

St. Paul, December 1st 2014.

MPB RESEARCH GRANT FINAL REPORT

I. Project Title: Investigating the epidemiology of *Mycoplasma hyorhinis* in sow farms

Principal Investigator: Dr. Maria Pieters

Institution: University of Minnesota

II. Abstract

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) and *M. hyorhinis* are important microorganisms affecting swine. *M. hyopneumoniae* causes chronic respiratory infections in pigs which generate significant losses to the swine industry. *M. hyorhinis* is a colonizer of the upper respiratory tract of the pig which can cause arthritis and polyserositis after weaning age, and can be commonly identified in pneumonic cases. Frequently, *M. hyopneumoniae* and *M. hyorhinis* are identified in the same pig, suggesting that a colonization relationship between these two microorganisms may exist. However, the epidemiology of these bacteria does not appear to be similar in the field. The level of piglet colonization with *M. hyopneumoniae* at weaning age has been suggested as a predictor of disease presentation in grower and finisher pigs, while *M. hyorhinis* piglet colonization at weaning does not seem to have a strong influence in post weaning transmission. Thus, we aimed at investigating the nasal swab colonization of dams and piglets with *M. hyopneumoniae* and *M. hyorhinis* in commercial sow farms, and to investigate their relationship with several environmental, farm and piglet factors. DNA was obtained from a set of nasal swabs collected from dams in piglets in 4 commercial sow farms and was tested for detection of *M. hyorhinis* with a species specific real time PCR. All samples were previously

tested for detection of *M. hyopneumoniae* genetic material. A logistic mixed model was used to test for the association of various factors with the risk of piglet PCR positivity to *M. hyorhinis* at weaning age. Also, the association between the two pathogens was evaluated. The prevalence of *M. hyorhinis* in dams and piglets was low in all farms studied. No association between *M. hyorhinis* positivity and environmental, farm and piglet factors was observed. The relationship between the two pathogens was not significant. Under the conditions of this study, *M. hyopneumoniae* and *M. hyorhinis* colonization at weaning does not seem to be associated, although they could be associated at later production stages.

III. Introduction

Several species of Mycoplasmas have been isolated from swine, among them, *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*), *M. suis* and *M. hyosynoviae* appeared, for a long time, to be the only pathogenic Mycoplasmas in pigs, while other species, like *M. hyorhinis*, *M. hyopharyngis* and *M. flocculare* were considered commensals (Thacker and Minion, 2012). However, during the last years, the advent of better diagnostic testing has allowed for more frequent identification of microorganisms that had been implicated in clinical outbreaks, but had remained underdiagnosed otherwise.

Mycoplasma hyorhinis was first described by Switzer in 1955 and was considered a commensal of the pig's nasal cavities since. In the pig literature it was usually referred as an "always present" microorganism with no clinical implications. Nevertheless, over the years several reports and manuscripts have described the isolation and detection of *M. hyorhinis* in pathologic conditions in pigs and as a cause of post-weaning polyserositis and arthritis (Potgieter and Ross, 1972). In the recent years, the diagnostic capabilities have improved and more specific and

accurate characterization of microorganisms is achieved, thanks in big part to molecular detection tools. In our research group, a real time PCR was developed by Clavijo and Oliveira (2010) that allows for the accurate identification of *M. hyorhinis* in clinical specimens. This new diagnostic method had been run as part of a battery of test in polyserositis cases at the Diagnostic Laboratory of the University of Minnesota and similar technologies have been adopted in other porcine diagnostic laboratories (Gomes Neto et al., 2012). Results from this testing have yielded a considerable proportion of samples positive for *M. hyorhinis*, in cases where other pathogens have not been identified (VDL-UofM internal reports). And on the other hand, an important proportion of samples have been found negative to this microorganism, contrary to the common word that *M. hyorhinis* is ubiquitous. Also, in other cases *M. hyorhinis* has been found to co-infect the host with other agents, for example, *Haemophilus parasuis*. The results of carefully designed diagnostic investigations to answer the need of swine practitioners, along with agreement with clinical disease observed in the field have allowed for the more understandable findings in otherwise difficult to explain diagnostic cases. Thus, *M. hyorhinis* is now recognized as an important emergent pathogen in swine populations (Murray, 2012).

