RESEARCH REPORT
SWINE HEALTH

Title: *Mycoplasma hyorhinis* and *Mycoplasma hyosynoviae* in US herds: Characterization of detection patterns in oral fluids and clinical presentation **– NPB #17-027**

Revised

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Industry Summary: *Mycoplasma hyorhinis* and *M. hyosynoviae* are important emerging pathogens in swine in the US. Disease caused by *M. hyorhinis* is clinically expressed in the form of polyserositis and arthritis in recently weaned pigs. *Mycoplasma hyosynoviae* causes arthritis and consequent lameness that affects adult pigs close to market age. Thus, infection with these two microorganisms represents a heavy burden for the swine producer, not only in the form of economic losses, but also in the poor welfare picture that the clinical condition can express. Little information is available about the epidemiology of these important pathogens, which makes establishing management and control strategies extremely difficult. Therefore, this research was performed to build the foundation for a more detailed understanding of *M. hyorhinis* and *M. hyosynoviae* detection and their correlation with clinical disease. Under the conditions of this investigation, *M. hyorhinis* was frequently detected in oral fluids in nursery and finisher sites regardless of the clinical presentation of lameness. Therefore, detection of *M. hyorhinis* in oral fluids may not be an informative tool for diagnosis of lameness in pig populations. However, a strong association was identified between pig lameness and *M. hyosynoviae* detection in oral fluids, which warrants detailed investigation, particularly based on pig age.

Keywords: Lameness; nursery; finisher; *Mycoplasma hyorhinis*; *Mycoplasma hyosynoviae*.

Scientific Abstract:

This study was designed to detect *Mycoplasma hyorhinis* and *M. hyosynoviae* in oral fluids and determine their correlation with lameness scores in pigs. Thirty-seven nursery and/or finisher herds were included in this study. Oral fluids were collected by pen. Using species specific real-time PCR *M. hyorhinis* was detected in 97% of sampled herds, whereas 70% were positive for *M. hyosynoviae*. Lameness scores were determined for all pigs in each pen where oral fluids were collected. Lameness was identified in 3.9% of pigs across all sampled pens. No correlation was observed between lameness in pigs in a pen and detection of *M. hyorhinis* in oral fluid samples ($p>0.05$), whereas a significant correlation was observed between *M. hyosynoviae* detection in oral fluids and lameness ($p<0.05$). A negative correlation was observed between the proportion of lame pigs in the pen and Ct values for *M. hyosynoviae* in oral fluids ($p<0.05$; $r = -0.27$). An age-related effect was observed with *M. hyosynoviae* detection in oral fluids, indicating an increased prevalence of the bacterium in finishers compared to nursery pigs. Under the conditions of this study, *M. hyorhinis* was frequently detected in oral fluids from nursery and finisher pigs regardless of the clinical presentation of lameness, whereas the detection of *M. hyosynoviae* varied depending on the age of sample pigs. Our results suggest that oral fluids may not be an informative diagnostic sample for *M. hyorhinis* associated lameness. However, the association of lameness and *M. hyosynoviae* detection in oral fluids warrants prospective population-based diagnostic studies.

Introduction: Lameness and abnormal gait are two major issues affecting swine welfare. Arthritic lameness restricts animal performance and has immense economic implications in production (Killbride et al., 1992). Lameness is also considered as one of the most commonly reported causes for premature culling of breeding sows (Anil et al., 2005). Concerns have increased in recent years about arthritis and lameness, particularly in the growing-finishing phase of swine production (Jensen and Toft, 2009; Neto, 2012). Mostly, etiologies associated with pig lameness are infectious or those in relation with osteochondriosis (Torrison, 2005).

Infectious arthritis in pigs are mainly bacteriological in etiology and associated with *Erysipelothrix rhusiopathiae*, *Streptococcus suis*, *Haemophilus parasuis*, *Actinobacillus suis*, *Arcanobacterium pyogenes*, *Staphylococcus spp.*, *Salmonella cholearasuis*, *Mycoplasma hyorhinis*, or *M. hyosynoviae* (Friis et al., 1992a; Hariharan et al., 1992; Magnusson et al., 1998; Turner, 1982). Among the aforementioned bacterial causes, *M. hyorhinis* and *M. hyosynoviae* are frequently reported in different swine herds in association with lameness and arthritis. Data from diagnostic laboratories in the United States (US) over the recent years have presented evidence supporting the increased frequency of *Mycoplasma spp.* detection in association with swine arthritis (Neto, 2012; Clavijo et al., 2017).

