

The effect of *Mycoplasma hyopneumoniae* infection on growth in pigs with or without environmental constraints

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Summary— To determine the effects of *Mycoplasma hyopneumoniae* on growth performance, 32 commercial barrows that were free of *M. hyopneumoniae* infection were assigned by litter and weight to four treatment groups. Group 1 pigs were left as unchallenged controls. Groups 2–4 were mixed with *M. hyopneumoniae*-infected seeder pigs at 8 weeks of age. Group 2 pigs' environment was otherwise the same as that of the control group (no diurnal temperature fluctuation and normal ammonia concentration). Group 3 was exposed to diurnal temperature fluctuation but no elevated ammonia concentration. Group 4 pigs were exposed to diurnal temperature fluctuation and elevated ammonia concentration similar to those of an enzootic pneumonia-infected commercial herd. Some intratracheally inoculated seeder pigs began coughing 2 weeks after inoculation and most continued coughing until they were necropsied 8 weeks later. Contact-exposed pigs began coughing 4 weeks after exposure. We observed no differences in any measures of *M. hyopneumoniae* infection among the three exposed groups of pigs ($P > .10$). Data for the groups of exposed pigs were merged and compared to that of the control pigs. Control pigs did not develop clinical signs or lesions of *M. hyopneumoniae*, whereas all pigs except one in the exposed groups coughed or had other evidence of *M. hyopneumoniae* infection. Accumulated weight gain tended to be less ($P \leq .10$) in exposed pigs than in control pigs from 4 weeks to 10 weeks after exposure to seeder pigs except at 6 weeks after exposure. After 10 weeks, exposed pigs gained faster than control pigs such that subsequent accumulated weight gain was not different ($P > .10$). These results indicated that the ambient environment imposed in this study did not influence development of pneumonia due to *M. hyopneumoniae*. *M. hyopneumoniae* induced pneumonia and reduced weight gain during the early coughing period, but had little influence on overall gain when measured at slaughter.

Enzootic pneumonia of swine can be initiated by *M. hyopneumoniae* and is exacerbated by secondary infections, particularly by *Pasteurella multocida* serotype A. Environmental stressors also may contribute to this syndrome, which is a major constraint to efficient pork production throughout the world.¹ *M. hyopneumoniae* seems to be a particular problem in continuous-flow herds. In pigs that were reared in all-in/all-out groups in the farrowing house, nursery, and growing-finishing unit, any mycoplasmas that were transmitted from sows to pigs or between pigs did not induce clinical signs or lesions of pneumonia, nor did they enable secondary infections to develop.² Pigs reared in an all-in/all-out system did not have lesions or had minimal lesions at slaughter and gained at a faster rate than littermate pigs reared in a continuous system.^{2,3} Whether the increased rate of gain was because the extent and effects of pneumonia were reduced or because all-in/all-out environments reduced the

clinical effect of pneumonia is unknown. In commercial pigs, low ambient temperatures (4°C) and a high aerosol concentration of ammonia (>50 ppm) reduces bacterial clearance from lungs^{4,5} and a high aerosol concentration of ammonia (>50 ppm) reduce daily gain.⁶ Additionally, ambient environments of commercial herds influence growth performance of pigs that have pneumonia.⁷ However, the environments in commercial herds did not influence the growth performance of pneumonia-free pigs.⁸

In this study, we investigated whether experimental infection with *M. hyopneumoniae* would induce pneumonia and reduce growth rate in commercial pigs. We also wished to determine whether elevated concentration (17.9 ± 0.6 ppm) of ammonia (NH₃) and fluctuating ambient temperature (18.3 ± 0.17 to 21.0 ± 0.20°C), in addition to this infection, influenced the severity of pneumonia and its effect on growth rate.

Materials and methods

Animals

We obtained pigs from a swine herd that had been accredited as specific pathogen-free for 12 years. Lungs from 6-week-

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old pigs had been cultured or declared negative for *M. hyopneumoniae* as recently as 5 months before the project was started. To ensure that the herd had remained free of mycoplasmal infection, we blood tested all pigs used in this study and their dams and found them to be negative by the Western blot test before the study began. The experimental pigs were born in November. After weaning, pigs were housed in an all-in/all-out nursery until they were transferred to isolation facilities at Purdue University.

