RESEARCH REPORT
SWINE HEALTH

Title: Development and Validation of Novel Diagnostic Assays and Investigation of Disease Pathogenesis for Infectious Arthritis in Growing Pigs **- NPB #16-107**

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Industry Summary:

Formerly a "fringe" issue, lameness in swine production has become a priority in the face of recent veterinary and producer-driven lameness investigations showing that losses attributable to lameness can reach $23 per growing pig, and culls or mortality due to lameness can routinely approach 1% to 5% of the total population. The two largest swine diagnostic laboratories in the United States have reported an increase in infectious arthritis due to *Mycoplasma hyorhinis* and *Mycoplasma hyosynoviae*. The Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) diagnosed 890 cases of arthritis between 2003 and 2014. *Mycoplasma*-associated arthritis accounted for an average of 26% of arthritis cases over that period, with an increasing frequency in recent years.

Despite the common occurrence of *M. hyorhinis*-associated disease and *M. hyosynoviae*-associated arthritis, practitioners' desire improved antemortem diagnostics as well as a better understanding of disease pathogenesis and the immunologic response that can help quantify the effect of potential interventions of *Mycoplasma*-associated arthritis. Accordingly, the goals of this project were to develop and validate novel diagnostic assays for infectious arthritis due to *M. hyorhinis* and *M. hyosynoviae* in growing pigs and to characterize the immunopathology of *M. hyorhinis*-associated disease. Prior to this project, the number, quality, and quantity of samples necessary to develop reliable antibody ELISA assays were not available to researchers. To address this, our group generated the necessary samples for researchers to develop new tests, developed ELISAs using new technology for the detection of *M. hyorhinis* and *M. hyosynoviae*, and established association between detection of either agent in oral fluids by PCR and clinical disease.

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Keywords: *Mycoplasma hyorhinis*, *Mycoplasma hyosynoviae*, *Mycoplasma hyopneumoniae*, ELISA, oral fluids, swine

Scientific Abstract:

Background:

M. hyorhinis and *M. hyosynoviae* are common causes of polyarthritis in swine. The overall goal of this study was to develop reliable antibody ELISA assays for the detection of *M. hyorhinis* and *M. hyosynoviae*. These assays will provide antemortem diagnostics, surveillance methods, and pertinent information to practitioners concerning vaccination or antibiotic intervention timing resulting in better management strategies for swine production systems that will enhance production efficiency, animal well-being, and improve animal health.

Methods:

To generate a panel of specimens (serum and oral fluid) and specific polyclonal antibodies against *M. hyorhinis*, *M. flocculare*, *M. hyopneumoniae*, and *M. hyosynoviae*, development of oral fluid and serum antibody assays specific against *M. hyorhinis* and *M. hyosynoviae*, and
establish an association between detection of either agent in oral fluids by PCR and clinical disease, 50 cesarean derived colostrum deprived (CDCD), crossbred, mixed-sex, 8-week-old pigs were inoculated with Friis media, *M. hyorhinis*, *M. flocculare*, *M. hyopneumoniae*, or *M. hyosynoviae* (n=10/group). Serum samples were collected on day post-inoculation (DPI) 0, 3, 7, 10, 14, 17, 21, 24, 28, 35, 42, 49, and 56. Pen-based oral fluids (5 pens, 2 pigs per pen) were collected daily throughout the study. To investigate the immunopathogenesis and bacterial dissemination pattern of *M. hyorhinis* in a single and multiple inoculation model, CDCD pigs were inoculated once or four times with *M. hyorhinis* or sham-inoculated. We then designed and produced a recombinant polyprotein (rVlpA-G) consisting of the chimeric variable lipoproteins of *M. hyorhinis* cloned in frame in a prokaryotic vector system (pET32a). For *M. hyosynoviae,* we obtained a bacterial protein extract from a pure culture of *M. hyosynoviae* treated with Tween 20 detergent. Both antigens were used to develop indirect ELISAs for antibody (IgG and IgA) detection in serum and/or oral fluids.

Results:

M. hyosynoviae was detected by PCR in oral fluids daily for 11 days starting at DPI 3. Seven out of ten animals developed clinical signs consistent with *M. hyosynoviae*. *M. hyorhinis* was detected intermittently in oral fluids by PCR throughout the study period starting at DPI 2. Clinical signs consistent with *M. hyorhinis*-associated disease started at DPI 11 and eight out of the ten inoculated animals developed clinical signs that lasted through DPI 56. Lesions consistent with *M. hyorhinis* were observed at necropsy in nine out of ten *M. hyorhinis*-inoculated animals. The diagnostic performance, i.e., diagnostic sensitivity and specificity, and analytical specificity (cross-reactivity) of the *M. hyorhinis* rVlpA-G and *M. hyosynoviae* T20 indirect ELISAs was evaluated by testing a panel of samples of precisely known immune status (*M. hyorhinis*, *M. hyosynoviae*, *M. hyopneumoniae*, and *M. flocculare*). *M. hyorhinis* or *M. hyosynoviae* inoculated animals showed specific seroconversion between DPI 14 (IgA) and 28 (IgG). No cross-reactivity (100% analytical specificity) was observed under experimental conditions.