Mycoplasma spp. are recognized as recurrent colonizers and opportunistic infectious agents in swine and other livestock species. Among the different *Mycoplasma spp.*, *M. hyorhinis,* and *M. hyosynoviae* are inhabitants of the respiratory tract of pigs and have been recovered mostly from the upper respiratory tract, particularly the nasal cavity and tonsils of colonized pigs. *Mycoplasma hyorhinis* is often associated with polyserositis and polyarthritis, generally occurring in freshly weaned pigs, although occasionally detected in finishers (Magnusson et al., 1998; Thacker, 2006). *Mycoplasma hyosynoviae* is known to cause non-purulent arthritis, mostly in growing pigs by invading the susceptible joints (Thacker and Minion, 2012).

Several sample types have been used to detect *M. hyorhinis* or *M. hyosynoviae* in swine herds. For instance, tonsil tissue and synovial fluid samples have been used for bacterial culture (Ross and Duncan, 1970; Hagedorn-Olsen et al., 1999), serum for immunological assays

(Nielsen et al., 2005), as well as joint fluid, nasal swabs, tonsillar swabs or oral fluids for genetic material detection by real-time PCR (Neto et al., 2015).

The collection of oral fluid samples was first described by Prickett et al (2008). Since the first report, oral fluid-based monitoring has been widely adopted by swine veterinarians and producers due to sensitivity, convenience and less labor-intensiveness. In the case of population surveillance, in detecting pigs positive for *M. hyorhinis* or *M. hyosynoviae,* previous studies have shown oral fluid PCR as a sensitive test (Neto et al., 2015). However, the potential association between the detection of arthritogenic mycoplasmas in oral fluids and development of lameness in pigs has not been clearly established. Furthermore, it is important to note that, mere detection or arthritogenic Mycoplasmas will not infer their active role in pathogenesis, as these microorganisms can colonize the host as either commensals or infectious agents. Thus, this study was designed with the objective to detect *M. hyorhinis* and *M. hyosynoviae* in oral fluids and determine their correlation with clinical lameness.

Objectives: The overall objective of this investigation is to determine the prevalence of *M. hyorhinis* and *M. hyosynoviae* in US herds and to correlate prevalence at the farm level with clinical presentation in the field. Specifically, we aim at: 1. Estimating the prevalence of *M. hyorhinis* and *M. hyosynoviae* in farms with and without history of clinical presentation associated with one or both of these microorganisms. 2. Evaluating the correlation of *M. hyorhinis* and/or *M. hyosynoviae* detection by PCR and clinical presentation measured at the farm level. 3. Performing molecular characterization of *M. hyorhinis* variants detected in all farms.

Materials & Methods:

All pigs in this study were enrolled and sampled following protocols approved by the University of Minnesota Institutional Animal Care and Use Committee, and handled according to farm standard procedures.

Thirty-seven commercial swine herds located in five geographically different states in the US were selected for this study. Nineteen herds were located in Minnesota, seven in Iowa, five in South

Dakota, five in Wisconsin, and one in Nebraska. Housing conditions, facility layout, pig density, ventilation, flooring, and management practices varied among herds. However, all herds followed recommended Pork Quality Assurance (PQA) practices and were attended by a veterinarian. Swine herds included in the study were selected based on the following criteria: 1. Herd size greater than 2,000 heads. 2. Herds with pigs in either nursery (>3≤10 weeks of age) or finisher (> 10 weeks of age). 3. Previous report of clinical lameness by attending veterinarian.

The study design was cross-sectional, in which approximately 10 oral fluid samples were collected from each herd. The sample size was estimated based on an expected prevalence of 50%, using a diagnostic assay with an assumed sensitivity of 0.95 and assumed specificity of 0.9 with a confidence level of 0.95 and desired precision of 0.05. The population size was set to a minimum of 2,000 pigs. Therefore, approximately 450 pigs were sampled from each herd. The sampling was performed during the period of November 2017 - November 2018.

Oral fluids were collected by pen, following the method described by Prickett and co-authors (2008). Briefly, a 70-cm length of 3-strand twisted rope was untwisted and suspended at shoulder height from a metal bar in the pen. The rope was left in the pen for pigs to chew on for 20-30 min. Subsequently, fluid was collected from the rope and refrigerated until processing. Gloves were changed between collections of samples from different pens to avoid cross-contamination.