Management

Pigs were placed in identical cleaned and sanitized (formaldehyde-fumigated) rooms, each with a 10 m² solid concrete floor and a drain in one corner. The rooms were ventilated by positive-pressure fans (12 air changes per hour) and heat was controlled by thermostats. Waste was washed from the rooms once per day. Personnel were not allowed to enter the rooms if they had been in contact with other pigs during the previous 12 hours. All personnel wore freshly laundered outerwear, and had to pass through two doors and step through two foot baths to care for the pigs. Personnel cared for pigs that were uninfected before those that were infected.

All pigs were fed corn-soy diets that met National Research Council requirements for concentration of nutrients.⁹ Each room had a free-choice feeder and a nipple waterer.

Treatments

Forty-eight middle-weight 4-week-old barrows were randomized by litter and weight to four groups of 12 pigs and identified by colored ear tags. When the pigs were 6 weeks old, four pigs from each of groups 2, 3 and 4 were placed in an isolation room. Four pigs from the control group were also removed from the group and placed in another isolation room. We prepared each pig from groups 2-4 as a seeder pig by inoculating them intratracheally with 3 mL of broth containing 10⁷ color-changing units/mL *M. hyopneumoniae* strain P-5722-3.¹⁰ We expected the seeders to transfer *M. hyopneumoniae* to the uninoculated (exposed) pigs. At 8 weeks of age, the pigs in each group were reunited and were placed by group into isolation rooms. Group 1 consisted of 12 uninoculated (control) pigs. Groups 2-4 consisted of eight uninoculated (exposed) and four seeder pigs. Seeder pigs were kept in contact with exposed pigs in all groups for 8 weeks, and then were euthanatized and necropsied. The exposed pigs remained in their respective isolation rooms until they were taken to a slaughter plant at 165 days of age.

Environmental conditions

For the duration of the experiment, we set the room temperatures for pigs in groups 1 and 2 at 21°C. Ammonia concentrations were within acceptable ranges (1.0-5.0 ppm). We adjusted the room temperatures for pigs in groups 3 and 4 at 12 hour intervals to mimic the high (mean 21.0 ± 0.20°C) and low (18.3 ± 0.17°C) temperatures we measured the previous day in a Purdue University hog finishing building (10 miles northwest

of the isolation facilities), in which the frequency of pigs with mycoplasmal pneumonia (diagnosed by histopathology and culture examination) at slaughter was >80%. We continuously added ammonia to the room that housed the group 4 pigs and adjusted these concentrations weekly to mimic the previous week's ammonia concentration (mean 17.9 ± 0.6 ppm, low 14 ppm, high 22 ppm) measured in the University's commercial finishing building. The ammonia concentrations reported were those measured in the isolation units.

Evaluation of pigs, sera, and lungs from pigs and statistical analyses

We observed all groups of pigs for 10 minutes every day and recorded the number of pigs we visually observed to cough. We also noted any other clinical signs of disease. We weighed all pigs every other week, and bled all pigs at necropsy. We tested all sera for the presence of *M. hyopneumoniae* antibodies by the Western blot method.¹¹ Seeder pigs were electrocuted and then exsanguinated. All other pigs were slaughtered in a packing plant after being stunned with CO₂ and then exsanguinated. We examined all organs from all pigs macroscopically for lesions. We removed the respiratory tract from the carcasses and took them to our laboratory.

Lungs from all pigs were subjected to the following procedures:

- we examined the dorsal surface of each pair of lungs and the ventral surface of the accessory lobe to ensure that any lesions were consistent with those caused by *M. hyopneumoniae*. We sketched the margin of each lesion on a standard sheet that showed the outlines of the lung lobes. We then used a computerized digitizer (Osteoplan, Zeiss, Thornwood NY) to determine the severity of lesions as a percentage of the total lung area, and that percent was used as the lesion score.
- we collected sections of the ventral margin of two anterior and two middle lobes, or portions of lesions in these areas, and performed fluorescent antibody tests (FATs) to *M. hyopneumoniae*.¹⁰
- After lightly searing lungs with a torch to destroy surface contaminants, we collected samples for bacterial culture; however, we only cultured and examined those lungs negative to FAT for *M. hyopneumoniae*. Using sterile techniques, we collected blocks of tissue approximately 1 cm³ from the edge of the lesion. If there were no lesions, we collected portions of the ventral margins of the anterior and middle lobes. We pooled the tissues from the lobes of each individual pig and macerated them in sterile Friis broth without antibiotics. We then cultured aliquots of this suspension for *M. hyopneumoniae* as described by Armstrong and Friis.¹²

We inoculated aliquots onto sheep blood agar plates with a streak inoculum of *Staphylococcus epidermidis*, and incubated them overnight at 37°C to grow and identify the bacteria.

