RESEARCH REPORT
SWINE HEALTH

Title: Characterization of *Mycoplasma hyorhinis* transmission and spread within endemically infected populations – **NPB #10-004**

Investigators: Simone Oliveira, Albert Rovira

Co-investigators: Maria Jose Clavijo, Deb Murray

Institution: University of Minnesota

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Industry Summary:

Bacterial diseases create a significant economic impact in today's swine industry by causing an increase in mortality rates and a reduction in feed efficiency and growth. Polyserositis is one of the main causes of mortality in nursery pigs. The bacterium *Haemophilus parasuis* is typically considered the main cause of polyserositis. However, during the last years we have identified another bacterium, *Mycoplasma hyorhinis,* as the main cause of polyserositis in many cases. In fact, 55% of polyserositis and 12% of arthritis cases received at the Minnesota Veterinary Diagnostic Laboratory test positive for this pathogen by PCR. Many of these pigs are actually coinfected with *H. parasuis* and *M. hyorhinis*. Although this pathogen was first described in 1955, very little research has been generated regarding the ecology and epidemiology of this organism, which is needed to design effective control and prevention protocols.

In order to begin to generate this vital information, our group at the University of Minnesota initiated a cross sectional study with the objective of estimating the colonization prevalence of M. *hyorhinis* in pigs of different age groups. Three 6000 farrow-to-wean herds, designated as herds A, B and C, and their nurseries were selected. Although all three herds had

history of *M. hyorhinis* disease, only herds A and B were experiencing high nursery mortality due to polyserositis at the time of sampling. The sampling of each herd included the collection of nasal swabs from 60 sows, 60 piglets in each group of 1, 7, 14 and 21 days of age as well as 30 pigs in each group of 28, 35, 42, 49, 56, 63, 70 and 77 days of age. Additionally, since *M. hyorhinis* can be detected in the oropharyngeal surface, oral fluid samples were collected from one pen per age throughout the nursery. In order to investigate the role of *M. hyorhinis* in polyserositis cases tissue samples were collected from ten clinically affected and ten clinically healthy pigs necropsied at the age of the peak of mortality in the nursery. Samples were tested for *M. hyorhinis* by a quantitative PCR developed in our laboratory.

M. hyorhinis was detected in the nasal cavity of 5/60 sows in herd A, 3/60 in herd B and none in herd C. In herd A and B, where clinical cases of *M. hyorhinis* were present, the colonization prevalence in suckling piglets was low (avg=8%) and high in post-weaning pigs (avg=98%). In contrast, in herd C, where *M. hyorhinis* clinical cases were absent, colonization in pigs was very low until the last week in the nursery. A total of 7/8 oral fluids collected from post-weaning pigs tested *M. hyorhinis* positive in herd A and B, while 1/8 tested positive in herd C. Polyserositis was not observed in any of the healthy animals from all three herds or in the diseased pigs from herd three. However, in herds A and B polyserositis was observed in 9/10 and 4/10 diseased pigs respectively (Figure 2). *M. hyorhinis* was detected by PCR in the pericardium of 8/10 diseased pigs in herd A and 3/10 in herd B. Isolation of *M. hyorhinis* from the pericardium was achieved only in herds A and B. In herd three *M. hyorhinis* was not detected by PCR in any of the necropsied pigs.

In summary, *M. hyorhinis* is an important cause of polyserositis and arthritis in post-weaning pigs. It can be detected by PCR in nasal swabs, tonsil swabs and oral fluids. Detection of this pathogen in the nasal cavity of an individual pig does not imply disease; however, testing nasal swabs of a group of pigs may be useful to determine the time of exposure in a herd. Colonization may occur in pigs as early as one day of age, but most of the pigs become colonized sometime in the nursery. High prevalence of *M. hyorhinis* nasal colonization in weaned pigs appears to be correlated to the presence of *M. hyorhinis* in polyserositis cases.

Keywords: swine, *Mycoplasma hyorhinis*, epidemiology, polyserositis, colonization.