Lameness scores (0-4) were determined for pigs in each pen where oral fluids were collected. The same evaluator determined the lameness score for all pens and in all herds. The gait and leg position of sampled pigs were assessed and lameness scores were recorded based on the movement pattern of pigs, as described by Nielsen et al. (2001). Briefly, a score of 0 reflected the movement pattern where pig gets up immediately from a lying position and moves freely in the pen with balanced weight on all four limbs. For score 1, pig rises immediately but a reluctant movement is observed, with short steps and uneven distribution of body weight. When pig moves slowly in the pen with short steps and reduced weight in the sore limb, or pig rises slowly and the affected limb is not weight bearing, then a score of 2 was assigned. For score 3, the pig is reluctant to rise, with muscle shivering when standing and lifts the sore limb from the floor, or

pig refuses to walk or walks on three limbs only. Score of 4 was assigned for a pig only rising when forced and standing with marked signs of pain (e.g. reluctance to move, limping and vocalization). Pigs with lameness score 2 or higher were considered lame, and the proportion of lame pigs in a pen was determined. The production records including treatments and mortality were examined to take into account clinical history of lameness, therapeutic and prophylactic treatment regimens and underlying production issues.

Sample processing and testing

Oral fluid samples were submitted to the Veterinary Diagnostic Laboratory at the University of Minnesota for DNA extraction and species-specific real-time PCR to detect *M. hyorhinis* and *M. hyosynoviae*. Samples were considered positive by real-time PCR for *M. hyorhinis* and *M. hyosynoviae* when Ct ≤ 37.

Statistical analysis

A generalized linear mixed model (GLMM) was used for the analyses fitting herds as a random effect to allow for correlated results within populations. Factors such as weeks of age, proportion of lame pigs in a pen, Ct values for detection of *M. hyorhinis* or *M. hyosynoviae,* and their interactions were considered as fixed effects. Models were fitted to estimate the association of a positive PCR detection in oral fluids to proportion of lame pigs in a pen along with pig age. The proportion of *M. hyorhinis* or *M. hyosynoviae* positive pens in a herd was determined by dividing the number of pens positive for *M. hyorhinis* or *M. hyosynoviae* by the total number of pens sampled in the herd. Similarly, the proportion of lame pigs in a pen was determined by dividing the number of pigs with lameness scores $\geq$2 by the total number of pigs in the pen. The correlation analyses were performed using the Spearman's method. The strength and direction of correlations were considered significant when a p value greater than 0.05 was obtained.

Results:

Among the 373 pens sampled in five different states in the United States, 54% of the samples were collected in Minnesota, 19% in Iowa, 13% in South Dakota, 13% in Wisconsin and 1% in Nebraska. All 37 sampled herds housed both nursey and finisher pigs. Twenty six percent of the sampled pens were nurseries and 73% were finishers. The age of pigs, number of pens positive

for *M. hyorhinis* or *M. hyosynoviae,* and the proportion of lame pigs in each production stage (nursery and finisher) within each herd are summarized in Table 1.

Mycoplasma hyorhinis was detected by real-time PCR from oral fluid in all sampled herds irrespective of the age groups in Minnesota (19/19), South Dakota (5/5), Wisconsin (5/5) and Nebraska (1/1). In Iowa, 6/7 sampled herds were positive for *M. hyorhinis* in oral fluids.

The level of detection of *M. hyosynoviae* varied depending on the herd. Ninety percent of the sampled herds were positive for *M. hyosynoviae* in Minnesota (17/19) whereas 40% (2/5) and 28% (2/7) were positive in South Dakota and Iowa, respectively. In Wisconsin 100% of herds (5/5) were positive, and the herd sampled in Nebraska was negative for *M. hyosynoviae.*

Additionally, age of the pigs was observed to have an effect on detection of *M. hyosynoviae* in oral fluids, irrespective of the herds or geographical location ($p<0.05$; Figure 1).