Discussion:

The newly developed antibody ELISA assays for the detection of *M. hyorhinis* or *M. hyosynoviae* are currently available at the ISU-VDL and will assist in the diagnosis and surveillance of *M. hyorhinis*-associated disease and *M. hyosynoviae*-arthritis.

Introduction:

Formerly a "fringe" issue, lameness in swine production has become a priority in the face of recent veterinary and producer-driven lameness investigations showing that losses attributable to lameness can reach $23 per growing pig.[1] Culls or mortality due to lameness and joint disease can routinely approach 1% to 5% of the total population.[2] The two largest swine diagnostic laboratories in the US have reported an increase in infectious arthritis due to *M. hyorhinis* and *M. hyosynoviae* over the last several years.[3,4] The Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) diagnosed 890 cases of arthritis between 2003 and 2014. *Mycoplasma*-associated arthritis accounted for an average of 26% of arthritis cases over that period, with an increasing frequency in recent years.

M. hyosynoviae has an affinity for joints, causing a severe arthritis in pigs more than 10 weeks old.[5,6] Disease may affect individual pigs or become an epidemic with 10 to 50% of pigs affected before reaching market weight.[7] *M. hyorhinis*-associated disease has recently been identified as one of the main concerns of the US pork industry[8] and has been diagnosed in nearly every production system in which it is sought.[9] Unlike *M. hyosynoviae*, *M. hyorhinis* also causes a polyserositis and is emerging as an important contributor to mortality in nursery pigs.[10]

The ISU-VDL diagnosed a total of 2949 cases with fatal bacterial septicemia or polyserositis due to *S. suis*, *H. parasuis*, or *M. hyorhinis* between 2005 and 2014. In 2010 *M. hyorhinis* accounted for 1.8% (4 of 226) of these cases. This percentage has increased in recent years, up to 28% (134 of 606) in 2014. Similarly, approximately 50% of the cases with polyserositis received at the MN-VDL from September 2009 to May 2010 are demonstrated to have involvement of this etiology.[11]

Despite the common occurrence of *M. hyorhinis*-associated disease and *M. hyosynoviae*-associated arthritis our understanding of immunopathogenesis and the epidemiologic implications that may exist between PCR results from oral fluids and clinical signs is limited. Swine veterinarians express a desire for improved antemortem diagnostics as well as a better understanding of disease pathogenesis and the immunologic response with a focus on the effect of potential interventions of *Mycoplasma*-associated arthritis. Currently, there are no available ELISAs for either *M. hyorhinis* or *M. hyosynoviae*, limited knowledge concerning the immunologic response, and no established correlation between detection of either agent in oral fluids by PCR and clinical disease. Swine producers and veterinarians are faced with the challenge of controlling *M. hyorhinis*-associated disease and *M. hyosynoviae*-associated arthritis without the tools necessary to prevent disease, monitor pathogen circulation, or evaluate interventions.

Objectives:

Objective #1: Generation of a panel of specimens (serum and oral fluid) and specific polyclonal antibodies against *M. hyorhinis*, *M. flocculare*, *M. hyopneumoniae*, and *M. hyosynoviae*.

Objective #2: Development of oral fluid and serum antibody assays specific against *M. hyorhinis* and *M. hyosynoviae*.

Objective #3: Investigate the immunopathogenesis of *M. hyorhinis*-associated disease.

Materials & Methods:

Animal study #1

Bacterial isolates and microbial techniques

A *M. hyorhinis* field isolate was obtained from a pig with fibrinous pleuritis and was cultivated in Friis medium. A sample of the seed of *M. hyorhinis* strain 38983 and inoculum was verified pure by PCR. *M. hyorhinis* 38983 was seeded into Friis broth and incubated at 37°C for 48 hours. A sample of the inoculum was retained for mycoplasma quantification. Estimation of the dose for *M. hyorhinis* 38983 revealed that it contained 3.2 x 10^8 colony forming units (cfu)/ml.