Measuring growth performance

Each biweekly weight measurement was compared to the baseline weight measurement to determine biweekly accumulated gain. We used analysis of variance (ANOVA) and the General Linear Models Procedure¹⁵ to determine whether biweekly accumulated gain among the treatment groups and between the control and merged treatment groups were significantly different. Accumulated weight gain among groups 2-4 did not significantly differ ($P > .05$), so we merged the accumulated weight gain of groups 2-4 and then compared the mean accumulated weight gain of the three treatment groups to that of the control group at 2-week intervals. We used Fisher's exact test¹⁵ to determine the significance of differences between control and merged treatment groups for:

- the number of pigs observed to cough;
- the number of pigs with serum antibodies; and
- the number of pigs with gross lesions.

Results

Seeder pigs

Some of the seeder pigs were coughing 2 weeks after intratracheal inoculation when they were reintroduced to their original groups of pigs. We observed all seeder pigs to cough before we removed them 8 weeks later. During the exposure period we observed the seeder pigs of each treatment group to cough a similar number of days. Most seeder pigs had

stopped coughing before necropsy. At necropsy, when the seeder pigs were 16 weeks old, only four of the 12 had lung lesions, all were FAT negative, and lungs from only two pigs yielded *M. hyopneumoniae*. *P. multocida* was not isolated from lungs of any seeder pigs.

Exposed pigs

Lungs of only two of the 24 exposed pigs had gross lesions of pneumonia. Because FAT for the presence of *M. hyopneumoniae* is rarely positive in our laboratory in lesion-free lungs, we chose to use cultural examination as our definitive test. However, lung samples from all exposed pigs were contaminated with ubiquitous organisms such as *Bacillus sp.*, coliforms, and α - and β -hemolytic *Streptococcus sp.*, which prevented us from isolating bacterial pathogens from the lungs of any of the exposed pigs. We found that these organisms were present in large numbers in the scald water of the packing plant. Thus, valid microbiological results could not be obtained from the lungs of the exposed pigs and were not reported.

After exposure to seeder pigs, we determined that groups 2-4 did not significantly differ in:

- the number of exposed pigs that were observed coughing;
- the number of exposed pigs that seroconverted; and
- the number that had pneumonia at slaughter ($P > .10$).

Accumulated weight gain was not different ($P > .10$) at any weighing period among the three groups of exposed pigs. Pigs in group 2 (in the same "low-stress" environment as the control pigs) had the lowest accumulated weight gain (Table 1). When data from pigs in the exposed groups were merged and compared with data from control pigs, measures of pneumo-

Table 1.— Data from pigs that had contact exposure (except for group 1) to seeder pigs

Group	Pigs per group	Weight gain	Pigs observed coughing	Pigs Western blot seropositive	Pigs with lung lesions	Ammonia	Temperature
1	8	94.5 ± 8.2	0(a)	2(a)	0	3.0 ± 0.5	21 ± 0
2	8	88.0 ± 5.0	8(b)	7(b)	1	3.3 ± 0.3	21 ± 0
3	8	89.8 ± 5.9	7(b)	5(a,b)	0	3.4 ± 0.4	21.0 ± 0.2 18.3 ± 0.2
4	8	95.1 ± 6.9	8(b)	6(a,b)	1	17.9 ± 0.6	21.0 ± 0.2 18.3 ± 0.2

Weight gain: Mean ± SEM accumulated weight gain (kg).

Pigs observed coughing: Numbers in this column with different letters in parentheses are significantly different ($P < .05$).

Pigs Western blot seropositive: Number of pigs that reacted with serum antibodies to *M. hyopneumoniae* cell membrane protein (Western blot). Numbers in this column with different letters in parentheses are significantly different ($P < .05$).

