RESEARCH REPORT
SWINE HEALTH

Title: Prevalence of *Mycoplasma hyopneumoniae* in different parity sows as a preliminary tool for eradication - **NPB # 02-081**

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Abstract: The objective of this study was to establish the prevalence of *Mycoplasma hyopneumoniae (M. hyopneumoniae)* in different parity sows using nested PCR (NPCR) and scanning electron microscopy (SEM). Additionally, blood samples were collected to analyze antibodies to *M. hyopneumoniae* with a monoclonal blocking enzyme-linked immunosorbent assay (ELISA). This study was conducted in 44 sows from a farm endemically infected with *M. hyopneumoniae.* Sows were assigned to groups of 15 animals each by parity: group I (parity 0-2), group II (parity 3-5) and group III (parity 6 or more). At slaughter, blood samples were collected to measure antibodies to *M. hyopneumoniae.* An approximately one-centimeter section of trachea at the bronchial bifurcation was obtained for SEM and a swab was collected from the bronchus at the right apical lobe of the lung for NPCR.

NPCR results showed statistically significant differences between groups I, II, and III, with group I having a higher prevalence of positive animals. SEM results between groups showed a tendency to have structures less compatible with *M. hyopneumoniae* with increasing parity (p: 0.0947 $p<0.05$). ELISA results showed a gradual decrease on the antibody levels with increasing parity. Group I sows presented a higher *M. hyopneumoniae* prevalence (18%) by SEM and NPCR compared to groups II and III sows, (2% and 9% respectively). In addition, this study showed a significant agreement ($p<0.05$) between NPCR and SEM in the detection accuracy of *M. hyopneumoniae*.

These results suggested that group of sows between 0 and 2 parities should be considered as a risk factor to disseminate mycoplasmal respiratory disease. Also, the study found the presence of positive sows in groups II and III by SEM and PCR, although no antibodies were detected in any sow from group III. These results confirm earlier hypothesis showing that ELISA negative results may be found in infected older animals, suggesting a probable cause for failures in some eradication programs. It also suggests that the 10 month-old cut-off point widely used in European eradication programs, may not work well in SEW farms.

Introduction: Control of infectious diseases has been a permanent challenge for swine veterinarians and producers around the world. *M. hyopneumoniae* plays a significant role in the Respiratory Disease Complex, which represents one of the most important chronic diseases of swine[1].

Use of SEW production systems has been described as the most effective way to control diseases but, SEW has not eliminated the problem.

It has been accepted for many years that older sows recovered from the infection are less likely to carry the organism[2]. Based on this assumption, an eradication program has been developed in Europe with variable success[3]. This program is based on removal of all animals younger than 10 months of age from the infected herds, combined with a period of 14 day with no farrowings and antibiotic medication of all breeding animals. The rationale for this protocol is based on the traditional epidemiology of the disease, characterized by absence of clinical signs in pigs older than 10 months, probably resulting from early infection and recovery seen in continuos flow systems. However there is no scientific data that documents that older sows are truly free of *M. hyopneumoniae* infection. The reason for this has been the lack of sensitive tests to demonstrate infection in live animals. Detection of infection has been usually based on clinical, pathological and serological tests, which are of low sensitivity. More recently a nested polymerase chain reaction (N-PCR), has been developed and has proven useful for the detection of *M. hyopneumoniae* using nasal or bronchial swabs[4,5]. In addition Scanning electron microscopy (SEM) has been considered a very useful technique to detect and understand *M. hyopneumoniae* pathogenesis and its attachment mechanism to the cilia[6,7]. NPCR and SEM combined with ELISA should prove useful tools for designing effective eradication programs against *M. hyopneumoniae* in specific herds. This study corroborated previous results showing a high *M. hyopneumoniae* in young sows and demonstrated the presence of some high parity positive sows constituting an obvious re-infection risk.

Objective: The objective of this study was to demonstrate the presence of *M. hyopneumoniae* by N-PCR and scanning electron microscopy (SEM) in the tracheal epithelium of different parity sows.

Materials and Methods: A three thousand-sow herd was used in this study and a previous visit confirmed the presence of positive sows with a nasal prevalence of 16%. This result confirmed the data obtained from the swine producer and the herd veterinarian who reported that the herd was clinically infected with *M. hyopneumoniae* based on obvious clinical signs. Also, a reduction in the productive parameters of finishing pigs was seen in some groups of animals.

Management practices in this farm included the use of all-in/all-out segregated production in the nursery and grow-finishing units. Good housing conditions and adequate ventilation systems were used to maintain a high health status in the farm. In addition, strict internal and external biosecurity measures were practiced. No vaccine or medications against mycoplasma were being used at the time of this study. The weaning age was 21 days on average.

Sampling

Samples were collected from cull sows from different parities grouped in 15 sows each of parities 0-2, 3-5 and 6 or more.