A *M. hyosynoviae* field isolate was obtained from a pig with arthritis and was cultivated in Difco with turkey serum (D-TS). A sample of the seed and final inoculum of *M. hyosynoviae* strain 34428 was verified pure by PCR. *M. hyosynoviae* 34428 was seeded into D-TS and incubated at 37°C for 48 hours. Tubes of

growth were pooled for inoculation. A sample of the inoculum was retained for mycoplasma quantification. Estimation of the dose for *M. hyosynoviae* 34428 revealed that it contained 2.1 x 109 cfu/ml.

A *M. flocculare* isolate was cultivated in Friis medium. A sample of the seed of *M. flocculare* strain 27399 and final inoculum was verified pure by PCR. *M. flocculare* 27399 was seeded into flasks containing Friis and incubated in a shaking water bath at 37°C for 48 hours. Flasks were pooled for inoculation

A lung homogenate containing *M. hyopneumoniae* strain 232 was diluted 1:100 in 10 ml Friis medium.

Animals

Fifty eight-week-old CDCD pigs were allocated to one of five groups housed separately based on inoculum and placed in one of five pens with two animals per pen.

Inoculation

Oral fluids (*M. hyorhinis*, *M. flocculare*, and *M. hyopneumoniae*) and tonsil scraping (*M. hyosynoviae*) collected prior to challenge were negative for detection of *M. hyorhinis*, *M. flocculare*, *M. hyopneumoniae*, and *M. hyosynoviae* by PCR. Negative control animals were inoculated intranasally with Friis medium (1 ml/nostril). *M. hyorhinis*-inoculated animals received an estimated 3.2 x 10^8 cfu/ml administered into the peritoneal cavity (2 ml) and painted on the tonsil (2 ml). *M. hyosynoviae*-inoculated animals received an estimated 2.1 x 10^9 cfu/ml administered into the nasal cavity (1 ml/nostril) and ear vein (1 ml), and painted on the tonsil (2 ml). *M. flocculare* was inoculated into the nasal cavity (1 ml/nostril) and trachea (1 ml) and painted on the tonsil (2 ml) on 0 and 4 days post inoculation (DPI). *M. hyopneumoniae*-inoculated animals received 1 ml of lung homogenate administered into the trachea. Animals were sedated with an intramuscular injection of Telazol 500mg, Ketamine 250mg, and Xylazine 250mg at 4.4 mg/kg prior to inoculation with the expectation of the control and *M. hyopneumoniae*-inoculated group.

Sample collection and necropsy

Whole blood was collected 0, 3, 7, 10, 14, 17, 21, 24, 28 days post inoculation and weekly thereafter. Blood samples were centrifuged (1,500 x g for 15 min), and serum was stored at -80ºC until assayed. Pen-based oral fluids samples (5 pens/group) were collected daily and stored at -80ºC until assayed. All animals in all groups were clinically evaluated daily At 56 DPI pigs were necropsied and gross lesions were evaluated by a veterinary diagnostic pathologist.

DNA extraction from oral fluids samples

High volume DNA extraction from oral fluid samples were performed at the Iowa State University Veterinary Diagnostic Laboratory per standard operating procedure.

Real-time PCR for M. hyopneumoniae, M. hyosynoviae, M. hyorhinis, and M. flocculare

The PCR assays for all four mycoplasmas were performed at the Iowa State University Veterinary Diagnostic Laboratory per standard operating procedure.

ELISA development and evaluation

M. hyorhinis Indirect ELISA- Sequences of *M. hyorhinis* surface variable lipoprotein (Vlp) A, B, C, D, E, F and G were expressed as a chimeric polypeptide using an *E. coli* expression system, purified using affinity chromatography and used to coat a 96-well polystyrene plates. The chimeric recombinant Vlp A-G indirect ELISA was performed using the same buffer and assay conditions as the *M. hyosynoviae* ELISA. *M. hyosynoviae* Indirect ELISA-To assess the presence of IgG in swine serum a Tween 20 ELISA was performed as follow. First, a pure culture of *M. hyosynoviae* was grown in Difco medium and the cells were harvested through centrifugation and washed three times in phosphate buffered saline (PBS 1X pH 7.4). The surface proteins were extracted mixing the bacterial pellet with a 2% solution of Tween 20, incubating the mix at 37ºC for 90 min, and centrifugation (30 min at 59,573 g). The supernatant was separated and used at 1:100 dilution to coat 96-well polystyrene plates. Serum specimens were diluted at 1:50 dilution and incubated for 1h at 37ºC, followed by three washes with PBS-T (0.1% Tween 20), a second incubation of 1 hour at 37ºC with an goat anti-pig IgG-Fc fragment antibody HRP conjugated at 1:20,000 dilution, followed by three washes and a final 15 minutes incubation at 25ºC with a TMB substrate. The reaction was stopped by the addition of 100 µl of stop solution to each well. Reactions were measured as optical density (OD) at

450 nm using an ELISA plate reader (Biotek® Instruments Inc.) operated with commercial software (GEN5™, Biotek® Instruments Inc.). The antibody response in serum and oral fluid were represented as sample-to-positive (S/P) ratios.

