ORIGINAL RESEARCH

Suh D, Rutten S, Dee SA, et al. Effect of nursery depopulation on the seroprevalence of *Mycoplasma hyopneumoniae* infection in nursery pigs. *Swine Health and Production.* 1998;6(4):151-155.

Effect of nursery depopulation on the seroprevalence of *Mycoplasma hyopneumoniae* in nursery pigs

Dongkyun Suh, DVM, MS; Stephanie Rutten; Scott A. Dee, DVM, PhD, Dipl. ACVM; Han Soo Joo, DVM, PhD; Carlos Pijoan, DVM, PhD

Summary

Objective: To examine seroprevalence of *Mycoplasma hyopneumoniae* in pigs of different age groups and retrospectively determine whether previous nursery depopulation (ND) was associated with the seroprevalence of *M. hyopneumoniae* in nurseries.

Methods: Sera of 4-, 8-, 12-, 16-, and 20-week-old pigs from seven herds were selected from a serum bank to serologically profile *M. hyopneumoniae* infections. Availability of representative sera in the serum bank was a major criterion for herd selection. Sera were tested for *M. hyopneumoniae* antibodies by ELISA using Tween-20 extracted antigen. Serum samples were also selected from 13 of 34 swine herds that previously participated in a ND study. To evaluate *M. hyopneumoniae* infection after ND, ELISA was performed for the 13 herds with 10 sera of 8- to 10-week-old nursery pigs collected prior to and three times after ND for up to 12 months.

Results: Serological profiles showed positive mean antibody titers (MAT) for two of seven herds at 8 weeks, four of seven herds at 12 weeks, six of seven herds at 16 weeks, and all of the herds at 20 weeks of age. Prior to ND, 10 of the 13 herds had positive MAT in sera of 8- to 10-week-old pigs. Sample seroprevalence by ELISA antibody titer ranged from 50%-100%. Following ND, six of the 10 herds were MAT positive in 8- to 10-week-old pigs; four of the 10 herds remained MAT negative for up to 12 months after ND.

Implications: Seroconversion to *M. hyopneumoniae* was detected between 10–16 weeks of age, indicating the occurrence of natural infection during the nursery age (4–12 weeks of age). Nursery depopulation appeared to be an effective method to control *M. hyopneumoniae* infection among nursery pigs in some herds.

Keywords: swine, *Mycoplasma hyopneumoniae*, nursery depopulation

Received: September 19, 1997
Accepted: March 31, 1998

Swine enzootic pneumonia, an economically important swine respiratory disease, is caused primarily by *Mycoplasma hyopneumoniae*. The incidence and severity of swine enzootic pneumonia are exacerbated by complex interaction among *M. hyopneumoniae*, environmental factors, management practices, and secondary pathogens such as *Pasteurella multocida* and *Actinobacillus pleuropneumoniae*.[1,2] Recently, a porcine respiratory disease complex (PRDC) has been described as one of the most significant problems in the United States swine industry.[3] The PRDC is caused by infection with multiple agents, including porcine reproductive and respiratory syndrome virus (PRRSV), *M. hyopneumoniae*, swine influenza virus (SIV), and others.

Clark, et al.,[4] have demonstrated that *M. hyopneumoniae* infection can be prevented using either modified medicated early weaning (MMEW) or isolated weaning techniques. Because of the potentially excessive cost per pig weaned, MMEW technology has not been routinely used on commercial herds. Studies investigating the effect of *Mycoplasma* vaccine on pig performance have yielded inconsistent results,[5,6] supporting the importance of an antibody serum profile for *M. hyopneumoniae* infection within the herd.[7] Recently, Dee, et al.,[8] have demonstrated the nursery depopulation (ND) method to be effective in controlling PRRSV problems in nursery pigs of infected herds.

This study had two objectives:

- to perform serologic profiles for *M. hyopneumoniae* infection in pigs of different age groups to establish a pattern of seroconversion (phase one), and
- to compare serum antibody concentrations for pigs in those "at risk" age groups from herds prior to and after ND (phase two) to determine whether ND could influence seroprevalence of *M. hyopneumoniae*.