As expected, emergent pathogens are difficult to deal with in great part due to the lack of knowledge of their ecology and behavior, and the host response or effect of environmental conditions. In regards to *M. hyorhinis*, Clavijo et al. (2012a; 2012b) have started to investigate the epidemiology of this bacterium in a selected number of swine herds showing clinical outbreaks associated with infection by *M. hyorhinis*. In their studies, the group has found that the level of *M. hyorhinis* colonization during the lactation period can be low in piglets and their mothers. In one herd, colonization was between 5-10% at the sow and piglet level. In a second herd, colonization was not detected in sows, and the colonization level in piglets was very low.

Interestingly, data from one study suggested a trend in parity effect for colonization (Clavijo et al 2012a); while in another study the parity effect was not evident (Clavijo et al., 2012b). However, sample collection for sows and piglets at weaning were completed one time in these farms, and no multiple groups were evaluated in the same herd. Moreover, no climatologic data has been evaluated in regards with the level of *M. hyorhinis* colonization at weaning age.

Studies investigating the epidemiology of *M. hyopneumoniae* in regards with prevalence at weaning have opened the door to new avenues of research by documenting the high variability in the level of positive animals in different weaning groups within the same farm, and among different farms. This is especially important knowing that the percentage of at weaning prevalence influences the severity of clinical disease in later stages of production. It is unknown whether the level of sow and piglet colonization with *M. hyorhinis* follows patterns similar to those observed in *M. hyopneumoniae* infections.

In both cases, pigs are born free of *M. hyopneumoniae* and *M. hyorhinis*. Colonization may occur sometime during the lactation period and amplification of the number of positive animals occurs after weaning. However, the peak of clinical disease observed with these two pathogens differs greatly. Infection with *M. hyorhinis* is usually observed between 3 and 10 weeks of age (Reviewed by Thacker and Minion, 2012), while *M. hyopneumoniae* clinical infections are evident during the last weeks of the finishing period (Maes et al., 2008). The growth rate of each one of these bacteria varies considerably and it can be speculated that the transmission rate among hosts colonized with this pathogens varies as well, maybe accounting for the different disease presentation pattern.

In this study we propose to investigate the epidemiology of *M. hyorhinis* in sows and piglets in several weaning groups in three different swine farms in order to determine whether the

colonization status at weaning is similar among different weaning groups within the same farm. Also, we aim to calculate the association of certain dam and piglet factors with their probability to carry *M. hyorhinitis* in the nasal cavities, and to estimate the association between climatic factors and the prevalence of *M. hyorhinitis* colonization at weaning.

IV. Objectives

The overall objective of this study was to investigate the epidemiology of *M. hyorhinitis* in sows and piglets in several weaning groups in three different swine farms. Therefore, the aims of the proposed study were:

1. To determine the prevalence of *M. hyorhinitis* colonization at weaning in **gilt and sow** nasal swabs.
2. To determine the prevalence of *M. hyorhinitis* colonization at weaning in **piglet** nasal swabs.
3. To determine if *M. hyorhinitis* colonization status at weaning is similar among different weaning groups within the same farm.
4. To calculate the association of certain dam and piglet factors with their probability to carry *M. hyorhinitis* in the nasal passages.
5. To estimate the association between climatic factors and the prevalence of *M. hyorhinitis* colonization at weaning.

V. Procedures

This investigation included the estimation of *M. hyorhinitis* prevalence of colonization at weaning in piglets and their mothers -as measured by real time PCR from nasal swabs- in various farrowing groups, in 4 farms, for a total of 15 weaning groups tested. **All the samples used in this investigation had been already collected and were stored in the form of extracted DNA**

at -20°C. The samples were collected during 2012 and were part of a larger investigation for the study of *M. hyopneumoniae* prevalence at weaning, which has been already published (Pieters et al., 2014).