Animal study #2

Bacterial isolates and microbial techniques

A *M. hyorhinis* field isolate was obtained from a pig with fibrinous pleuritis and was cultivated in Friis medium and triple cloned. A sample of the seed of *M. hyorhinis* strain 38983 and inoculum was verified pure by PCR. *M. hyorhinis* 38983 was seeded into Friis broth and incubated at 37°C for 48 hours. A sample of the inoculum was retained for mycoplasma quantification. Estimation of the dose for *M. hyorhinis* 38983 revealed that it contained 4.5 x 10^7 colony forming units (cfu)/ml.

Animals

Twenty four six-week-old CDCD pigs were allocated to one of three groups and placed in pens with two animals per pen.

Inoculation

Animals were inoculated once (n=12; Mhr1) or four (n=8; Mhr2) times with *M. hyorhinis* or sham-inoculated (n=3; controls). Animals were inoculated via tonsillar painting (3 mL) and intranasally (1 mL) with either *M. hyorhinis* or Friis.

Sample collection and necropsy

Clinical signs were evaluated daily. Whole blood was collected DPI 0, 7, 14, 21, 28, 35 and 42. Blood samples were centrifuged (1,500 x g for 15 min), and serum was stored at -80ºC until assayed. Pen-based oral fluids samples, nasal swabs, and oropharyngeal swabs were collected at similar intervals. At DPI 42 animals were euthanized and evaluated grossly for lesions consistent with *M. hyorhinis* by a veterinary diagnostic pathologist.

DNA extraction from oral fluids samples

High volume DNA extraction from oral fluid samples were performed at the Iowa State University Veterinary Diagnostic Laboratory per standard operating procedure.

Real-time PCR for M. hyorhinis

The PCR assay for *M. hyorhinis* was performed at the Iowa State University Veterinary Diagnostic Laboratory per standard operating procedure.

Results:

Objective #1: Generation of a panel of specimens (serum and oral fluid) and specific polyclonal antibodies against *M. hyorhinis*, *M. flocculare*, *M. hyopneumoniae*, and *M. hyosynoviae*.

Clinical signs

Clinical signs consistent with *Mycoplasma*-associated disease were not observed at any point during the study in the control or *M. flocculare*-inoculated group. Clinical signs consistent with *M. hyorhinis*-associated disease including lameness, joint swelling, rough hair coat and loss of condition that lasted through DPI 56 were observed in eight out of the ten *M. hyorhinis*-inoculated animals. Animals 13 and 14 never developed clinical signs. At DPI 11 roughen hair coats and loss of condition and mild front and hind limb lameness were noted in Pen A and D, respectively. At DPI 13, Animal 11 (Pen A) lameness of the right front limb with a reluctance to move and rough hair coat was noted, Animals 15 and 16 (Pen C) mild lameness, loss of condition and rough hair coats was noted, Animals 17 and 18 (Pen D) depression and reluctance to move and front and hind limb lameness was noted, respectively, and Animals 19 and 20 (Pen E) lameness and joint swelling was noted. At DPI 24 Animal 12 (Pen A) was non-weight bearing on the right front limb. Two animals (18 and 19) were euthanized at DPI 24 due to an inability to ambulate and anorexia as a result of polyarthritis and polyserositis. Lameness, rough hair coat, joint swelling, and loss of condition in all affected pens remained relatively unchanged from initial observations through the conclusion of the study. In the *M. hyosynoviae*-inoculated group seven out of ten pigs developed clinical signs consistent with *M. hyosynoviae*-arthritis that remained until DPI 28. Clinical signs consisted of swollen hocks, leg stiffness, and reluctance to move and altered gait. Clinical signs were first noted DPI 8. On DPI 10 clinical signs were also

noted in Animals 33, 34, 37, 39, and 40. Animal 31 died during blood collection on DPI 10. In the *M. hyopneumoniae*-inoculated group coughing was intermittently noted.

