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Project Update: Detection of *Mycoplasma hyopneumoniae* using flocked and bud swabs

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Background

- *Mycoplasma hyopneumoniae* is the causative agent of enzootic pneumonia, a chronic respiratory condition in pigs.
- Sterile swabs are used to collect clinical samples from the pig's respiratory tract.
- Research studies have shown that the sensitivity of respiratory pathogens detection can vary depending on the type of swab used for sample collection.
- The effect of swab type on *M. hyopneumoniae* detection has not been evaluated to date.

Objective

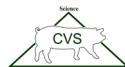
To compare two types of commercial swabs for *M. hyopneumoniae* detection by real-time PCR

Material and methods

- In order to compare swab absorption, 10 nylon-flocked and 10 rayon-bud swabs were individually weighted and dipped into one matrix, either 200 μ L of PBS or 0.600g \pm 0.002g of lubricant (as a surrogate for respiratory secretions). Swabs were dipped in the matrix and forty comparisons were made, including 10 repeats for each combination (type of swab and matrix).
- The detection of *M. hyopneumoniae* by real-time PCR was compared using tracheal and bronchial mucous from *M. hyopneumoniae* negative pigs mixed with *M. hyopneumoniae* strain AP414 at a final concentration of 650 ng/ μ L, equally distributed into 40 aliquots. In each tube, one nylon-flocked and one rayon-bud swab were dipped alternating the dipping order. Twenty repeats per swab type and dipping order were performed (n=80). DNA was extracted and samples run by PCR individually in duplicate.
- Paired t-test was used to compare absorption of PBS or lubricant based on swab type, and a linear mixed model was performed to assess differences in Ct values between swab types.



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Results:

- The absorption of PBS and lubricant was significantly higher ($p < 0.05$) in nylon-flocked than in rayon-bud swabs (data not shown).
- Detection of *M. hyopneumoniae* by real-time PCR of each swab type is shown in Figure 1. Overall, the mean Ct value of *M. hyopneumoniae* detection in nylon-flocked and rayon-bud swabs was significantly different, and was significantly influenced by dipping order (25.9 ± 0.48 and 26.4 ± 0.78 for nylon-flocked swabs, and 27.6 ± 0.84 and 26.9 ± 0.57 for rayon-bud swabs dipped first and second, respectively).
- No differences in the percent of positive samples detected by each swab type were observed, which could be attributed to the bacterial load used for testing.

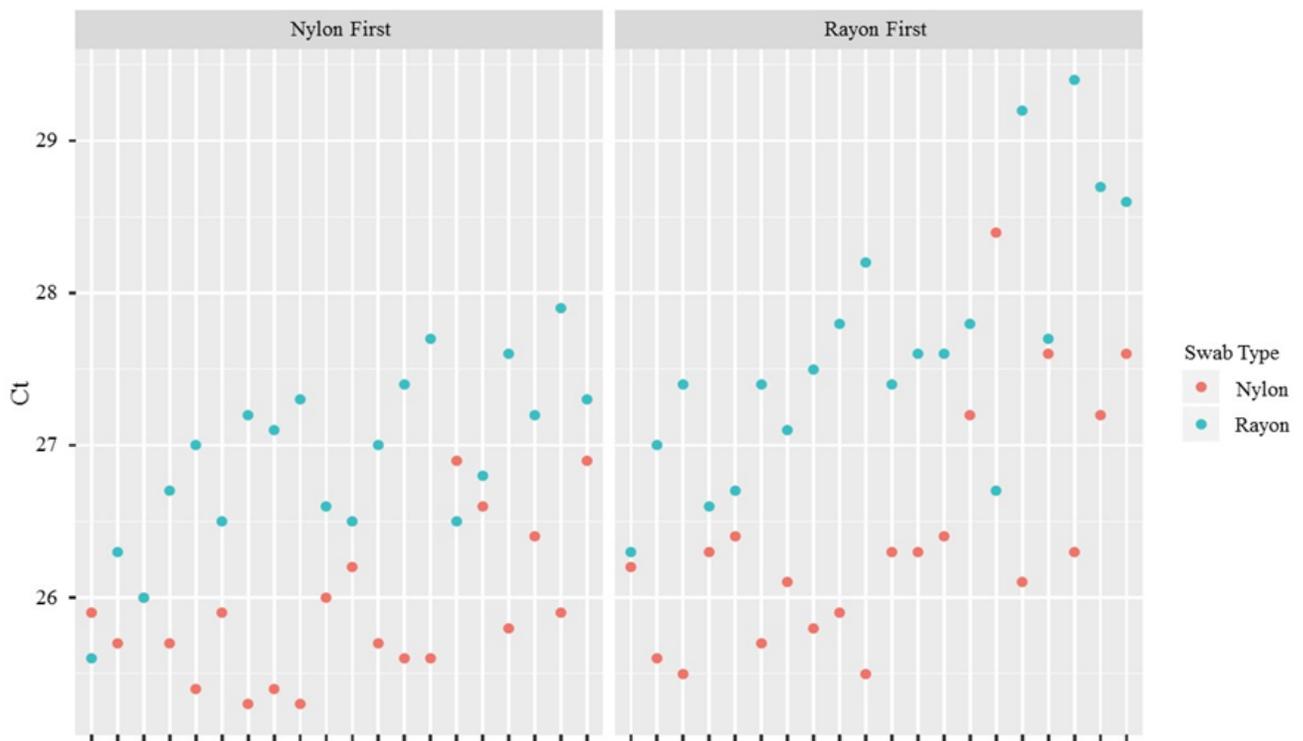


Figure 1. *Mycoplasma hyopneumoniae* detection by real-time PCR. Samples were tested based on dipping order into a bacterial culture (panels) and on swab type (nylon-flocked and rayon-bud). All results are shown as Ct values. Red dots: Nylon-flocked swabs. Blue dots: Rayon-bud swabs.

Conclusions and Implications:

- Nylon-flocked swabs are made of short fibers arranged perpendicularly, working as a brush, while rayon-bud swabs consist of a small wad wrapped around one end of a rod. It is speculated that the swab design may have been responsible for the differences observed in this study. Although the influence of the material cannot be ruled out.
- Absorption and detection of *M. hyopneumoniae* in nylon-flocked swabs was significantly higher, although mean Ct differences were only 0.5 to 1.7, which may not be of significant importance from the biological and diagnostic perspective. Nevertheless, it can be suggested that nylon-flocked swabs are used in cases of *M. hyopneumoniae* chronic infections, when bacterial loads may be low and therefore more difficult to detect.
- Overall, our results highlight the potential influence of the material and shape of the sampling device used for *M. hyopneumoniae* detection by PCR.