Lameness (scores 2 or higher) was observed in 3.9% (1,139/28,729) of pigs across all sampled herds. No correlation was observed between pig lameness and Ct values for *M. hyorhinis* detection in oral fluid samples ($p>0.05$; Figure 2A). A high proportion of pigs were scored lame in pens from which oral fluids were detected positive for *M. hyosynoviae* with low Ct values. As shown in Figure 2B, a negative correlation was observed between the proportion of lame pigs in a pen and Ct values for *M. hyosynoviae* detection in oral fluids ($p<0.05$; $r = -0.27$). The proportion of lame pigs in a herd was positively correlated with the proportion of pens detected positive for *M. hyosynoviae* in oral fluid samples. The mixed model explained significant main effects for Ct values of *M. hyosynoviae* detection and weeks of age, on the proportion of lame pigs in a pen. There was no effect for the age of animals on pig lameness or on the Ct values for *M. hyorhinis* detection ($p>0.05$; Figure 3A). However, the statistical analysis supported the observation that as the weeks of age increased the proportion of pigs positive for *M. hyosynoviae* increased with greater risk of lameness (Figure 3B).

Molecular characterization of *M. hyorhinis* was attempted, however, the nature of the sample did not allow for molecular typing, regardless of the use of various methods for DNA extraction.

Discussion:

The current study intended to identify the potential of oral fluids, a sample type facilitating population level surveillance and detection, in determining the prevalence of arthritogenic mycoplasmas in swine production herds and their association with the development of lameness in pigs. Under the conditions of this investigation, *M. hyorhinis* was frequently detected in oral fluids in nursery and finisher herds regardless of the clinical presentation of lameness. However, an increased detection of *M. hyosynoviae* in the finisher compared to the nursery herds was observed, along with its significant correlation to increased proportion of lame pigs in the herd.

Mycoplasma hyorhinis has been associated with a diverse range of clinical signs in pigs including pneumonia, polyserositis, polyarthritis, pericarditis, eustachitis, and otitis, among others (Ennis et al., 1971; Morita et al., 1999; Straw et al., 2006). However, polyarthritis and lameness have emerged as increasing concerns in different swine productions systems (Neto, 2012). Previous studies have identified a very low prevalence of *M. hyorhinis* in pre-weaning piglets, whereas in the nursery period, the pervasiveness increases significantly (Clavijo et al., 2017; Roos et al., 2019). Similar to *M. hyorhinis, M. hyosynoviae* colonizes the upper respiratory tract with transmission occurring typically after 4-8 weeks of age (Ross and Spear, 1973; Roos et al., 2019). The main clinical signs observed in wean-to-finish pigs include lameness, joint swelling and pain resulting in gait alteration (Scheiber and Thacker, 2012). Moreover, both bacteria are ubiquitous and can exist in pigs simultaneously as commensals. Henceforth, the mere detection will not infer their active role in pathogenesis. This necessitates population level investigations to identify the potential association between the detection of arthritogenic mycoplasmas and development of lameness in pigs, which will eventually aid to improve diagnostics, prevention, and control strategies in the field.

From 2010, oral fluids based tests have been used for the detection and monitoring of several swine pathogens including vesicular stomatitis virus, porcine respiratory and reproductive syndrome virus and porcine circovirus type 2 in swine populations (Prickett et al., 2008;

Prickett and Zimmerman, 2010). However, the testing for *M. hyosynoviae* and *M. hyorhinis*, using oral fluid samples gained interest only since 2015 (Gomes Neto et al., 2015b).

Although, oral fluid is considered as aggregate sampling method, which facilitates monitoring, surveillance and detection of disease in animal populations, the test could be limited by the individual status of pen mates participating in the collection. For instance, in this study, the contribution of a clinical lame pig in the pen to the oral fluid sample is biologically dubious. Therefore, while the results presented in this study are intriguing, it has been taken into account that several confounding factors like true prevalence, dynamics of infection, individual host immunity, management practices and nutrition, could have influenced results.

Considerations have been made to include nursery and finisher stage pigs to portray the dynamics of infection targeting several pig production states in the US. Nevertheless, under the conditions of this investigation, *M. hyorhinis* was detected in oral fluids in almost every nursery and finisher herd regardless of the clinical presentation of lameness. On tracing back the production practices of the identified negative herd for *M. hyorhinis,* it was of notice that pigs were administered an autogenous vaccine against the bacterium prior to sample collection. These findings shed light into how informative would an oral fluid sample be as a diagnostic tool for detection of *M. hyorhinis* from a lame pig population. It is also important to emphasize that the only clinical sign that was evaluated in the present study was lameness. Therefore, *M. hyorhinis* detection in oral fluids warrants prospective studies in relation with polyserositis and other clinical signs exhibited along with infection.