Materials and methods

Phase one: Determining *M. hyopneumoniae* seroconversion patterns

To evaluate age patterns of *M. hyopneumoniae* seroconversion, seven farms were selected based on the following criteria:

DKS, SR, HSJ, CP: College of Veterinary Medicine, Department of Clinical and Population Sciences, University of Minnesota, St. Paul, Minnesota, 55108. SAD: Swine Health Center, Morris, Minnesota

This article is available online at http://www.aasp.org/shap.html

- Sera from the herd were stored in a serum bank. The serum bank consists of sera submitted for PRRSV antibody profiles during the 5 years prior to the start of this study;
- Sera represented pigs at 4-, 8-, 12-, 16- and 20-weeks old; and
- *Mycoplasma hyopneumoniae* had been established in the herd.

Of these farms, six met the above criteria, while a seventh contained sera only from pigs ages 4- through 16-weeks of age. Ten sera from each age group of the selected farms were evaluated for antibodies to *M. hyopneumoniae*, and a pattern of seroconversion was established.

Phase two: Determining the effect of ND

Based on results of the seroconversion pattern we established in phase one of the study, we compared seroprevalence of *M. hyopneumoniae* prior to and after ND using 13 of 34 farms involved in a previous study.[9] Farm selection was based on the following criteria:

- representative serum samples were collected prior to and after ND for up to 12 months;
- sera from the herd were stored in a serum bank;
- ND had been performed in the herd in conjunction with previous study; and
- there was no history of previous vaccination against *M. hyopneumoniae* in the herd.

Ten sera were selected from 8- to 10- week-old nursery pigs for each farm prior to and 2–4, 6–8, and 10–12 months after ND. Sera were evaluated for antibody concentration to *M. hyopneumoniae*.

Enzyme-linked immunosorbent assay (ELISA)

Mycoplasma ELISA antigen was prepared as described previously.[10] Briefly, *M. hyopneumoniae* was grown in Friis medium at 35°C. Cells were pelleted by centrifugation at 10,000 rpm for 30 minutes. The pellet was washed three times and suspended in phosphate buffered saline (PBS, pH 7.2). The suspension was mixed with an equal volume of 2% Tween-20 in PBS, stirred 90 minutes at 37°C, and centrifuged at 10,000 rpm for 1 hour. Supernatant was then passed through a 0.2-µm membrane filter and stored at -70°C in aliquots.

For ELISA, 96-well microplates (Immulon II, Dynatech Laboratories, Inc., Alexandria, Virginia) were coated by incubating at 4°C overnight with 100 µL of antigen in carbonate/bicarbonate buffer (pH 9.6). Antigen was removed, and plates were incubated at 37°C for 3 hours with blocking solution containing 1% bovine serum albumin (BSA) and 3% rabbit serum in PBS.

Before use, plates were washed three times with PBS containing 0.05% Tween-20. Fifty µL of each test serum diluted 1:50 in blocking solution was added to duplicate wells. Plates were incubated at 37°C for 30 minutes. After washing, 50 µL of peroxidase-conjugated rabbit anti-swine IgG (1: 3,000, Organon Teknica-Cappel Co., Pennsylvania) were added to each well and incubated at 37°C for 30 minutes. Wells were washed again, and 50 µL of substrate containing o-phenylenediamine (Sigma Chemical Co., St. Louis, Missouri) and H_2O_2 was added. After 10 minutes at room temperature, the reaction was stopped by adding 50 µL 0.5N sulfuric acid. Optical density (OD) values were measured with an ELISA reader at 490 nm. OD values of 0.300 or greater were regarded as positive in this study.

Results

Phase one

Profiles of *M. hyopneumoniae* seroconversion patterns demonstrated that antibody titer prevalence increased with age (Figure 1). Evaluation of the seven herds found none with positive MAT at 4 weeks of age; however, by 16 weeks, six of seven farms had positive titers, and by 20 weeks, all had positive MAT.

