RESEARCH ABSTRACT

SWINE HEALTH

Title: Optimization and viability of lung homogenate for successful *Mycoplasma hyopneumoniae* exposure in gilts during acclimation – NPB #19-120

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Scientific Abstract

Gilt acclimation has become a common practice in the United States swine industry to control infections caused by *M. hyopneumoniae*. The goal of gilt acclimation is to achieve uniform exposure of gilts prior to entry to the breeding herd, to create a "Day 0" for disease elimination purposes. Swine practitioners utilize lung homogenate (LH) containing *M. hyopneumoniae* for exposure, which has shown to be effective in a timely manner. However, the characterization, preparation, handling, and storage of the LH has not been tested experimentally, which poses a risk for failure of successful exposure. Therefore, the purpose of this study was to establish methods of LH preparation and storage to optimize the process and result in a successful exposure of gilts during acclimation.

Three aims were completed in this study including: 1) To evaluate the *M. hyopneumoniae* concentration in different anatomical lung sections of naturally infected pigs, 2) To evaluate the effect of the LH homogeneity on *M. hyopneumoniae* detection by real-time PCR, and 3) To evaluate the viability of *M. hyopneumoniae* in the LH under various storage and dilution conditions.

For aim one, a total of nine lung donor pigs were selected from three different farms, and categorized into the following Ct value groups: low < 24 Ct, medium 25-30 Ct, and high 31-39 Ct based on deep tracheal catheter (DTC) samples. Selected pigs were humanely euthanized, followed by observation of lung lesion scores and collection of bronchial swabs (BS) from each lung lobe. For each lobe, tissue was blended at a 70:30 proportion of tissue and Friis medium to create lung lobe-specific homogenate samples. Real-time PCR for *M. hyopneumoniae* detection was performed on BS and lung lobe homogenates (5 replicates each).

For aim two, lungs were collected from four donor pigs, and blended whole at a 70 tissue: 30 Friis ratio. The resulting LH was used to obtain 50:50 and 30:70 dilutions. All three LH dilutions were further diluted at a 1:10 ratio and treated as follows: i) No filtration, ii) Gross filtration using a nylon stocking and iii) Fine filtration (100 µm). Filtered and diluted products were tested for *M. hyopneumoniae* via real-time PCR (5 replicates each).

For aim three, whole lungs were obtained from two *M. hyopneumoniae*-positive donors and blended separately. For each donor lung, two preparations were created including a 70:30 tissue:Friis medium dilution and a 70:30 tissue:saline dilution. From those dilutions, four dilution aliquots were created and stored in different conditions: 1) Fresh at 25ºC, 2) 1 week frozen at -20ºC, 3) 1 month frozen at -20ºC and 4) 6 months frozen at -20ºC. There were four challenge groups based on the length of storage, with eight gilts each, resulting in 32 challenged gilts. For each challenge group, two gilts were randomly assigned a treatment of either LH prepared with saline or medium from donor pig one or two resulting in four gilts for both saline and medium. For all challenge groups, DTC and sera were collected at the start of the trial and four weeks post-inoculation. At necropsy, lung lesions were evaluated, and a BS was collected.

The results obtained from aim one suggested that selecting a pig with a low DTC Ct value provided a relatively consistent load of *M. hyopneumoniae* throughout the whole lung, as well as a higher bacterial load, compared to gilts in the medium and high DTC Ct groups. In addition, lung lesion scores were not a suitable method for selecting a donor gilt, as *M. hyopneumoniae* was detected in lobes without any lung lesions and *vice versa*. Results from aim two indicated that dilution and filtration of the lung homogenate generated conflicting results, without a clear indication of their effect on *M. hyopneumoniae* detection by real-time PCR. Lastly, aim three results showed that both solutions, Friis medium and saline, were adequate for dilution of LH, and the capability of the LH to induce disease appeared to be slightly lower after six months of storage, compared to homogenate frozen for less than or equal to one month. Overall, conclusions drawn from this pilot study help optimize the preparation of LH to result in successful gilt exposure for *M. hyopneumoniae* acclimation.