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Title: Minimizing sample cross-contamination for the detection of *Mycoplasma hyopneumoniae*

Introduction

Cross-contamination between samples is one of the most common pre-analytical errors and can cause misleading diagnostic results. Recent studies illustrating the issue of sample contamination emphasize DNA's significant resistance to degradation when on environmental surfaces, which is undoubtedly an important problem for PCR testing and posterior result interpretation.¹ Current sampling recommendations include measures that aim at minimizing specimen cross-contamination.² For example, materials used to collect tracheal secretion samples for the detection of *Mycoplasma hyopneumoniae*, including mouth speculums and laryngoscopes, should be disinfected between pigs. However, the disinfection method is not standardized, and although most commercially available disinfecting wipes may have bactericidal effects, it is currently unknown if they prevent DNA carryover between samples. Therefore, the objective of this study was to compare various disinfecting compounds in order to minimize sample cross-contamination in swine tracheal secretion collection.

Materials and Methods

Tracheal secretions were collected with various material sets from nursery pigs that had been experimentally inoculated with *M. hyopneumoniae* (n=36), for natural contamination. In parallel, 1:10 diluted *M. hyopneumoniae* culture was sprayed onto six separate material sets for artificial contamination. The sets included a mouth speculum, a laryngoscope blade, and a pair of scissors. Swabs were collected from each material. The material sets were thoroughly cleaned with one of the following treatment-based wipes: hydrogen peroxide, alkyl dimethyl benzyl ammonium chloride, bleach, alcohol, or phosphate-buffered saline (PBS). Following the disinfecting wipe, a PBS wipe was used to collect a post-cleansing sample from each material. The DNA was extracted from the pre-cleansing swabs, disinfecting wipes, and post-cleansing samples for both natural and artificial contamination, and a species-specific PCR was performed to detect *M. hyopneumoniae*. Ct values were compared with Kruskal-Wallis tests to determine statistical significance.

Results

Statistical difference between sampling materials was not achieved, with a p-value of 0.09 and 0.06 for the natural and artificial contamination respectively. Thus, data from various materials were grouped as replicates for each treatment group for this study. Starting Ct values were not statistically different between treatment groups for natural ($p = 0.50$) or artificial ($p = 0.79$) contamination. Of the naturally contaminated treatment groups, bleach and hydrogen peroxide were the only disinfectants to produce negative PCR results ($p = 0.04$). Bleach was the only treatment to generate negative PCR results for the artificially contaminated materials ($p=0.02$).

Discussion and Conclusions

Results of this investigation suggested that bleach was the disinfectant that successfully removed all *M. hyopneumoniae* genetic material. These results seemingly agree with other studies on different bacteria.^{3,4} These results can be further applied for the development of a protocol for minimizing or eliminating sample cross-contamination with the use of bleach.

References

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