Pen-based oral fluids PCR

Oral fluids from the control group were negative by PCR for *M. hyorhinis*, *M. flocculare*, *M. hyosynoviae*, and *M. hyopneumoniae* at all sampling time points. *M. hyorhinis* was detected intermittently in oral fluids by PCR throughout the study period starting at DPI 2; ten days prior to clinical signs. The number of pens positive by PCR and average Cq with standard deviation is presented in Figure 1. *M. hyosynoviae* was detected by PCR in oral fluids daily for 11 days starting at DPI 3. Detection of *M. hyosynoviae* occurred four days prior to clinical signs and continued through DPI 15 with all pens positive at DPI 10. The number of pens positive by PCR and average Ct with standard deviation is presented in Figure 2. *M. flocculare* was detected in oral fluids by PCR from Pen D at DPI 50. *M. hyopneumoniae* was first detected in oral fluids at DPI 12. *M. hyopneumoniae* was detected in oral fluids by PCR intermittently until DPI 48 with a total of 16 positive oral fluid samples during the duration of the study.

Serum PCR

M. hyorhinis was detected by PCR in serum from Animal 13 at DPI 10 (Cq 35.9). All other testing time points (DPI 0, 3, 7, 10, 14, 17, 24, and 28) were negative. *M. hyosynoviae* was detected by PCR in Animals 36 (Cq 33.3) and 39 (Cq 34.4) at DPI 3 and 7, respectively. All other testing time points (DPI 0, 3, 7, 10, and 14) were negative.

Necropsy

Gross lesions were not noted in the control or *M. flocculare*-inoculated groups. Lesions consistent with *M. hyorhinis* were observed at necropsy in all pigs with the exception of Animal 13. This animal never developed clinical signs consistent with *M. hyorhinis*-associated disease during the study. Lesions included fibrosing epicarditis, fibrosing pleuritis, fibrinous and fibrosing arthritis, and fibrosing peritonitis (Figure 3). In the *M. hyosynoviae*-inoculated group increased joint fluid was noted in Animals 31, 38 and 39 at DPI 56. Mild multifocal cranioventral consolidation was noted in Animals 41, 43, 46, and 48 in the *M. hyopneumoniae*-inoculated group.

Objective #2: Development of oral fluid and serum antibody assays specific against *M. hyorhinis* and *M. hyosynoviae*.

ELISA development and evaluation

The diagnostic performance, i.e., diagnostic sensitivity and specificity, and analytical specificity (cross-reactivity) of the *M. hyorhinis* rVlpA-G and *M. hyosynoviae* T20 indirect ELISAs were evaluated by testing a panel of samples of precisely known immune status (*M. hyorhinis*, *M. hyosynoviae*, *M. hyopneumoniae*, and *M. flocculare*) generated by experimental inoculation of CDCD pigs. Antibodies against *M. hyorhinis* or *M. hyosynoviae* as determined by the *M. hyorhinis* ELISA and whole cell *M. hyosynoviae* ELISA were not detected in any other group. Seven out of ten pigs seroconverted based on a *M. hyopneumoniae* ELISA available at the ISU VDL. Seronegative animals included 44, 45, and 50. All seronegative animals were in different pens. All *M. hyorhinis* or *M. hyosynoviae* inoculated animals showed specific seroconversion between DPI 14 (IgA) and 28 (IgG) (Figure 4 and 5, respectively). No cross-reactivity (100% analytical specificity) was observed under experimental conditions.

Publication: The complete results of Objective #1 and #2 are in preparation for submission as a full manuscript during the next 6 months. A portion of the results will be presented at the IPVS 2018 as an oral presentation titled, "Chimeric variant membrane surface lipoprotein (VlpA-G) as biomarker for early diagnosis of active *Mycoplasma hyorhinis* infection" by Giménez-Lirola *et al*.

Objective #3: Investigate the immunopathogenesis of *M. hyorhinis*-associated disease.

Clinical signs

Clinical signs consistent with *M. hyorhinis*-associated disease were not observed in any group.

Pen-based oral fluids PCR

M. hyorhinis was not detected in any oral fluid sample during the duration of the study in control animals. *M. hyorhinis* was detected only in one pen on DPI 42 in the Mhr1 group. *M. hyorhinis* was detected DPI 35 and DPI 42 in a single and different pen in the Mhr2 group.

Nasal swab and oropharyngeal swab PCR

M. hyorhinis was not detected in any nasal swab or oropharyngeal swab during the duration of the study in control animals. *M. hyorhinis* was detected by PCR on nasal swabs in 6 out of 12 pigs and at each sampling point in 2 pigs in the Mhr1 group. *M. hyorhinis* was detected by qPCR on nasal swabs at each sampling point in 3 pigs in the Mhr2 group.

Necropsy

Gross lesions consistent with *M. hyorhinis*-associated disease were not observed in any group.

rVlpA-G IgG ELISA

IgA was detected in oral fluids and serum at DPI 14 in animals from the Mhr2 group; however, in animals from the Mhr1 group, IgA was only detectable in oral fluids after 35 dpi. Significant levels of IgG were detected in the Mhr2 group after 28 and 35 dpi in serum and oral fluids, respectively ($p < 0.05$). Finally, no significant levels of IgG were detected in either serum or oral fluids in pigs from the Mhr1 group ($p > 0.05$). Animals in the Mhr2 group developed an earlier and stronger humoral response compared to animals in Mhr1.