IV. Scientific Abstract:

Mycoplasma hyorhinis is a common inhabitant of the respiratory tract of pigs, which can cause polyserositis in animals of 3 to 10 weeks of age, as well as arthritis in finishing pigs. Approximately 50% of the cases with polyserositis received at the Minnesota VDL in 2009 had the involvement of this pathogen. The objective of this study was to characterize the pattern of *M. hyorhinis* colonization in endemically infected herds. Three 6000 sow farrow-to-wean herds, defined as A, B and C, as well as their nurseries located in MN and SD were selected. These herds had a diagnostic history of recurrent mortality associated with *M. hyorhinis* isolation from systemic sites. Nasal swabs were collected from 60 sows, 60 piglets in each group of 1, 7, 14 and 21 days of age as well as 30 pigs in each group of 28, 35, 42, 49, 56, 63, 70 and 77 days of age. Oral fluids were also collected from the same post weaning pigs. In order to investigate the role of this pathogen in polyserositis cases tissue samples were collected from ten clinically affected and ten clinically healthy pigs necropsied at the age of the peak of mortality. All nasal swabs were tested by a real time PCR developed in our laboratory. *M. hyorhinis* was detected in the nasal cavity of 5/60 sows in herd A, 3/60 in herd B and none in herd C. In herd A and B where clinical cases of *M. hyorhinis* were present, the colonization prevalence in suckling piglets was low (avg=8%) and high in post-weaning pigs (avg=98%). In contrast, in herd C where *M. hyorhinis* clinical signs were absent, colonization in pigs was very low until the last week in the nursery. A total of 7/8 oral fluids tested *M. hyorhinis* positive in herd A and B, while 1/8 tested positive in herd C. Polyserositis was not observed in any of the healthy animals from all three herds or in the diseased pigs from herd C. However, in herds A and B polyserositis was observed in 9/10 and 4/10 diseased pigs respectively. Isolation of *M. hyorhinis* from the pericardium was achieved only in herds A and B. *M. hyorhinis* was detected by PCR in the pericardium of 8/10 diseased pigs in herd A and 3/10 in herd B. In the healthy pigs only one sample tested PCR positive. In herd C *M. hyorhinis* was not detected in any of the necropsied pigs. In summary, *M. hyorhinis* can be detected by PCR in nasal swabs, tonsil swabs and oral fluids. The pathogen can colonize pigs at day one of age; however, most of the pigs become colonized sometime in the nursery. High prevalence of *M. hyorhinis* nasal colonization in weaned pigs appears to be correlated to the presence of *M. hyorhinis* associated disease and the detection of the agent in polyserositis cases in nursery pigs.

Introduction:

In recent years *Mycoplasma hyorhinis* has been recognized as an important cause of mortality in nursery pigs. This pathogen is usually found in the upper respiratory tract of colonized pigs, more specifically in nasal secretions and in the oropharyngeal surface. Piglets become colonized by contact from their sows and it is transmitted through nose-to-nose contact among pigs afterwards. *M. hyorhinis* causes polyserositis in 3-10 week old pigs, where animals show mainly fever, dyspnea, reluctance to move and unthriftiness. Infection in finishing stage pigs is usually characterized by arthritis; nonetheless, most *M. hyorhinis* infections are generally subclinical. Other clinical presentations including rhinitis, pneumonia, otitis and conjunctivitis have been reported for this organism. However, the role of *M. hyorhinis* in these disease presentations is unclear.

Mycoplasma hyorhinis lesions are very similar to those caused by *Haemophilus parasuis*. Interestingly, the timing of mortality caused by *M. hyorhinis* and *H. parasuis* in affected herds also appear to coincide. A survey of detection of *M hyorhinis* by PCR in polyserositis and arthritis cases submitted to the Minnesota Veterinary Diagnostic Laboratory (MVDL) in 2009 revealed that this pathogen was detected in 55% of polyserositis and 12% of arthritis cases. In addition, concomitant detection *M hyorhinis* and *H. parasuis* was observed in 40% of the cases tested. These data suggests that these pathogens have a similar epidemiology and dynamics of infection and raises the question whether control measures that have been implemented for *H. parasuis* can be applied for *M. hyorhinis*.