Pigs with lung lesions: Number of pigs with lesions typical of mycoplasmal pneumonia. In each case, lesions occupied less than 2% of the lung surface area.

Ammonia: Mean ± SEM for weekly ammonia concentration (ppm) within each group.

Temperature: Mean ± SEM for temperature in degrees Celsius for each group. The mean daily high and low temperatures for the period of exposure are shown for groups 3 and 4.

nia (coughing and seroconversion, but not lesion scores) were different ($P < .05$) between exposed and control pigs. At 4 weeks after exposure, pigs in groups 2-4 had lower weight gain ($P = .06$) than control pigs, even though at this point we had observed only 1 exposed pig to cough (Fig 1). Accumulated weight gain among the exposed pigs continued to lag behind that of control pigs: there tended to be significant differences among the weight measurements of the groups at 4, 8, and 10 weeks after exposure ($P < .10$), although there was no significant difference in the measurements at 6 weeks after exposure. Between 4 and 10 weeks after exposure, most of the exposed pigs were observed coughing. Although coughing did not subside until the 14th week after exposure, after the 10th week these clinical signs were not associated with retarded growth (exposed pigs gained faster than control pigs between 10 and 12 weeks after exposure), and accumulated weight gain was not different ($P > .10$) from 12 to 16 weeks after exposure. From the 10th to the 16th week post-exposure, pigs in groups 2-4 were able to fully compensate for the previous growth lag so that by 12 weeks of age, the growth of the exposed pigs did not differ significantly from that of the control pigs.

Discussion

We used only 48 pigs in this study and, because of costs, performed no replicates. It is possible, then, that had we included more animals or repeated the experiments, we would have found significant differences among the treatment (exposed) groups, indicating that environmental stressors do, in fact, exacerbate the problems associated with *M. hyopneumoniae*. The clinical signs and lesions of pneumonia we observed in the seeder pigs were similar to those reported by others.¹⁴ In the present study, we believed that the seeder pigs infected with *M. hyopneumoniae* were the source of these organisms for contact transmission to exposed pigs, even though at necropsy we noted lesions in only four pigs and could isolate the pathogen from only two. We believe that these seeder pigs were infected and did develop lesions, but that these lesions had resolved by the time the animals were necropsied, as observed in other studies.^{15,16} We expected to find no evidence of pneumonia at slaughter in exposed pigs, either, because slaughter was about 105 days after exposure and other researchers,^{15,16} have reported that healing would have been nearly complete at that time.

Because our cultural techniques for exposed pigs were compromised by the presence of many bacteria in the scald water, we had to rely on differences in coughing and seroconversion between exposed and unexposed pigs to determine whether *M. hyopneumoniae* were transmitted. The clinical pattern of pneumonia in exposed pigs was typical of *M. hyopneumoniae* infections that we¹⁷ and others^{15,16} have observed. Also, once the data were merged, seroconversion to *M. hyopneumoniae* occurred in more exposed pigs than unexposed pigs ($P < .05$). The reported seroconversions in the two unexposed pigs (Table 1) were very weak responses and may have been the result of *M. flocculare* cross reactions. Although the method we used to detect previous exposure to *M. hyopneumoniae* was unexpectedly unsuccessful, we believe that the coughing and seroconversion in the exposed pigs and the lack of similar results in control pigs were satisfactory to substantiate infection.

Under the general environmental conditions of this study, neither the magnitude of fluctuations in temperature nor the elevated concentration of NH_3 was sufficient to influence the pigs' susceptibility to mycoplasmal or enzootic pneumonia or their growth performance.⁴⁵ The ammonia concentrations and temperature fluctuations used in this experiment are commonly found in finishing units in the midwest. Because clinical signs and lesions of pneumonia did not differ among the exposed groups of pigs, we conclude that the elevated ammonia concentrations and variable ambient temperatures found in the field do not appear to exacerbate *M. hyopneumoniae* infection.