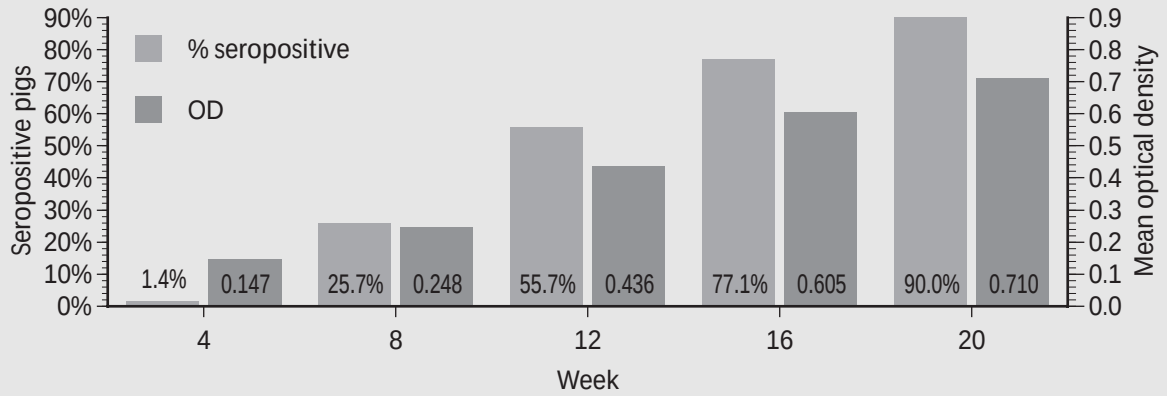
Phase two

Three herds of thirteen (farms 11–13) had negative MAT prior to ND and maintained MAT-negative status throughout the study (Figure 2). The remaining 10 herds were MAT positive prior to ND.

Following ND, four of ten herds continued to be MAT-negative, while the remaining six herds were MAT positive both prior to and after ND (Figure 2).

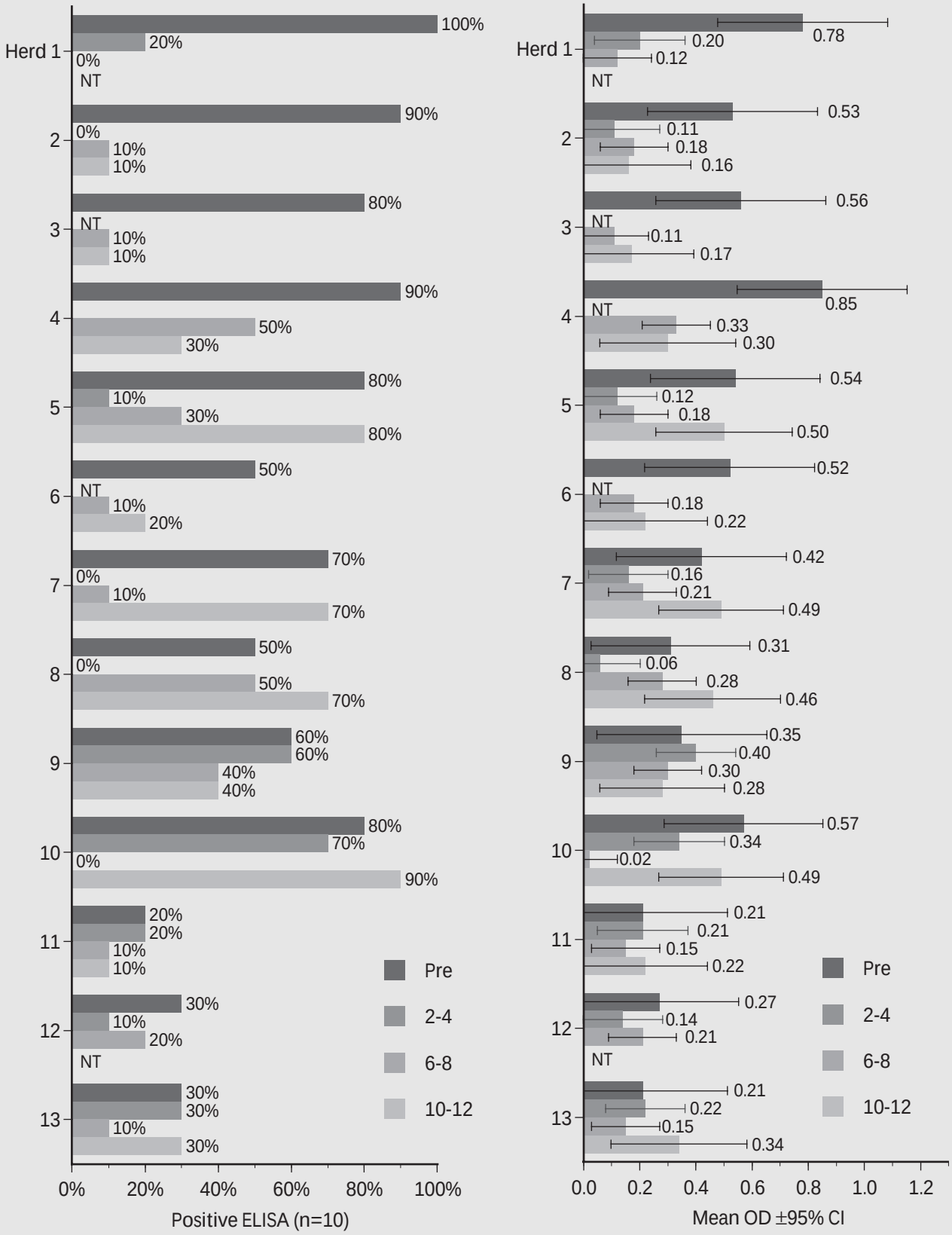
There was no significant difference on the 13 farms between antibody