Publication: The complete results of Objective #3 are in preparation for submission as a full manuscript during the next 6 months. A portion of the results will be presented at the IOM 2018 as an presentation titled, "Colonization and serological response of *Mycoplasma hyorhinis* in experimentally infected swine" by Merodio *et al*.

<u>Discussion</u>:

Despite the common occurrence of *M. hyorhinis*-associated disease and *M. hyosynoviae*-associated arthritis our understanding of immunopathogenesis and the epidemiologic implications that may exist between PCR results from oral fluids and clinical signs is limited. Swine veterinarians have also expressed a desire for improved antemortem diagnostics as well as a better understanding of disease pathogenesis and the immunologic response with a focus on the effect of potential interventions of *Mycoplasma*-associated arthritis.

The detection of *M. hyorhinis* and *M. hyosynoviae* in oral fluids by PCR, presence of consistent clinical signs, development of consistent gross lesions, and detection of serum antibodies indicates that the experimental infection was successful in Animal Study #1. Both *M. hyorhinis* and *M. hyosynoviae* were detected in oral fluids by PCR prior to clinical signs. However, *M. hyosynoviae* was only detected in oral fluid by PCR for a short duration of time in this study. All *M. hyorhinis* or *M. hyosynoviae* inoculated animals showed specific seroconversion between DPI 14 (IgA) and 28 (IgG). No cross-reactivity (100% analytical specificity) was observed under experimental conditions.

The lack of clinical signs and presence of a humoral response and bacterial colonization in Animal Study #2, indicates that the multiple inoculation experimental model may mimic subclinical natural infection in the field. Based on this observation, animals would have to be exposed multiple times to mount a detectable immune response.

This work has provided the necessary known-status samples to develop assays for the detection of antibodies against *M. hyorhinis* and *M. hyosynoviae*. The development and evaluation of serologic tools as reported in this study and investigation of potential uses of samples for diagnostics and surveillance are critical for disease control and intervention strategies and to achieve a better understanding of *M. hyorhinis* and *M. hyosynoviae* epidemiology in industrial herds.

The newly developed antibody ELISA assays for the detection of *M. hyorhinis* and *M. hyosynoviae* are currently available at the ISU-VDL and will assist in the diagnosis and surveillance of *M. hyorhinis*-associated disease and *M. hyosynoviae*-associated arthritis.

Figures:

Figure 1. The number of pens positive by *Mycoplasma hyorhinis* PCR and average Cq with standard deviation.