Alternatively, the level of detection of *M. hyosynoviae* varied between herds. Interestingly, in line with the epidemiology and disease transmission patterns previously reported, oral fluid samples from the pens in finisher herds were observed to have a high burden of *M. hyosynoviae.* Our results identified a close association in detection of *M. hyosynoviae* in oral fluids to the proportion of pigs lame in the pen. Moreover, there was an increase in the proportion of lame pigs in the pens as weeks of age increased. As mentioned, although various production systems were evaluated in this study, several other confounding factors like

dynamics of infection, individual host immunity, density of pigs in the herd or nutrition, could also impact the results.

It is also important to report that all the sampled herds in the study were administered with generic antibiotics as part of the regular farm practice, however, potential effects on detection on *M. hyorhinis* or *M. hyosynoviae* were not evaluated. Previous investigations have suggested no effect of antimicrobial medication on detection of arthritogenic Mycoplasmas in pigs (Roos, 2016).

Population level investigations using oral fluid samples would help identify potential association between the detection of arthritogenic mycoplasmas particularly, *M. hyosynoviae* and development of lameness in pigs. However, as the data on the accuracy of oral fluid PCR for the detection of *M hyosynoviae and M hyorhinis* is limited on different age groups, longitudinal population studies delineating the effect of systemic transmission patterns of the bacteria would be needed.

Table 1. Summary of data collection.

Herd group	Age of pigs (weeks)	State*	No. of pens positive for *M. hyorhinis*/No. of pens sampled	No. of pens positive for *M. hyosynoviae* /No. of pens sampled	Proportion of lame pigs
Nursery	4	MN	4/4	0/4	0.00
	6	IA	0/10	1/10	0.00
	7	MN	10/10	4/10	0.01
	7	IA	10/10	0/10	0.01
	8	MN	10/10	9/10	0.01
	8	SD	10/10	0/10	0.02
	8	WI	10/10	4/10	0.01
	9	MN	10/10	9/10	0.01
	10	MN_1	4/4	0/4	0.05
	10	MN_2	10/10	0/10	0.04
	10	IA	10/10	0/10	0.02
Finisher	12	MN	10/10	10/10	0.04
	14	MN	10/10	10/10	0.07
	16	MN_1	4/4	2/4	0.05
	16	MN_2	10/10	10/10	0.06
	16	IA	10/10	0/10	0.06
	16	WI	10/10	10/10	0.06
	17	MN_1	10/10	10/10	0.07
	17	MN_2	10/10	10/10	0.06
	17	MN_3	10/10	0/10	0.05
	18	MN	10/10	9/10	0.05
	18	IA	10/10	0/10	0.05
	18	SD	10/10	0/10	0.05
	18	WI	10/10	10/10	0.03
	20	MN_1	6/6	6/6	0.03
	20	MN_2	10/10	5/10	0.04
	20	WI	10/10	10/10	0.07
	22	MN_1	4/4	2/4	0.10
	22	MN_2	9/9	9/9	0.06
	22	SD	9/9	0/9	0.06
	24	MN	6/6	6/6	0.02
	24	IA	10/10	4/10	0.05
	24	WI	10/10	10/10	0.05
	25	MN_1	3/4	3/4	0.10
	25	MN_2	10/10	10/10	0.02
	26	SD_1	10/10	10/10	0.05
	26	SD_2	10/10	8/10	0.05
	28	MN_1	10/10	10/10	0.03
	28	MN_2	9/9	9/9	0.08
	28	MN_3	10/10	10/10	0.05
	28	IA	10/10	0/10	0.03
	28	NE	4/4	0/4	0.05

*Multiple herds sampled from the same state at similar ages. Thus, a sequential number is assigned to each new sampled herd.

