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# SDEC Partners Research Update

## Evaluation of the feasibility of *Mycoplasma hyopneumoniae* detection in processing fluids

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### Background

- The use of processing fluids (PF) to detect and monitor PRRSv and other pathogens is increasing among producers and veterinarians.
- Preliminary data from our research team identified *Mycoplasma hyopneumoniae* in PF at the litter level, using a species-specific real-time PCR, in a *M. hyopneumoniae* endemically infected farm.

### Objectives

- To investigate the detection of *M. hyopneumoniae* in non-respiratory tissues and fluids collected from suckling pigs at processing age.
- To develop an *in situ* hybridization (ISH) assay to further identify *M. hyopneumoniae* in non-respiratory tissues.

### Material and methods

- Freshly farrowed litters were sampled at two sow farms with previous detection of *M. hyopneumoniae* in PF.
- The following samples were obtained from:
  - Dams: Whole blood, serum, colostrum, whole placenta and vaginal swab.
  - Stillborn: Individually bagged and submitted for full diagnostics *M. hyopneumoniae* workup at the UMN-VDL. Whole blood was also collected during sampling.
  - Viable piglets: New born piglets were processed prior to suckling . Tails and testicles were collected individually per piglet and gender was recorded. Whole blood and laryngeal swabs were collected for all piglets.

  (PPE and sampling supplies were changed or disinfected between collection for each piglet)
- Daily aggregated PF were collected at a sow farm over a 10-week period
- A novel RNA-based ISH was developed using hybridization-coupled signal amplification system in histological tissue sections. To aid visualization of transcriptionally active bacterial organism expressing ribosomal and adhesin proteins.

**Results:**

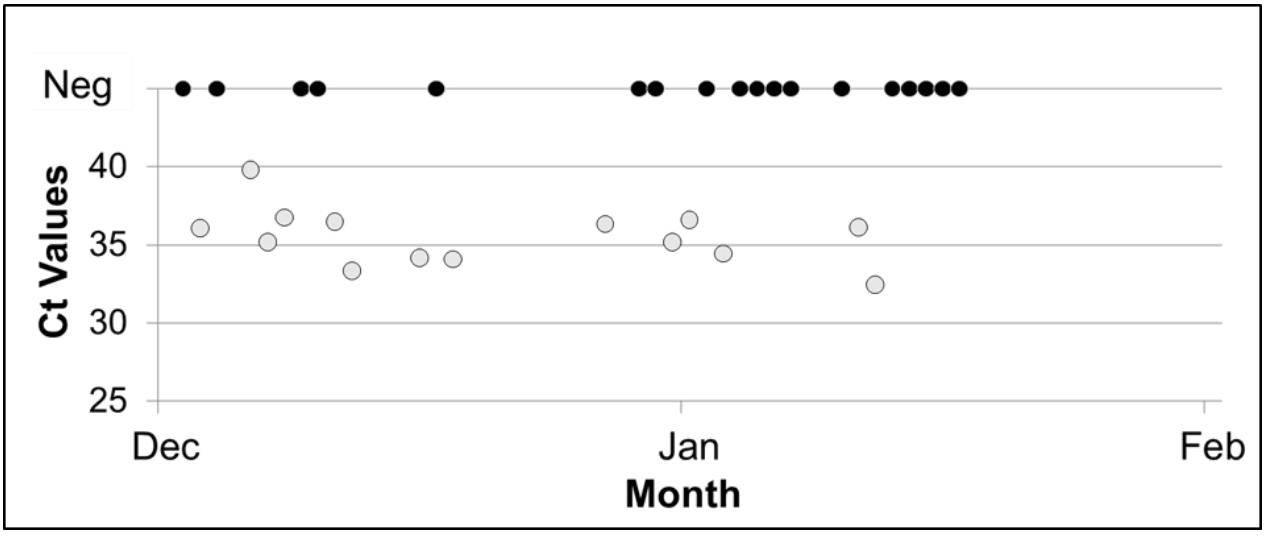
***Mycoplasma hyopneumoniae* detection in non-respiratory tissues or fluids**

- All dams tested negative for *M. hyopneumoniae* by RT-PCR in blood, serum, colostrum, placenta, and vaginal swabs
- Fifty percent of dams were seropositive by Oxoid™ *Mycoplasma hyopneumoniae* ELISA.
- All blood samples from stillborn and piglets resulted negative to *M. hyopneumoniae* by RT- PCR.
- *Mycoplasma hyopneumoniae* was detected in 2/54 individual fluid samples (tails and testicles). *M. hyopneumoniae* was detected (Ct<40) over the 10-week period by RT-PCR (Figure 1).
- PF and their associated testicles were collected individually at the litter level. All PF were tested by *M. hyopneumoniae* by RT-PCR. Samples were fixed in formalin to perform ISH on positive samples.

**Development of an *In situ* hybridization assay**

- The ISH-RNA technique established the distribution of *M. hyopneumoniae* in affected tissues in association with histological lesions, characterized by lymphoplasmocytic peribronchiolitis and/or hyperplasia of the bronchoassociated lymphoid tissues.
- In *M. hyopneumoniae* positive lungs, hybridization signals were observed in the apical membrane of the respiratory epithelium of bronchi and bronchioles.
- Positive signals were also observed in inflammatory cells and degenerative epithelial cells within the bronchial and bronchiolar lumen.
- The ISH-RNA technique provided molecular detection of *M. hyopneumoniae* cells expressing mRNA of proteins and elucidated the localization patterns by visualization in tissue.

**Figure 1.** *Mycoplasma hyopneumoniae* RT-PCR results of the daily aggregated processing fluids (PF). Cycle threshold (Ct) values <40 and ≥40 are represented in grey and black, respectively.




**Conclusions and Implications**

- *Mycoplasma hyopneumoniae* was detected intermittently in aggregated PF.
- In this investigation, *M. hyopneumoniae* was not detected in piglet tissues or samples, regardless of *M. hyopneumoniae* detection in aggregated PF.
- Regardless of the fact that environmental contamination can not be ruled out, aggregated PF could be a good indicator of *M. hyopneumoniae* at farm level.
- A specific *In situ* hybridization assay for *M. hyopneumoniae* was developed, which will be applied to non-respiratory piglet tissue samples.