A total of 15 farrowing groups were included in the study (4 farms). For each farrowing group, 54 randomly selected piglets and their dams were sampled one day prior to weaning and swabs will be processed for detection of *M. hyorhinis* DNA.

Farms: Four commercial >900-sow farrow-to-wean farms, A, B, C and D, were selected for this study. The farms status for *M. hyorhinis* is unknown. However, history of *M. hyorhinis* disease has been reported in only one of the farms.

Sample size calculation: Assuming that *M. hyorhinis* prevalence at weaning is 5% and a farrowing group of ~40 sows, each weaning group consisted of 360 piglets approximately. A sample size of 54 piglets was determined by applying binomial approximation, with a 95% confidence and 5% maximum tolerable error.

Piglet and sow sampling: All animals were sampled following protocols approved by the University of Minnesota Institutional Animal Care and Use Committee. Each farm was visited for sample collection at least 2 weeks apart, except farm D, which was sampled only once. This sampling protocol allowed for all farms to be visited during the same seasons of the year and for biosecurity measures (e.g. downtime) to be kept, except for farm D. Nasal swab sample collection was performed in the farrowing rooms. Each nasal swab sample was collected by swabbing both nostrils of the animal, one day prior to weaning, and at the same time nasal swabs were collected from their mothers. Sampling was stratified by parity, and randomized within

litter. In other words, the sampling was based on the parity structure for that week, and piglets to be sampled were randomly selected out of the litter.

***M. hyorhinis* PCR:** Nasal swabs were immediately frozen and taken to the laboratory where DNA was extracted. A real-time PCR test was run for the identification of a fragment of DNA of the 16S ribosomal gene specific to *M. hyorhinis* (Clavijo et al., 2010).

***Prevalence of M. hyorhinis* colonization:** In order to estimate the prevalence at weaning for each farrowing group, a total of 54 samples per weekly group were tested. In order to obtain the estimated prevalence for each group, the number of PCR positive animals was divided by the total number of animals tested in the weaning group. The colonization prevalence was estimated for the dams and for the piglets, separately.

Weaning group and piglet information: For every weaning group in this study certain data was collected, which included: parity distribution and percentage of gilts in the farrowing group, acclimation procedure for gilts, source of replacement gilts, average weaning age, concurrent diagnosed infections, percentage of cross-fostering, maximum age at cross-fostering, piglet gender, piglet weight category, use of antimicrobial drugs during gestation, use of antimicrobial drugs during lactation, vaccination protocols in place (for dams and piglets), farrowing dedicated personnel, biosecurity, all in/all out per room, pre-weaning mortality rate, season of the year.

Capture of environmental data: As part of this study, temperature, relative humidity, wind speed, and solar radiation were periodically recorded for each one of the farms involved in the study. Climatologic data for the area where the farm was located was obtained from weather stations installed *on site*.

Statistical Analysis: A logistic mixed model was used to test for the association of various factors with the risk of piglet PCR positivity to *M. hyopneumoniae* at weaning age. These factors included piglet age, piglet weight category, piglet gender, litter cross-fostering status, and five weather variables, namely relative humidity, radiation, temperature, wind speed, and wind direction. Farm and sampling event nested within farm were included as random effects. Terms with a p -value < 0.05 were considered significant. Analyses were performed in R, version 3.0.1, using the lme4 package.

VI. Results

In farms A and C, all dams were negative for *M. hyopneumoniae* and *M. hyorhinae* at all sampling times. Piglets in farm A were negative for *M. hyopneumoniae*, and 1/216 piglets (0.46%) was positive for *M. hyorhinae*. In farm B, 49/317 dams (15.45%) were positive for *M. hyopneumoniae* and 9/317 (2.83%) were positive for *M. hyorhinae*, while 64/317 piglets (20.18%) were positive for *M. hyopneumoniae*, and 9/317 piglets (2.83%) were positive for *M. hyorhinae*. All piglets in farm C were negative for *M. hyopneumoniae*, and 23/21 (10.55%) were positive for *M. hyorhinae*. In farm D, 1/25 dams (4%) resulted positive for *M. hyopneumoniae*, while all dams were negative for *M. hyorhinae*, as shown in Fig. 1.