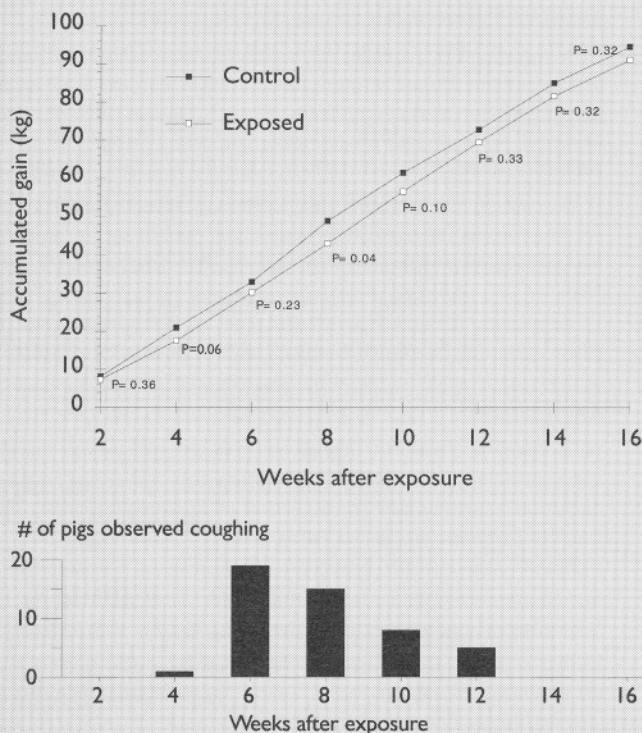


Fig 1.—(Top) Relationship between coughing and accumulated gain: 24 pigs exposed to seeder pigs and 8 pigs not exposed. Accumulation of gain was from 8 weeks of age. P values $> .10$ are not significant. (Bottom) The number of exposed pigs observed to cough. During the trial, 23 of 24 of the pigs that were exposed to seeder pigs were observed to cough. (Seeder pigs were inoculated intratracheally with *M. hyopneumoniae*.) None of the unexposed (control) pigs were observed to cough.

Nor do these environmental conditions have an effect on accumulated weight gain of *M. hyopneumoniae*-infected pigs: pigs in group 2 had the same environmental conditions as the control pigs, yet tended to have the least accumulated weight gain. These findings contradict those of Straw, who reported that environmental differences between groups of pigs with enzootic pneumonia resulted in growth performance differences.⁷ It is possible that the difference in growth performance she noted was due to the presence of different infectious agents in her study: enzootic pneumonia is considered to be a combination infection. It is possible, however, that our challenge model (seeder pigs) did not provide a consistent challenge among the treatment groups, and that this obscured any environmental effects on *M. hyopneumoniae* transmission and accumulated weight gain. The final weight difference between exposed and control pigs may have been the true residual effect of *M. hyopneumoniae* on pigs and this could have been determined with a larger sample size. However, this model (albeit without secondary agents) was as similar to field conditions as we could make it and, according to previous studies, would ensure that the disease was transmitted.¹⁷ Perhaps environmental insults compounded the effect of secondary agents on growth performance in other studies, but did not influence the effect on growth performance of the primary agent, i.e. *M. hyopneumoniae*. Perhaps the temperature fluctuation or NH₃ concentration used in this study was too low to influence growth performance.⁵ It appears that before environmental constraints have lasting influence on performance of pigs with enzootic pneumonia, secondary infections must occur.

The influence of *M. hyopneumoniae* on weight gain in the early growth phase in this experiment was similar to that observed by others.¹⁸ However, Pointon¹⁸ observed that weight gain was consistently reduced throughout the growth phase in exposed pigs, whereas in our study, we observed compensatory weight gain so that at the end of the study, weight gain did not differ between exposed and non-exposed pigs. In Pointon's study,¹⁸ secondary bacteria were isolated in some exposed pigs and lesions in most pigs had not regressed at the time of necropsy, whereas in our study coughing had subsided, and lesions were regressing or had regressed, which is more typical of uncomplicated *M. hyopneumoniae* infections.^{15,16} Thus, we conclude that the results we observed in this study more likely represented the effects of *M. hyopneumoniae* infection by itself than those in Pointon's study.¹⁸

Although *M. hyopneumoniae* infection is necessary to establish secondary bacterial pneumonias, and is thus of economic significance to the swine industry, this organism alone or in the presence of environmental stressors (but not secondary pathogens) may not be responsible for the reduced daily gains attributed to enzootic pneumonia in commercial swine herds.¹

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