Parameters measured

For each group the following parameters were measured:

Prevalence of N-PCR positive dams

Prevalence of SEM positive dams.

Prevalence and extent of lung lesions at slaughter.

A blood sample was obtained at the slaughterhouse from every sow to evaluate seroconversion to *M. hyopneumoniae* by Tween-ELISA. Lung lesions were evaluated using the described PigMon schedule for slaughter inspections and performed in a blinded fashion.

A one-cm section of tracheal tissue at the bronchial bifurcation was obtained for SEM and a swab was collected from the bronchus at the right apical lobe of the lung for N-PCR.

Blood, SEM, and swab samples were kept refrigerated (4 ^{0}C) until submitted to the laboratory.

N-PCR

The first reaction amplified a region of 649 pb from the 16S ribosomal gene using the primers previously described[8]. The forward primer as 5'-ACT AGA TAG GAA ATG CTC TAG T-3'(nucleotide positions 463-484) and the reverse 5'-GTG GAC TAC CAG GGT ATC T-3' (nucleotide positions 797-815).

Five microliters (μl) of the DNA were used in the first reaction and 0.5 μl for the second reaction. Twenty-five μl mixture was used for reactions, containing 0.2 mM of each primer 20 pmol of each primer, 1 x PCR-buffer, 5% glycerol, 3mM $MgCl_2$ and 1U of Taq DNA polymerase. A thermocycler was used under the same program in both reactions, 30 cycles denaturalization at 94^{0}C for 30 sec, primer annealing at 60 ^{0}C for 45 sec and extension at 72 ^{0}C for 30 sec.

Once the samples were amplified, 6 μl of each sample was analyzed by electrophoresis in a 1 % agarose gel with 0.5 μg/ml of ethidium bromide. Final results were obtained and photographed using the eagle eye system and Adobe PhotoShop.

SEM

Samples were processed as previously described[6]. Briefly, 0.5 cm^2 of bronchus section was fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (PB) pH 7.2 for 48 h at 4 ^{0}C. All tissues were washed three times (15 min) in PB, dehydrated in graduated alcohol-water solutions at 10 min intervals and in 100% ethanol solution for 15 min, then placed in acetone and dried at critical point in a Balzer CPD type 10 (40 ^{0}C, 80 bar). The samples were adhered to brass stubs using silver-print conductive paint and were coated with gold in a vacuum evaporator.

Field trachea visualization was performed to detect the microorganisms attached to the cilia applying 10 observation fields per sow at 10,000X. Several structural conditions were used to identify *M. hyopneumoniae* including shape, size and localization[6].

Serology

Antibodies against *M. hyopneumoniae* were detected using the Tween-ELISA test previously described[9]. The antigen for the test was obtained from Iowa State University and the ELISA was performed according to industry standards at the Diagnostic Veterinary Laboratory at the University of Minnesota.

Statistical analysis

The chi-square test was used to analyze the distribution of NPCR, SEM and ELISA results among groups I, II, and III . ELISA results were analyzed by ANOVA with a 95% confidence interval ($P<0.05$). NPCR, SEM and ELISA results among groups were analyzed using Kruskal-wallis one-way ($P<0.05$) (Statistix for Windows®, Analytical Software, Tallahassee, Florida).

Results: Forty-four sows were sampled for NPCR, SEM and serology. NPCR results showed statistically significant differences between groups I, II and, III. SEM results showed a tendency (p: 0.0947, $p<0.05$) to have structures less compatible with *M. hyopneumoniae* as parity increased. ELISA results showed a gradual decrease on

antibody levels with an increase in parity. Fifty seven percent of the group I sows (parities 0-2) were both SEM and NPCR positive to *M. hyopneumoniae* and 35 % of this group I were SEM, NPCR and ELISA positive to *M. hyopneumoniae*. Six percent of the group II sows (parities 3-5) were both SEM and NPCR positive. Twenty percent were only SEM positive, 13% were only NPCR positive and 13% were only ELISA positive. Twenty six percent of the group III sows (parities 6 or more) were both SEM and NPCR positive. Six percent were SEM positive and 12% ELISA positive.
Four percent of the sows were found with gross lesions typical of mycoplasmal pneumonia.

Discussion: The presence of *M. hyopneumoniae* in young animals with high antibody levels confirmed results found in previous studies[10,11]. Antibody levels detected by ELISA against *M. hyopneumoniae* have been correlated with contact with the pathogen and/or as a immunological response after vaccination but may not indicate protection circulating throughout the system [12]. Gilt vaccination against *M. hyopneumoniae* is considered an effective practice to control the respiratory disease; elevated antibodies can be detected until several months later[10] and this could explain the presence of higher antibodies found in younger sows which could be derived from vaccination during gilt development. Results showed a decrease in antibodies as parity increased with a statistically significant difference between groups I, II, and III. ($p < 0.05$).