Two of 25 piglets (8%) were positive for *M. hyopneumoniae* and *M. hyorhinae* (data not shown). The proportion *M. hyorhinae* and *M. hyopneumoniae* colonized dams and piglets appeared to be independent of the colonization with either microorganism (Fig. 2). Environmental factors did not have an influence on the proportion of *M. hyorhinae* positive dams and piglets (data not shown).

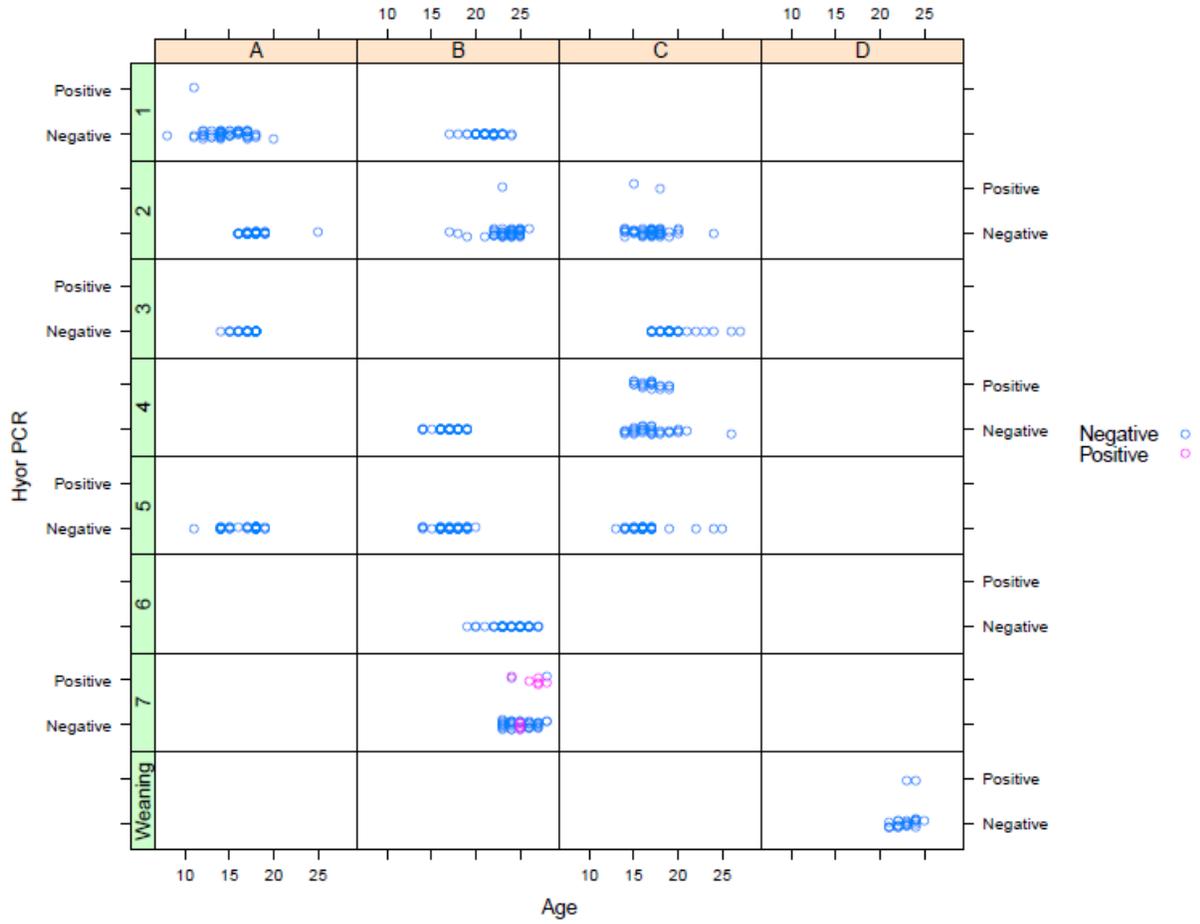


Figure 1. Nasal swab prevalence of *M. hyorhinis* by real time PCR by farm.