Figure 1



Mean seroprevalence and mean antibody profiles of *M. hyopneumoniae* in pigs of different ages; ten sera per herd from seven farms included for each age group except 20 weeks which had only six farms. OD values greater than 0.300 were considered positive.

Figure 2



Mean antibody titers for 13 herds performing nursery depopulation. OD values greater than 0.300 were considered positive.

titers and farms ($P = .9299$). However, significant relationships were found between antibody titers and time from ND ($P < .0001$). Further evaluation found the difference between pre- and post-ND was highly significant ($P = .0002$).

Discussion

Although several serologic tests, including complement fixation[11] and indirect hemagglutination,[12] have been used to detect antibodies to *M. hyopneumoniae*, ELISA using Tween-20 extracted antigen was chosen for this study. ELISA was found to be highly sensitive and specific in comparative studies among different serologic tests.[13,14,15] No significant cross reaction was reported when sera with high concentrations of antibodies against *Mycoplasma flocculare* were tested using the Tween-20 *M. hyopneumoniae* ELISA.[15]

For this study, ten sera were used from each herd sampling to have 95% confidence of detecting a herd as positive if the prevalence was at least 30%. In the first part, seroconversion to *M. hyopneumoniae* infection was detected at 8 weeks of age in four of the seven herds, and pigs from all herds became seropositive by 10–16 weeks of age. These results are consistent with those reported by Sheldrake, et al.,[16] who found that seroconversion was more likely at 12–16 weeks of age. Given that it takes ≥4 weeks for pigs to seroconvert after *M. hyopneumoniae* challenge,[13] natural infection in those herds could have occurred in the nursery. This seroconversion pattern appears to correlate with the time at which passive antibodies become undetectable. Morris, et al.,[17] suggested that passive antibodies wane at 1–2 months, depending on initial antibody concentrations in the piglets.

Transmission of *M. hyopneumoniae* commonly occurs between carrier sows and their piglets, infected and naive pigs in farrowing and nursery rooms, and among pigs in growing and finishing units.[18] It has been reported that gilts are more apt to transmit *M. hyopneumoniae* to their pigs during the lactation period than are sows.[19] Therefore, piglets originating from herds with large numbers of gilt litters would be expected to have a higher risk of infection prior to weaning. Serologic profiles from this study showed that most *M. hyopneumoniae* infection occurred among pigs in the nursery phase. These data indicate that newly infected nursery pigs are a high risk group for *M. hyopneumoniae* transmission.

All-in–all-out (AIAO) management of nursery pigs with vaccination and/or medication of the potential risk group may be helpful to control *M. hyopneumoniae* infections within herds. Vaccination of replacement gilts at 5 and 2 weeks prior to farrowing may help to boost maternal immunity and reduce piglet contamination during lactation. Further investigation of vaccination use to reduce gilt litter contamination is necessary.

Since none of the farms included in phase two of the study weaned beyond 30 days (4 weeks), and seroconversion to *M. hyopneumoniae* antigen typically requires 4 weeks, seroconversion would be expected in 8- to 10-week-old pigs. Nursery age sera were most relevant in this study.

Dee and Joo[8] reported ND to be an effective method to eliminate PRRS virus in endemically infected herds. Elimination was successful in herds that demonstrated a high seroprevalence of PRRS antibodies in 8- to 10-week-old pigs with no or low seroprevalence among sows and recently weaned piglets.

In this study, we found mean antibody titers to *M. hyopneumoniae* were significantly reduced immediately and up to 12 months after ND. It is not clear how the seroconversion pattern was stopped and the seronegative status maintained; however, as with PRRSV, ND may have prevented spread of *M. hyopneumoniae* infection from older, previously infected nursery pigs to those recently weaned.

Results of our study suggest that ND may be effective in preventing horizontal transmission of *M. hyopneumoniae* in nurseries. While it appears that seroconversion has been prevented in certain cases, further studies are necessary to assess the ability of ND to eliminate the organism.