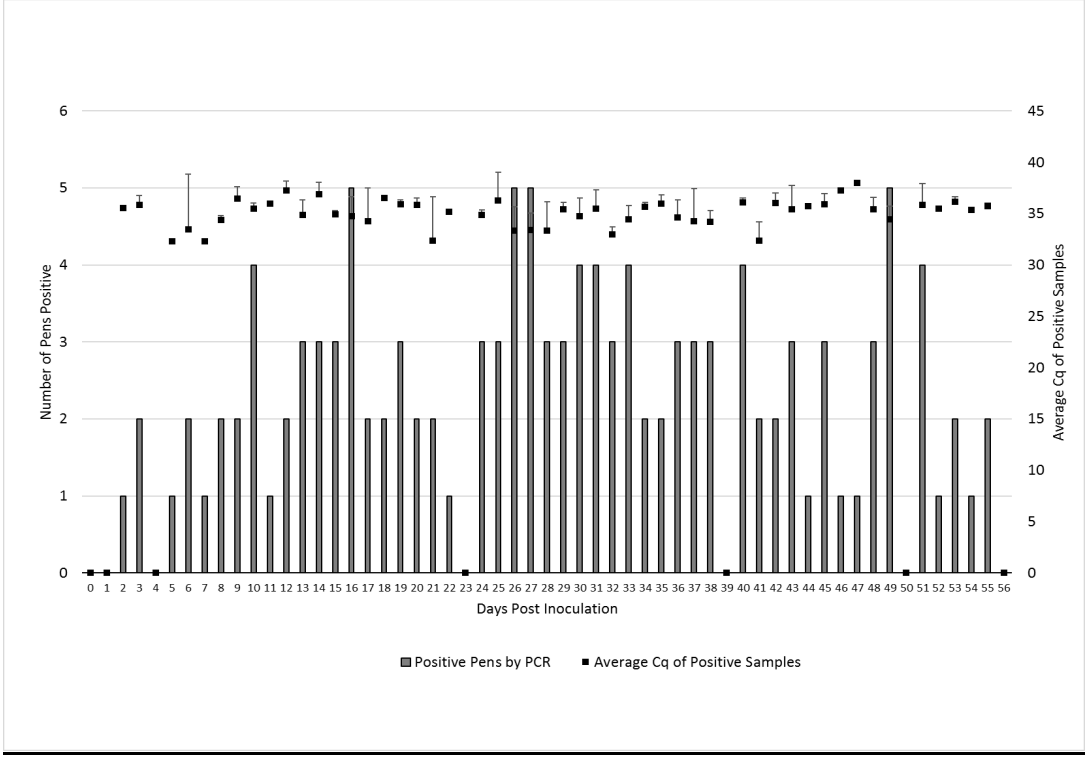


Figure 2. The number of pens positive by *Mycoplasma hyosynoviae* PCR and average Cq with standard deviation.

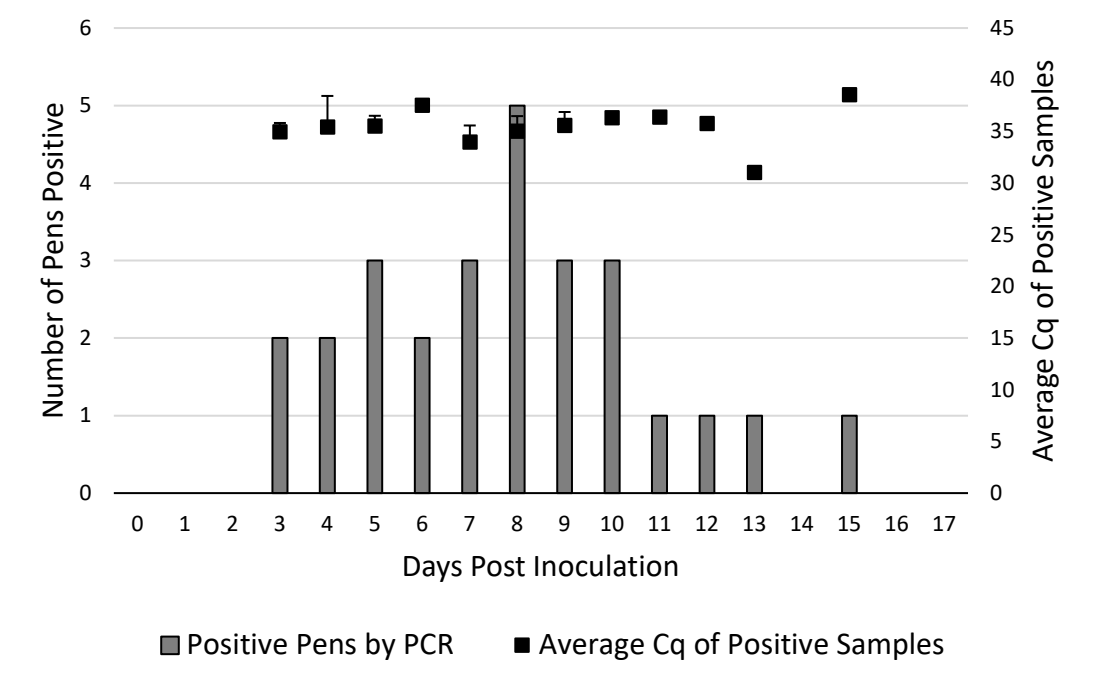


Figure 3. Fibrosing pleuritis (A), fibrinous arthritis (B, C), fibrosing serositis (D), fibrosing epicarditis (E), and fibrosing serositis (F) in *Mycoplasmahyorhinis*-inoculated animals.