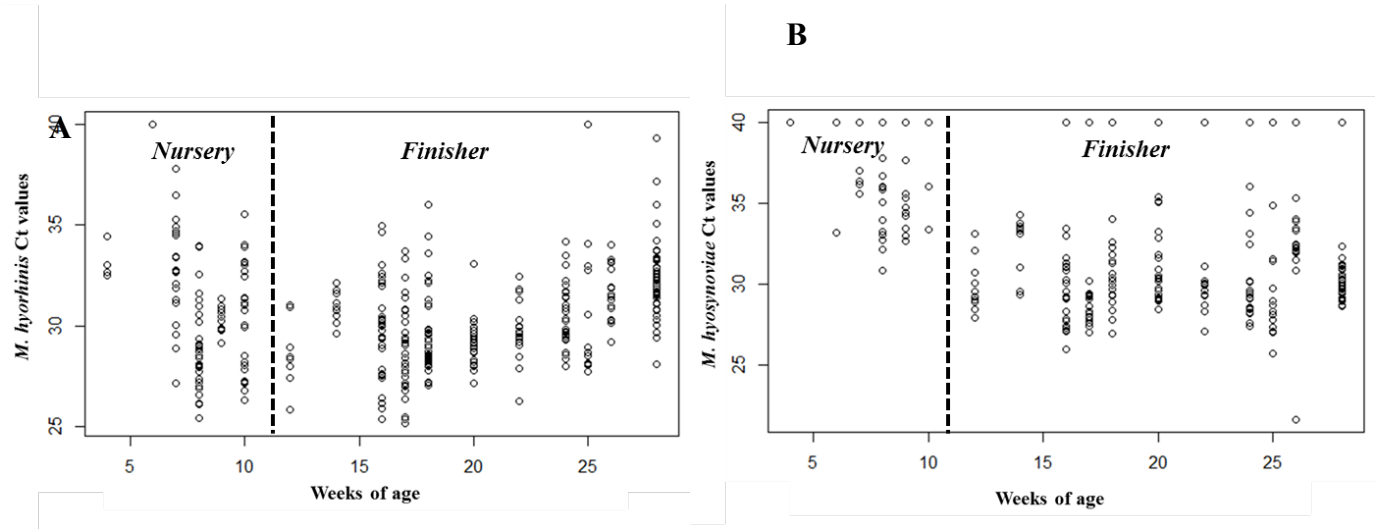

Figure 1. Detection of *Mycoplasma hyorhinis* and *M. hyosynoviae* and weeks of age of pigs sampled (by pen) in different herds. Each open sphere on the figure represents an oral fluid sample from a pen. Y-coordinates refer to the Ct values of *M. hyorhinis* or *M. hyosynoviae* detection whereas the X-coordinates correspond to the age of the pigs sampled in the pen. Herds with pigs ≤10 of weeks of age were grouped as nursery and those with >10 weeks of age were finishers. Samples were considered positive by real-time PCR for *M. hyorhinis* and *M. hyosynoviae* when Ct ≤ 37. **A.** No age effect was observed between *M. hyorhinis* detection and pigs age ($p>0.05$). **B.** Age of the pigs was observed to have an effect on the detection of *M. hyosynoviae* in oral fluids ($p<0.05$).

