Guidelines for sample selection from replacement gilts and growing pigs for successful *Mycoplasma hyopneumoniae* culture and isolation

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INTRODUCTION

Mycoplasma hyopnuemoniae (Mhp) has historically been challenging to culture from lung tissue samples of naturally infected swine. The most recent reported success rate for culture of Mhp was 8%. Many factors influence the success rate of culturing Mhp including the quality of the sample, presence of other bacteria in the sample and/or media, and laboratory growing conditions. Due to these obstacles, there was a need for work to determine best practices for successful culture of Mhp from field samples.

MATERIALS AND METHODS

This work was performed in 4 continuous flow growing pig barns identified as Mhp clinically active, confirmed by observation of clinical signs and positive PCR on lung tissue. Prior to sample collection, a pre-screening protocol was designed and implemented. **The first stage** of the protocol consisted of a cross sectional sampling of ≥ 10 tagged pigs per age group (≥ 3 age groups) for laryngeal swab collection, 3 – 5 weeks after the onset of Mhp clinical signs. Groups with results ≥ 70 % Mhp PCR positive were identified for further sampling. **The second stage** of the protocol consisted of selecting 2 – 4 Mhp PCR positive pigs with clinical signs including dry coughing and labored breathing, along with the lowest Mhp PCR Ct value in laryngeal swabs. Selected pigs were humanely euthanized and necropsied. Acute enzootic pneumonia like lesions were verified on gross lung tissue. The entire lung pluck (larynx to lungs) was collected and placed into clean bags. Bagged lungs were immediately placed on ice for transport to a freezer. Samples were placed in a - 20 °C freezer. Frozen samples were transported and delivered ≤ 24 hours post-collection to the Mycoplasma Lab, University of Minnesota. It is hypothesized that freezing aids microorganism detachment from tissue resulting in higher bacterial recovery.

RESULTS

Using the pre-screening protocol and methods described above, the authors have experienced a 100 % culture and isolation success rate on the first collection attempt at all 4 barns sampled.

CONCLUSION AND DISCUSSION

Under the conditions of this investigation, improvement the success rate of Mhp culture from lung tissue of naturally infected swine populations was achieved. This protocol can be used as guidelines for increased culture and isolation success. Utilization of the pre-screening protocol allowed for identification of the ideal specimen in terms of timing and degree of infection. This allowed the lab to process reliable samples with enough microbial load for culture and isolation of Mhp. Increasing the Mhp library of diagnostic and research laboratories will allow for expansion of research in Mhp diagnostics, characterization, antibiotic resistance and gilt acclimation procedures, among other research areas.

Figure 1: Laryngeal swab collection from naturally infected gilts during the First Stage of the Protocol.

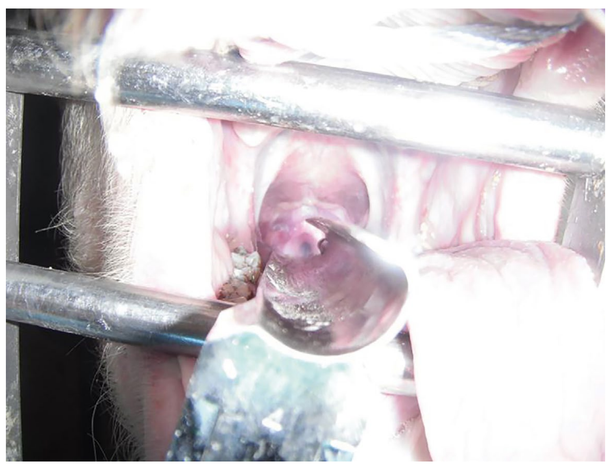


Figure 2: Selection of the final specimen during the Second Stage of the Protocol. Final selection criteria: Mhp PCR postive pig, clinical signs, the lowest PCR Ct value and acute enzootic pneumonia like lesions.

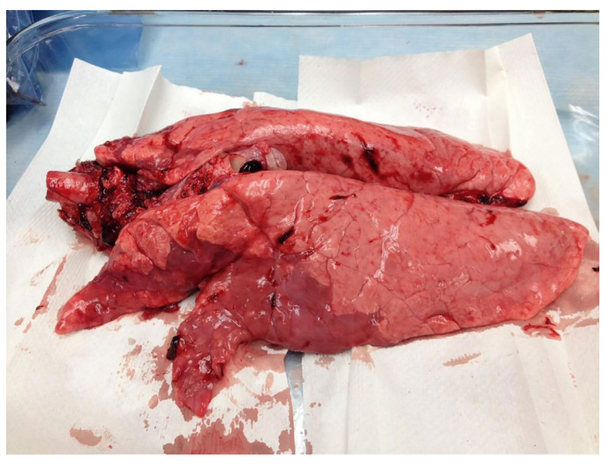
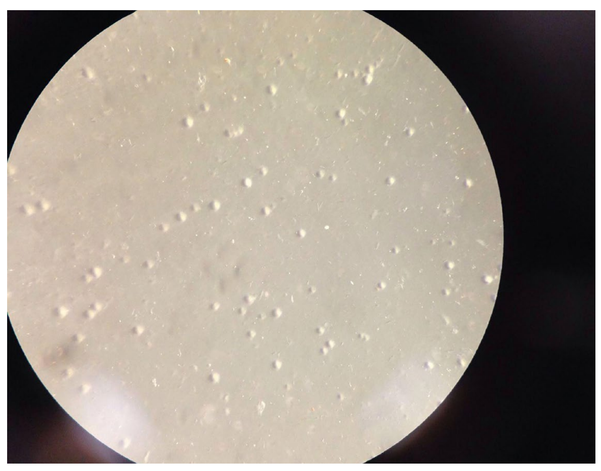


Figure 3: Mhp culture and growth in agar plate. Colonies observed after 4 weeks of incubation of one of the cases of this investigation.



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