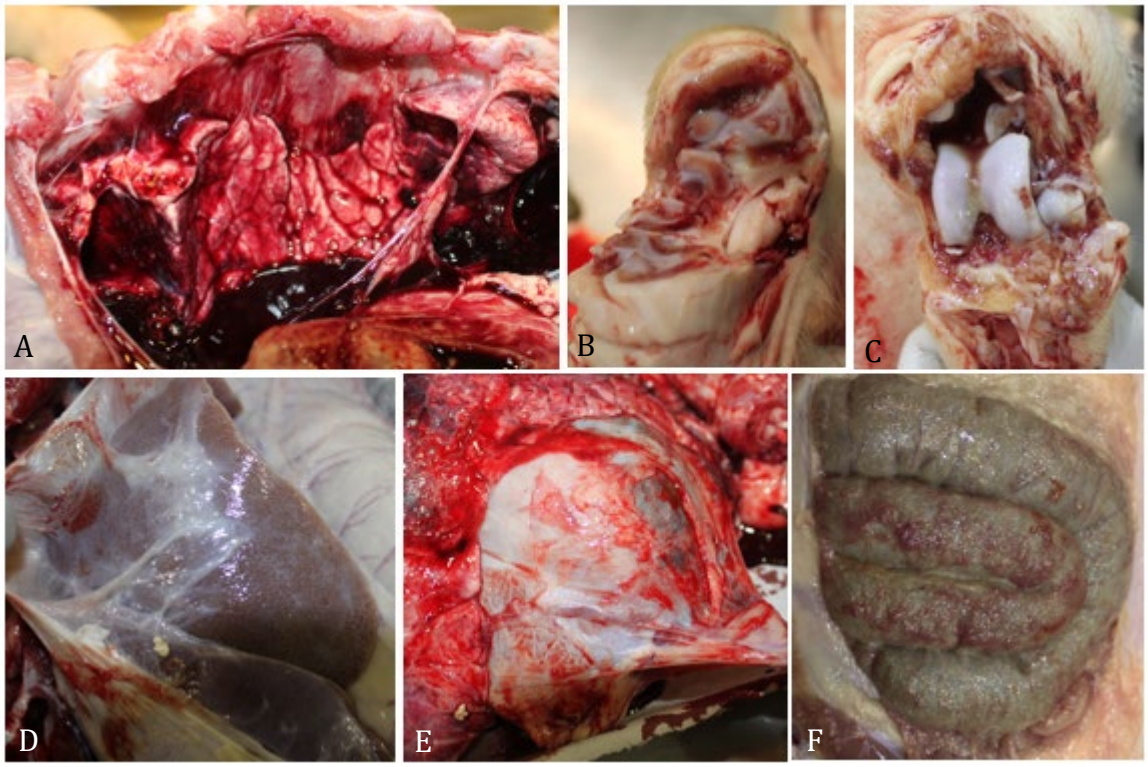


Figure 4. Serum IgG in animals inoculated with *Mycoplasma hyorhinis*.

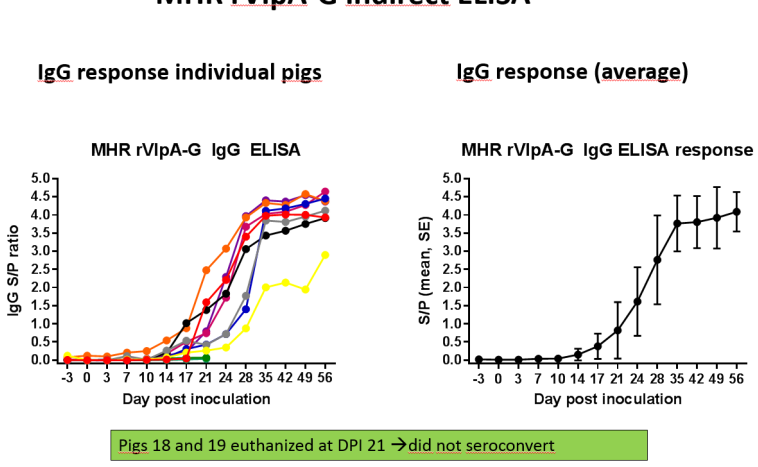
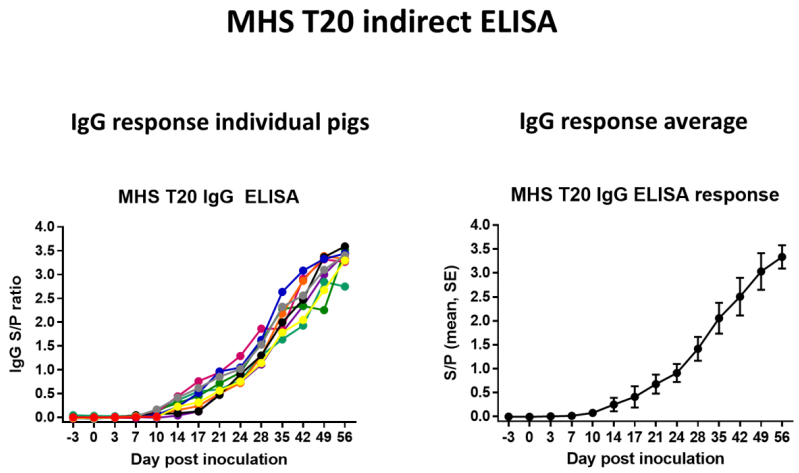


Figure 5. Serum IgG in animals inoculated with *Mycoplasma hyosynoviae*.



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