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SDEC Partners Research Update

Project Update: Effect of sample pre-treatment on *Mycoplasma hyopneumoniae* detection in oral fluids

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Background

- Oral fluids are commonly used to monitor swine pathogens on a pen basis
- Detection of *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) in oral fluids has low sensitivity, especially during the very early stages of infection
- It has been suggested that the oral fluid matrix proposes a challenge towards pathogen detection by partially inhibiting DNA/RNA extraction and PCR reactions

Objective

To determine the effect of thawing, sonication, or holding temperature, on the detection of *M. hyopneumoniae* in oral fluids from experimentally and naturally infected pigs

Material and methods

Pre-treatments in samples from experimentally infected pigs:

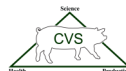
- Oral fluid samples collected from *M. hyopneumoniae* experimentally infected pigs (EIP) at 5, 9, 14, 21, and 28 days post-infection (DPI) were aliquoted into 17 sub-samples and pretreated as depicted in Fig. 1.
- Briefly, each sub-sample was either thawed at 4°C or 25°C for 2.5 h, sonicated during 10 min at either 4°C or 25°C, and held at 4°C or 25°C for 2 h
- Results from this testing were used to identify the two pre-treatments with the strongest influence on PCR detection.

Pretreatments in samples from naturally infected pigs:

- Oral fluid samples from *M. hyopneumoniae* naturally infected pigs (NIP) were obtained from the University of Minnesota Veterinary Diagnostic Laboratory.
- Samples were aliquoted into 4 sub-samples and pretreated by either thawing at 4°C or 25°C for 2.5 h, or holding at 4°C or 25°C for 3 h.
- All pretreated sub-samples were processed for DNA extraction using MagMAX[®] and *M. hyopneumoniae* real-time PCR using VetMAX[®] (Life Technologies).



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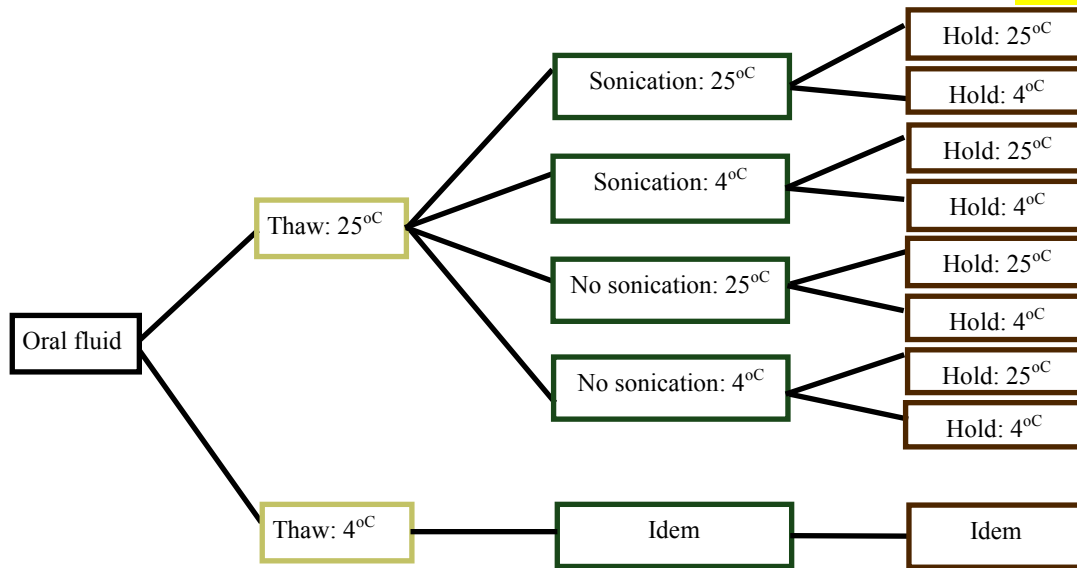


Figure 1. Experimental set-up for pre-treatment application (3 pre-treatments) for each of the 17 subsamples.

Results:

- Samples collected at 5, 14, and 21 DPI for EIP tested negative for *M. hyopneumoniae*.
- In a proportion of oral fluids collected from EIP with high Ct values ($\geq 35 \leq 38$), *M. hyopneumoniae* detection was numerically lower (higher Ct values) when samples were thawed at 25°C (Table 1).
- The use of sonication, and sonication and holding temperature did not seem to affect *M. hyopneumoniae* detection in oral fluid samples.
- The implementation of pre-treatments (thaw and holding temperature at 25 or 4°C) on samples from NIP did not seem to improve *M. hyopneumoniae* detection compared to each sample original Ct value (Wilcoxon signed-rank test; p value= 0.3-0.9).

Table 1. Effect of thawing temperature (4 or 25°C) on *M. hyopneumoniae* detection in oral fluids from experimentally infected

	5 DPI		9 DPI		14 DPI			21 DPI		28 DPI		
Thaw: 4°C	-	-	37.8	-	-	-	-	-	-	35.6	39.2	39.2
Thaw: 25°C	-	-	38.3	-	-	-	-	-	-	-	-	39.2
Δ Ct value	-	-	0.51	-	-	-	-	-	-	5.4	1.8	0

* - = *M. hyopneumoniae* was undetected by real-time PCR. pigs (mean Ct values).

Conclusions and Implications:

- Under the conditions of this investigation, the use of pre-treatments in oral fluids did not affect *M. hyopneumoniae* detection significantly, either in experimentally or naturally infected pigs. However, the number of *M. hyopneumoniae* positive oral fluid samples from EIP used in this investigation was small.
- The effect of pre-treatments on *M. hyopneumoniae* detection was not consistent across samples.
- Exploring the use of pre-treatments designed to purify oral fluid samples should be considered to increase *M. hyopneumoniae* detection in oral fluids.