .001$ ) within each treatment group at day 12 than in the same pigs at day 56 of the study.

The mean optical densities (ODs) to *M. hyopneumoniae* at day 12 were not positive for any of the groups (Figure 4). The mean ODs to

Table 1

Recovery of pathogens in the respiratory tract of 56-day-old pigs

Group	<i>S. suis</i>	<i>H. parasuis</i>	<i>A. pleuropneumoniae</i>	<i>B. bronchiseptica</i>	<i>P. multocida</i>	<i>M. hyopneumoniae</i> (FA)
CEW treated	1	2	0	0	0	5
CEW untreated	1	1	0	0	0	3
EW controls	1	4	0	0	0	0
LW treated	0	10	0	0	0	0
LW untreated	4	9	0	0	0	0

*M. hyopneumoniae* were highest at day 56 for the CEW treated and untreated pigs; however, the mean ODs did not differ significantly ( $P > .05$ ) between these two groups. The mean ODs of all three unchallenged groups showed evidence of decay, and the mean OD of the LW treated group was significantly lower ( $P < .06$ ) at 56 days of age than at 12 days of age. Two pigs in the LW control group had seroconverted, whereas none of the pigs in the LW treated and the EW control groups had seroconverted.

## Discussion

Differences in the mean weights of groups of pigs were not observed at either the beginning or the end of this experiment. Clark, et al., observed that *M. hyopneumoniae* does not effect growth of pigs during the first 28 days after challenge.<sup>5</sup> The sample size used in this study was not calculated to detect small differences in weight gain in this experiment, but rather to detect lesion score differences. We were, therefore, unable to determine whether tilimicosin has a growth-enhancing effect on mycoplasmal-infected early-weaned (EW) or late-weaned (LW) pigs in this experiment.

The significant difference we observed in coughing between the CEW treated and untreated groups suggests that tilimicosin postponed and minimized coughing in pigs when present in the feed during challenge with *M. hyopneumoniae*. This conclusion was supported by the absence of coughing and lung lesions in the EW controls and the presence of lung lesions in the CEW treated pigs. However, we observed that some coughing occurred as a result of the *M. hyopneumoniae* challenge when the tilimicosin was removed from the feed of the treated group. These data suggested that tilimicosin might be used strategically in pig rearing programs to delay *M. hyopneumoniae* infections until vaccination or isolation procedures are in place to prevent or eliminate this infection in growing/finishing pigs.

Our observations of lung lesions in challenged animals (with or without tilimicosin) along with the coughing data further supports our hypothesis that tilimicosin most likely delayed the onset of *M. hyopneumoniae* after challenge rather than eliminating the disease. Perhaps our sample size was inadequate to detect a difference in the lung lesion scores between CEW treated and untreated pigs that received the *M. hyopneumoniae* challenge. Our observations, nonetheless, do not warrant the expectation that tilimicosin would eliminate *M. hyopneumoniae*-induced respiratory disease.

Whether tilimicosin had an effect on isolation of *S. suis* could not be determined in this study. However, fewer *S. suis* isolations were made in the EW groups of pigs and in the LW treated pigs (Table 1). The EW groups had fewer pigs from which *H. parasuis* could be isolated than the LW groups. The reason that nearly all pigs in the LW groups were culture-positive for *H. parasuis* at necropsy was not determined. Tilimicosin did not appear to have an influence on the isolation of *H. parasuis* at necropsy. In the CEW treated and untreated groups of pigs, *M. hyopneumoniae* was identified as the cause of pneumonia in eight pigs. Interestingly, most of the pigs' lungs that were FA-positive to *M. hyopneumoniae* had the highest lesion scores. Tilimicosin treatment in the feed did not influence the proportion of FA-positive pigs in the two CEW groups.

The declining *A. pleuropneumoniae* antibody titers from 12- to 56-day-old pigs suggest that the passive antibodies that had been transferred from dams to their pigs decayed with age, which is consistent with the findings of Bosse, et al.<sup>9</sup> Consequently, the pigs in this trial were not infected with *A. pleuropneumoniae* by the time they were euthanized and thus the effect of tilimicosin on *A. pleuropneumoniae* could not be determined.

The mean optical densities (ODs) we observed in serum samples collected at 12 days from all pigs in this experiment were consistent with that of 14-day-old pigs from an infected herd investigated earlier.<sup>6</sup> It takes about 4 weeks for a challenge with *M. hyopneumoniae* to result in detectable seroconversion using the test applied in this study.<sup>8</sup> Thus, the ODs observed in this study—from pigs 4 weeks after challenge, when they were euthanized—were expected to be turning positive at this time. In the CEW treated and untreated groups of pigs, seroconversion had occurred in the CEW treated pigs whereas only two individual pigs in the CEW untreated group had seroconverted. Because the mean titers of these two groups were not significantly different, we hypothesized that both groups would have seroconverted, given enough time, between challenge and sampling. The EW control pigs were all seronegative when 56 days old, as expected.<sup>6</sup> The LW treated pigs were all seronegative with a significant decline in mean OD, whereas two pigs had seroconverted in the LW control group, indicating exposure. The limited duration of this study did not allow enough time to elapse for disease to develop in the exposed pigs or to be transferred to the unexposed pigs in this group, or possibly for pigs in the EW control group or the LW treated group to seroconvert.

## Implications

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- Although lesions of pneumonia at necropsy were not different in early-weaned challenged pigs, tilmicosin reduced the clinical expression (coughing) and delayed the onset of pneumonia in these pigs.
- Tilmicosin did not influence seroconversion in early-weaned challenged pigs.
- Future research should be conducted to determine whether feeding tilmicosin in pigs weaned at 21 days into AIAO facilities will result in increased performance and reduced clinical disease similar to SEW systems.
- Overall, tilmicosin appeared to delay clinical expression of mycoplasmal pneumonia and possibly enhanced the health of 21-day weaned pigs.

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