Regarding grouping by parity, 57 % of the group I sows (parities 0-2) were both SEM and NPCR positive to *M. hyopneumoniae* and 35 % of this group I were SEM, NPCR and ELISA positive to *M. hyopneumoniae*. Only 6% of group II sows (parities 3-5) were both SEM and NPCR positive. In addition, 20% were SEM positive, 13% were ELISA positive and, 13% were NPCR positive. Twenty six percent of the III group sows (parities 6 or more) were both SEM and NPCR positive. Six percent of the sows in this group were SEM positive and 13% ELISA positive. Results therefore showed a trend for resolution of the infection with age, although even some older sows were found to be infected.

These results confirmed our previous hypothesis and we showed that older sows are not necessarily truly negative even when ELISA negative. Therefore this result could explain the reason for failures in eradication programs of large herds where re-infection could be wrongly attributed to lateral transmission.

The study showed that NPCR and SEM had a good correlation in detecting *M. hyopneumoniae*. Although ELISA has been used as an important tool, it should be considered as an indicator of previous contact with *M. hyopneumoniae,* but not of infectious status. However the presence of high antibodies is an important factor in disease control, since they are transferred to the progeny and may confer a measure of protection.

ELISA antibody levels to *M. hyopneumoniae* were not correlated with pneumonic lesions, considering that only two sows presented lung lesions. In contrast, older sows (group III) were found to be NPCR and SEM positive despite ELISA negative results and the absence of gross lesions, indicating the thy were probably in the final resolution stage.

Based on these findings, this study concluded that field observations including prevalence and extent of pneumonia at slaughter, must be complemented with test such as NPCR not only to establish the real health status of the herd, but also as a monitoring plan to accomplish eradication programs with success.

References

1. Ross RF. Mycoplasmal diseases. In Straw BE, D'Allaire S, Mengeling WL, Taylor DJ, eds. *Disease of swine*. 8th ed. Ames, Iowa: Iowa State University Press; 1999:495-510.

2. Goodwin R. F. W. The phenomenon of suppressed respiratory disease in the litters of older sows. Vet. Rec. 1965;**77** (14): 383-387.

3. Zimmerman W, Odematt W, Tshudi P: Enzootische pneumoniae (EP): Die Teil sanierrung EP-reinfizierter Schweinezuchtbetribe als alternative zur Totalsanierung. (Swine enzootic pneumonia; partial depopulation in eradication of the infection) Schweiz. Arch. Tierheilk 1989,131 179-191.

4. Casalmiglia M, Pijoan C, Trigo A. Application of a nested polymerase chain reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. J Vet Diagn Invest. 1999;11:246-251.

5. Vicca J, Maes D, Thermote L, Peeters J, Haesebrouck F, de Kruif A. Patterns of Mycoplasma hyopneumoniae infections in Belgian farrow-to-finish pig herds with diverging disease-course. J Vet Med B Infect Dis Vet Public Health 2002;49(7):349-53

6. Blanchard, B., M. M. Vena, et al. "Electron microscopic observation of the respiratory tract of SPF piglets inoculated with *Mycoplasma hyopneumoniae*." Veterinary Microbiology 1992;**30**(4): 329-41.

7. Ruiz A. Galina L, Pijoan C *Mycoplasma hyopneumoniae* colonization of pigs sired by different boars Can J. Vet Res 2002; 66: 79-85.

8. Mattson JG, Bergstrom K, Wallgreen P, Johansson K. Detection of *Mycoplasma hyopneumoniae* in nose swabs from pigs by in vitro amplification of the 16S rRNA gene. J Clin Microbiol 1995;33:893-897.

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

10. Casalmiglia M, Pijoan C. Colonization state and colostral immunity to *Mycoplasma hyopneumoniae* of different parity sows. Vet Rec. 2000;146:530-532.

11. Yagihasshi T, Kazama S and Tajima M. Seroepidemiology of mycoplasmal pneumoniae of swine in Japan as surveyed by an enzime-linked immunosorbent assay. Veterinary Microbiology 1993; 34:155-166.

12. Sheldrake, R. F., Gardner, I. A., Saunders, M.M and Romalis L. F., Serum antibody response to *Mycoplasma hyopneumoniae* measured by enzyme-linked immunosorbent assay after experimental and natural infection of pigs. Aust. Vet J. 67:39-42

Lay Interpretation: Cull sows from a large (3000 sow) SEW farm were investigated at slaughter for the presence of *M. hyopneumoniae* using scanning electron microscopy (SEM) PCR (NPCR) and serology. Sows were allocated to groups based on parity.
As expected, younger sows had a significant higher prevalence of infected animals and their antibody titers were higher, maybe as a result of acclimation vaccination or more probable due to late infection characteristic of SEW systems.

However, a significant number of older sows were also infected, some with negative ELISA serology, suggesting that the antibody titers drop before infection is completed resolved.

These results suggest that the European Mycoplasma eradication program, which uses a 10-month of age cut-off for depopulation, may fail in SEW farm commonly found in the US.