Although the general aspects of infection of *M. hyorhinis* are reported in the literature, the details of horizontal transmission, vertical transmission, and how these patterns influence infection dynamics in nursery pigs is unknown.

Objectives:

The general objective of this study was to characterize the dynamics of *M. hyorhinis* infection in endemically infected populations. Specific aims of this study where: a) To estimate the colonization prevalence of *M. hyorhinis* in pigs of different age groups, b) To estimate the bacterial load in the nasal cavity from pigs of different age groups, and c) To investigate the role of *M. hyorhinis* and other pathogens in polyserositis.

Materials & Methods:

Experimental design: A total of three 6000 farrow-to-wean herds and their nurseries located in southern Minnesota and south east South Dakota were included in this study. In order to determine the prevalence of *M. hyorhinis* colonization a cross-sectional nasal sampling was performed in randomly selected sows, piglets of 1, 7, 14 and 21 days of age and pigs at weaning through 8 weeks post-weaning. All nasal samples were tested by a quantitative PCR assay.

Herd selection: The participating herds defined as A, B and C had a history of at least 5% nursery mortality with recurrent involvement of *M. hyorhinis* based on isolation or detection by PCR from clinical samples submitted to the Minnesota Veterinary Diagnostic Laboratory. At the time of the study herd C had no clinical cases of *M. hyorhinis.* These breeding herds were part of one production system, and were under the same genetic, nutrition and management protocols. Pigs were weaned at 21 days of age in off-site nursery facilities with all-in/all-out flows. None of the selected herds were under *M. hyorhinis* vaccination protocols during the time of this study. Medication protocols were consistent amongst all three herds. Herds A and B were PRRS virus positive unstable.

Sampling: In each herd nasal swabs were collected from 60 sows for detection of *M hyorhinis* by PCR. One piglet/sow in each herd was randomly selected and a nasal swab

was collected at days 0, 7, 14 and 21 of age, with a total of 60 piglets sampled per age group in each herd. The calculated sample size allowed for the detection of prevalence as low as 5%, a precision of 95% and a 95% confidence (http://epitools.ausvet.com.au/). Similarly, 30 post-weaning pigs were sampled in each group at 28, 35, 42, 49, 56, 63, 70 and 77 days. Sample size was calculated expecting 95% colonization prevalence, a precision of 95%, with 95% confidence (http://epitools.ausvet.com.au/). A total of 540 nasal swabs were collected in each herd during the months of August-October 2010. All nasal swabs were collected utilizing a BBL™ CultureSwab™ Liquid Stuart Medium (Franklin Lakes, NJ USA). A previous pilot study performed by our group confirmed that this pathogen can be easily detected from the oropharyngeal surface; therefore, a total of 8 oral fluid samples were collected from one pen with approximately 70 animals at 28, 35, 42, 49, 56, 63, 70 and 77 days of age.

Clinical/pathological evaluation: With the purpose of confirming the involvement of *M. hyorhinis* in nursery mortality, a total of 10 post-weaning pigs with clinical signs suggestive of polyserositis (prostration, cough, lameness, abdominal breathing, and fever of at least 105 °F), as well as 10 clinically healthy pigs were necropsied and sampled in each herd. Samples from lungs, bronchial lymph nodes, heart, spleen and joint were collected from each pig and submitted to the Minnesota Veterinary Diagnostic Laboratory for complete work up.

DNA extraction protocol and quantitative PCR assay: Extracted DNA from all nasal swabs, as well as oral fluid samples from sows and pigs, was obtained using the DNeasy Blood & Tissue kit (QIAGEN, Germany). The qPCR assay employed to test all nasal swabs and oral fluids is based on the 16S rRNA gene and was previously developed by our lab. It has the capability of detecting as little as 100 CFU/reaction. Reactions were carried out in the ABI

7500 fast real-time PCR system at 95°C for 3 min, 40 cycles of 95°C for 15 sec and 54°C for 50 sec.