A B

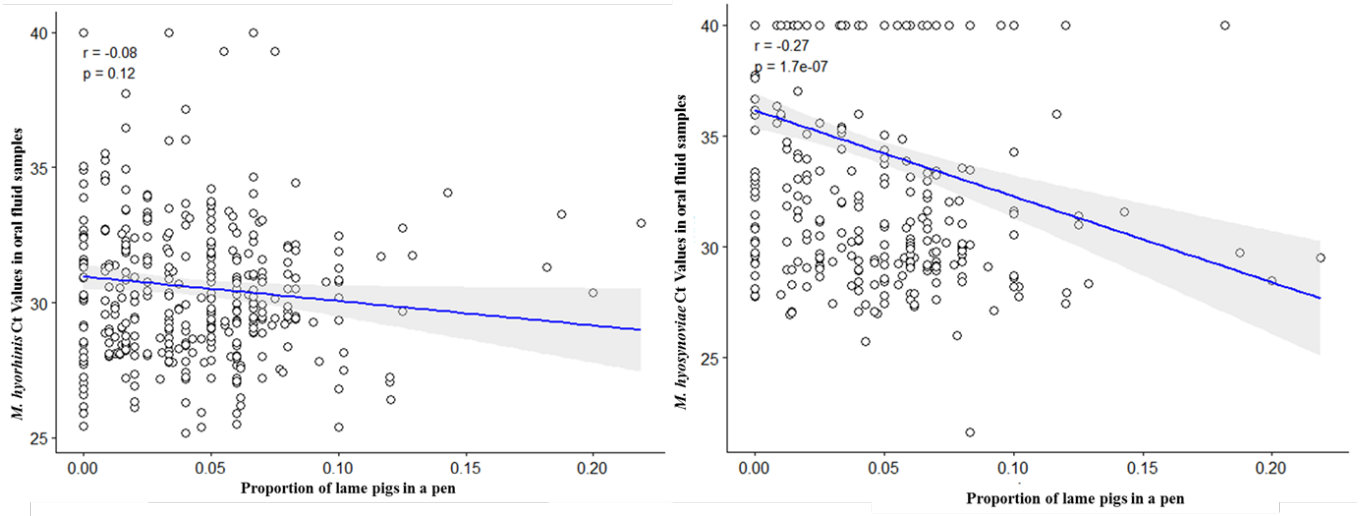

Figure 2. Correlation of *Mycoplasma hyorhinis* or *M. hyosynoviae* Ct values and proportion of lame pigs in a pen. The scatterplot shows the relationship between Ct values of *M. hyorhinis* or *M. hyosynoviae* detection and the proportion of lame pigs in the pen for each oral fluid sample collected. The Ct values of detection are presented on the Y axis and the proportion of lame pigs was shown in the X-axis. Samples were considered positive by real-time PCR for *M. hyorhinis* and *M. hyosynoviae* when Ct ≤ 37. **A.** No significant correlation was observed between the lameness in pigs and detection of *M. hyorhinis* in oral fluid samples ($p>0.05$). **B.** A significant negative correlation was observed between proportion of lame pigs in the pen and Ct values for *M. hyosynoviae* PCR detection in oral fluids ($p<0.05$; $r = -0.27$).

A B

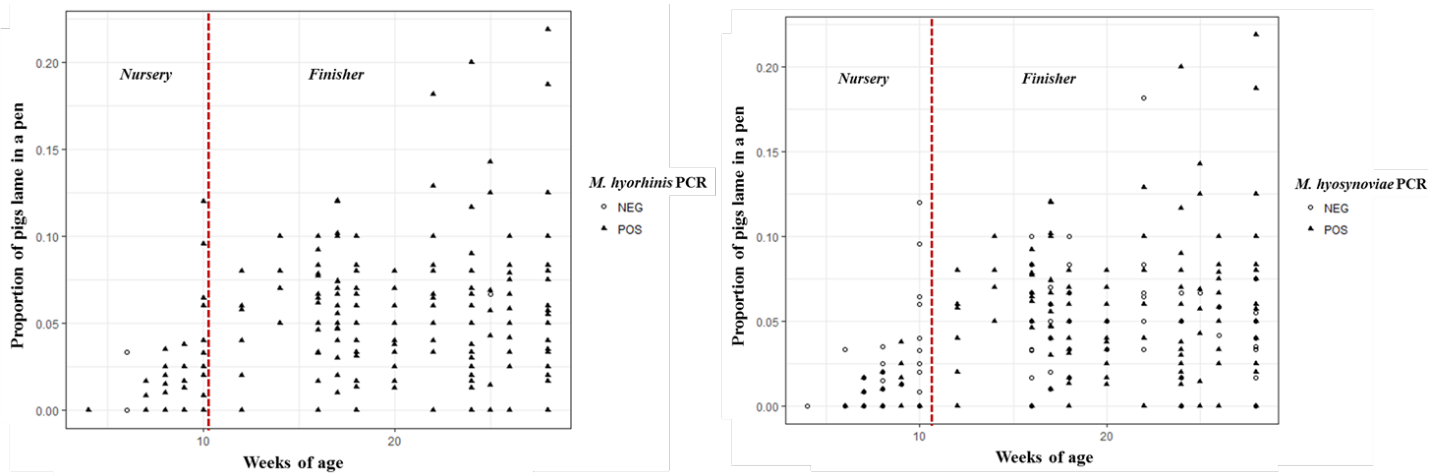

Figure 3. Relationship between the proportion of lame pigs in a pen and the age of the pigs. Each scatter dot represents an oral fluid sample and its shape indicates the positive (▲) or negative (○) detection of *M. hyorhinis* or *M. hyosynoviae.* Samples were considered positive by real-time PCR for *M. hyorhinis* and *M. hyosynoviae* when Ct values were ≤ 37. **A.** No effect of the age on pig lameness or on *M. hyorhinis* detection was observed ($p>0.05$). **B.** As the weeks of age increased, the proportion of pigs positive for *M. hyosynoviae* increased with greater risk to lameness ($p<0.05$).