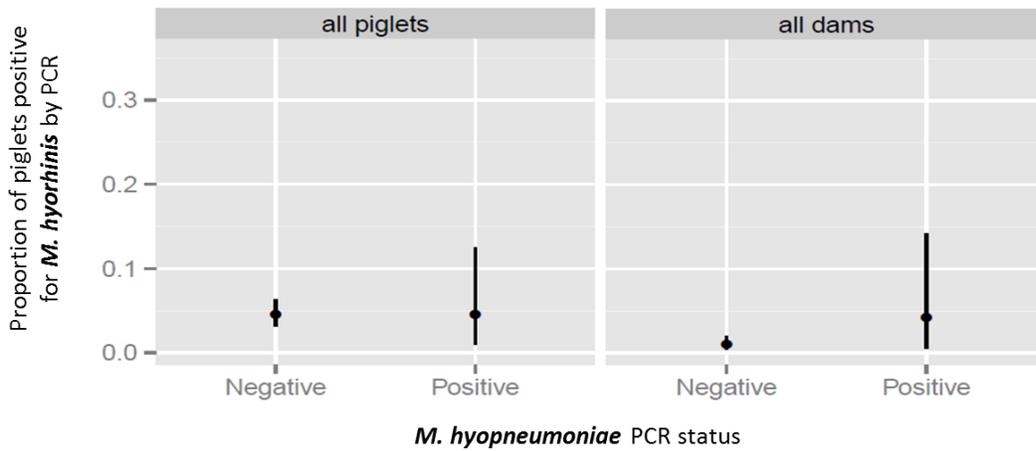


Figure 2. Proportion *M. hyorhinis* positive animals by real time PCR based on the PCR status for *M. hyopneumoniae*.

We then fit a model using only dam PCR status; this used 776 observations; the variability of the random effects and the coefficients in the model are shown below. We also tested pairwise combinations between the three status levels and dam positive was statistically significantly different than both other dam positive and no dam positive. The difference between other and none was not statistically significant. We also computed odds ratios and 95% confidence intervals for these differences but they were very large.

Random effects: Groups Name Variance Std.Dev.

Visit:Farm (Intercept) 5.166e+00 2.273e+00

Farm (Intercept) 7.399e-11 8.602e-06

Number of obs: 776, groups: Visit:Farm, 15; Farm, 4

To compare the association between *M. hyopneumoniae* and *M. hyorhinis* positivity in the piglets, a chi-squared test with p-value computed by simulation was used, due to small cell counts. It was not significant (p=1.0). The proportion of *M. hyopneumoniae* negative piglets that were *M. hyorhinis* positive was 0.045 (0.031, 0.063) and for *M. hyopneumoniae* positive piglets it was 0.045 (0.009, 0.125). Confidence intervals for proportions were computed using the Clopper-Pearson method.

Piglet <i>M. hyorhinis</i>	Piglet <i>M. hyopneumoniae</i>	
	Negative	Positive
Negative	677	64
Positive	32	3

Dam <i>M. hyorhinis</i>	Dam <i>M. hyopneumoniae</i>	
	Negative	Positive
Negative	721	46
Positive	7	2

p.value = 0.1054

In summary, results from this investigation suggest no association between *M. hyorhinis* positivity and environmental, farm, dam, and piglet factors at weaning age. The association between *M. hyorhinis* and *M. hyopneumoniae* PCR status at the dam and the piglet level was not significant. Under the conditions of this study, *M. hyopneumoniae* and *M. hyorhinis* colonization at weaning does not seem to be associated, although they could be associated at later production stages, however this needs to be further investigated.

VII. Results disclosure

Results of this investigation will be summarized, written and submitted in the form of an abstract to the Swine Leman Conference in September 2015. Also, a manuscript will be written and submitted for peer-review publication in a scientific journal. All publications will recognize proper funding by the Minnesota Pork Board and the Pork Checkoff.