Results:

Colonization prevalence and bacterial load: *M. hyorhinis* was detected in low proportions amongst sows in farms A and B. In farm C all sows tested negative. In herds A and B, where clinical cases of *M. hyorhinis* were present, the colonization prevalence was low in suckling piglets (avg=8%) and high in post-weaning pigs (avg=98%) (Figures 1 & 2). In contrast, in herd C, where *M. hyorhinis* clinical cases were absent, colonization in pigs was very low until day 77, when 100% of sampled pigs were colonized (Figure 3). Bacterial load in pre-weaning pigs and sows was variable in all three herds. In contrast, bacterial load in post-weaning pigs in all herds was relatively consistent (Figures 1, 2 & 3).

Oral fluids: *M. hyorhinis* was detected by PCR in all oral fluid samples from herds A and B with the exception of samples collected from pigs at 35 days (herd A) and 28 days (herd B). In both cases the animals did not show interest in the cotton rope and the sample obtained was minimal. In herd C, *M. hyorhinis* was detected in oral fluids only at 77 days, which coincides with the results obtained from the nasal cavity qPCR.

Clinical/pathological findings: Polyserositis was not observed in any of the healthy pigs from all three herds or in the diseased pigs from herd three. However, in herds A and B polyserositis was observed in 9/10 and 4/10 diseased pigs respectively. *M. hyorhinis* was detected by PCR in the pericardium of 8/10 diseased pigs in herd A and 3/10 in herd B (Figures 4 & 5). Isolation of *M. hyorhinis* from the pericardium was achieved only in herds A and B. In herd C, *M. hyorhinis* was not detected by PCR in any of the necropsied pigs (Figure 6). Results of *Haemophilus parasuis* PCR detection in the pericardium of all necropsied animals from all three herds are shown in figures 4, 5 and 6.

Discussion:

Recently *M. hyorhinis* has emerged as an important cause of polyserositis and arthritis in post-weaning pigs. This study estimated the prevalence of *M. hyorhinis* colonization in pigs from different age groups. *M. hyorhinis* can be detected in the nasal cavity of newborn piglets a few hours after birth and results show that prevalence prior to weaning was low with a noticeable increase detected after weaning. The sharp increase of *M. hyorhinis* detection after weaning may be due to decay of maternal immunity, commingling of pigs at weaning or co-infections with other pathogens such a PRRS virus. In herd C, where *M. hyorhinis* cases were absent, the colonization profile was very different that those observed in herds A and B. In this herd pigs became colonized only at 77 days, proving that this pathogen is not present in all pigs. Although it is not clear what are the mechanisms that enable *M. hyorhinis* to cause systemic disease, necropsy findings suggest that *M. hyorhinis* is continuously found within polyserositis lesions, sometimes independently of the presence of *H. parasuis.* Therefore, *M. hyorhinis* should be taken into account when designing control and prevention protocols for farms with mortality due to polyserositis.

In summary, *M. hyorhinis* is an important cause of polyserositis and arthritis in post-weaning pigs. It can be detected by PCR in nasal swabs, tonsil swabs and oral fluids. Detection of this pathogen in the nasal cavity of an individual pig does not imply disease; however, testing nasal swabs of a group of pigs may be useful to determine the time of exposure in a herd. Colonization may occur in pigs as early as one day of age, but most of the pigs become colonized sometime in the nursery. High prevalence of *M. hyorhinis* nasal colonization in weaned pigs appears to be correlated to the presence of *M. hyorhinis* associated disease and the detection of the agent in polyserositis cases in nursery pigs.