Implications

- Antibody titer prevalence increased with age.
- Nursery depopulation may be an effective method to control transmission of *M. hyopneumoniae* infection in nurseries.

References

1. Morrison RB, Pijoan C, Leman AD. Association between enzootic pneumonia and performance. *Pig News Inf*. 1986;7:23–31.

2. Straw BE. A look at the factors that contribute to the development of swine pneumonia. *Vet Med*. 1986; 8:747–756.

3. Boeckman S. Grow-finisher herds hit hard by porcine respiratory disease complex. *Swine Practitioner*. February 1996;.4–8.

4. Clark LK, Hill MA, Kniffen TS, et al. An evaluation of the components of medicated early weaning. *Swine Health and Production*. 1994;2(3):5–12.

5. Carlier P, Jambers B, Martinod S, Legrand A. Efficacy of stellamune™ mycoplasma in European trials. *Proc IPVS*. 1994;136.

6. Morrow MWE, Igesia G, Stanislaw C, Stephenson A, Erickson G. Effect of a *Mycoplasma* vaccine on average daily gain in swine. *SHAP*. 1994;2(6):13–18.

7. Blaha T, Delbeck F, Beilage E. Decision making on vaccinating against *Mycoplasma hyopneumoniae* infection. Proc. *Allen D. Leman Conf. St. Paul, Minnesota.* 1996; 190–192.

8. Dee SA, Joo HS. Prevention of the spread of porcine reproductive and respiratory syndrome virus in endemically infected pig herds by nursery depopulation. *Vet Rec*. 1994;135:6–9.

9. Dee SA, Joo HS, Polson DD, Park BK, Pijoan C, Molitor TW, Collins JE, King V. Evaluation of the effects of nursery depopulation on the persistence of porcine reproductive and respiratory syndrome virus and the productivity of 34 herds. *Vet Rec*. 1997; 140:247–248.

10. Nicolet J, Paroz P, Bruggmann S. Tween 20 soluble proteins of *Mycoplasma hyopneumoniae* as antigen for an enzyme linked immunosorbent assay. *Res Vet Sci*. 1980;29:305–309.

11. Boulanger P, Lecuyer C. Enzootic pneumonia of pigs: Complement-fixation tests for the detection of mycoplasma antibodies in the serum of immunized rabbits and infected swine. *Can J Com Med*. 1968;32:547–554.

12. Lam KM, Switzer WP. Mycoplasmal pneumonia of swine: Development of an indirect hemagglutination test. *Am J Vet Res*. 1971;32:1731–1736.

13. Amstrong CH, Freeman MJ, Sands-Freeman L, Lopez-Osun M, Young T, Runnels LJ. Comparison of the enzyme-linked immunosorbent assay and the indirect haemagglutination and complement fixation tests for detecting antibodies to *Mycoplasma hyopneumoniae*. *Can J Comp Med*. 1983;47:464–470

14. Kobisch M, Nicolet J. Comparison of enzyme- linked immunosorbent assay (ELISA) and indirect haemagglutination (IHA) in experimental *Mycoplasma hyopneumoniae* infection of pigs. *Isr J Med Sci*, 1987;23:644–646.

15. Bereiter M, Young TF, Joo HS, Ross RF. Evaluation of the ELISA and comparison to the complement fixation test and radial immunodiffusion enzyme assay for detection of antibodies against *Mycoplasma hyopneumoniae* in swine serum. *Vet Microbiol*, 1990; 25:177–192.

16. Sheldrake RF, Gardner IA, Saunders MM, Romalis LF. Serum antibody response to *Mycoplasma hyopneumoniae* measured by enzyme-linked immunosorbent assay after experimental and natural infection of pigs. *Aust Vet J.* 1990;67:39–42.

17. Morris CR Gardner IA, Hietala SK, Carpenter TE, Anderson RJ, Parker KM. Persistence of passively acquired antibodies to *Mycoplasma hyopneumoniae* in a swine herd. *Prev Vet Med*. 1994;21:29–41.

18. Ross RF. Mycoplasmal diseases. In: Diseases of Swine. Leman AD, et al ed. 7th ed. Ames, Iowa: *Iowa State University Press*, 1986:436–444.

19. Baekbo P, Masen KS, Larson LP, Szancer J. Eradication of *Mycoplasma hyopneumoniae* from infected herds without restocking. *Proc AASP*. 1994:457–459.