Figures

Figure 1. Prevalence of M. hyorhinis colonization and bacterial load at different age groups (Herd A)

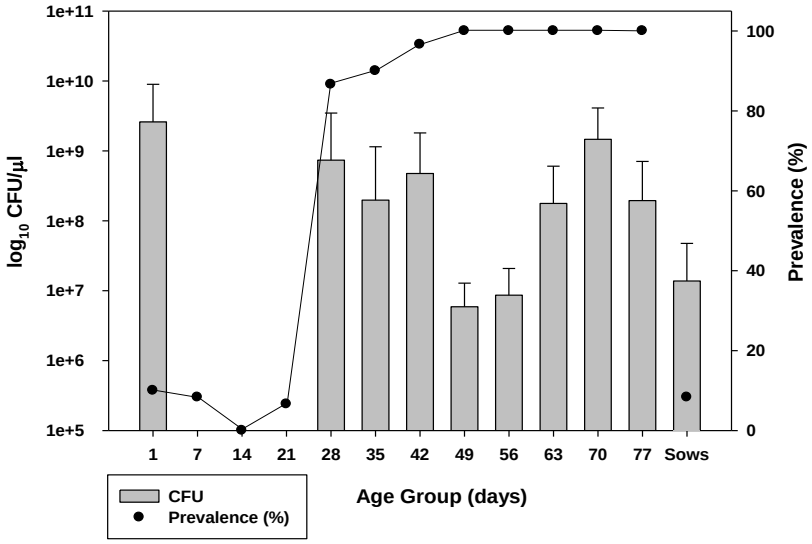

Figure 2. Prevalence of M. hyorhinis colonization and bacterial load at different age groups (Herd B)

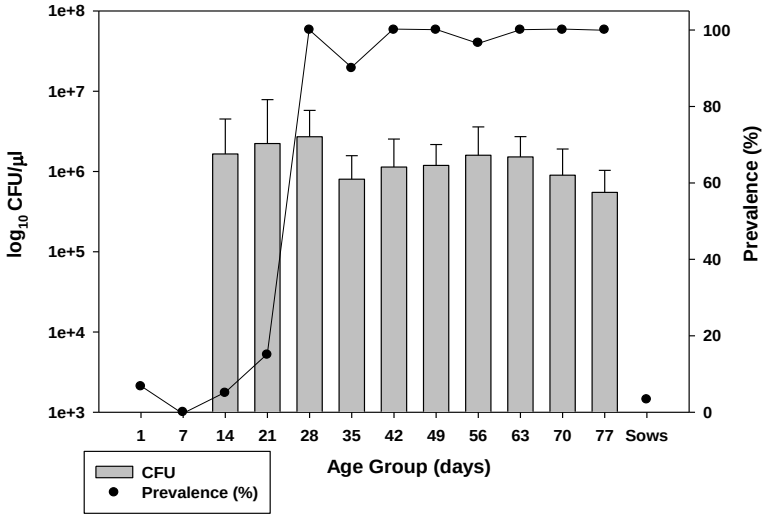

Figure 3. Prevalence of M. hyorhinis colonization and bacterial load at different age groups (Herd C)

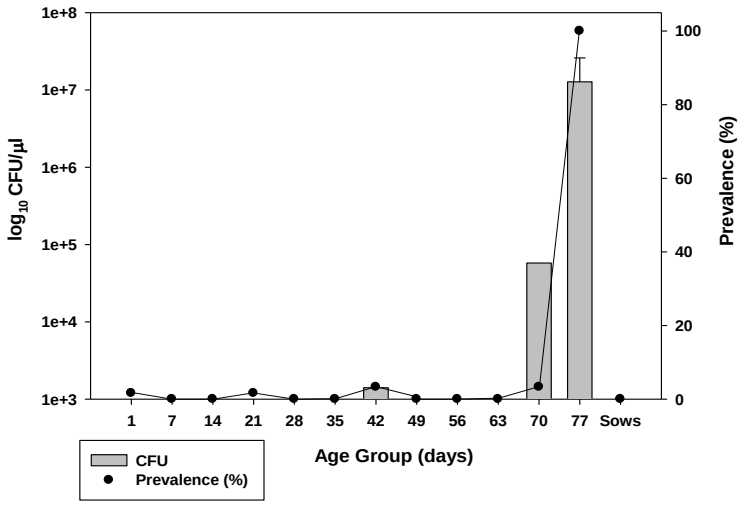

Figure 4. Polyserositis cases and pericardium PCR results from herd A.

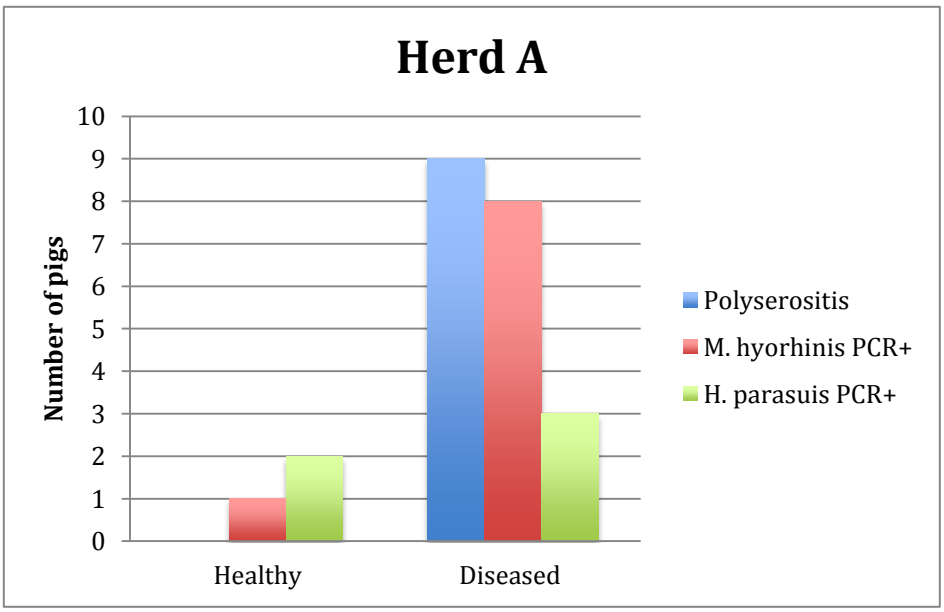

Figure 5. Polyserositis and pericardium PCR results from herd B.

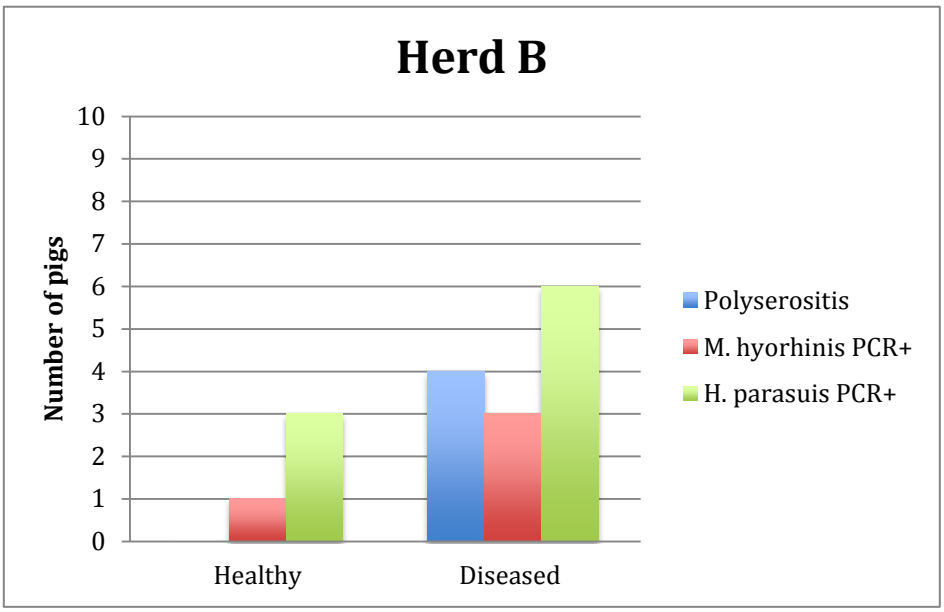

Figure 6. Polyserositis cases and pericardium PCR results from herd C.