

# Effect of Joint Sampling Technique on Bacterial Load in Synovial Fluid Samples of Swine in a Commercial Setting

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### Summary and Implications

In this trial sampling technique did impact levels of bacterial contamination present in synovial fluid. The differences in contamination levels were highly variable between sampling techniques, ranging from no bacteria present to bacterial concentrations of  $10^6$ . Needle aspiration proceeded by skinning of the joint resulted in the lowest level of contamination in samples indicating the skin is a major source of contamination and the use of needle aspiration over swab collection significantly reduces contamination levels.

### Introduction

Sampling joints is an important diagnostic tool for the detection of infectious agents present in synovial fluid. There are several methods utilized in the field to collect synovial fluid, but research to compare these sampling protocols is lacking. One parameter for comparing these procedures is analysis of bacterial contamination associated with each collection technique. High levels of bacterial contamination compromise synovial fluid diagnostics by lowering the quality of results practitioners receive from testing. Diagnostic testing has costs in terms of laboratory fees, animals sacrificed, practitioner time, packaging and delivery. Submitting a quality sample is necessary to provide the best service and value to swine producers (Schwartz, 2001). The objective of this study was to evaluate joint sampling techniques conducted in a farm environment for levels of bacterial contamination in synovial fluid.

### Materials and Methods

**Preliminary Evaluation:** A preliminary evaluation was conducted to refine an appropriate quantitative approach to measuring contamination. The preliminary study evaluated typical standard plate counts and semi-quantitative plating

on samples obtained from two swabbing techniques, one preceded by skinning of the joint and the other without. These techniques were performed on opposite hock joints of five late-nursery aged pigs.

**Primary Evaluation:** In the primary study, a comparison of three joint sampling techniques commonly used in the field was conducted. These techniques compared open joint swabbing vs. needle aspiration and skinned joints vs. non-skinned joints. In every comparison, two techniques were compared at one time by sampling opposite hock joints of the same animal at necropsy. This was replicated for ten animals per comparison in the first round and sixteen animals per comparison in the second round. Samples were collected by the same individual in all evaluations to maintain consistency in sampling technique and techniques were randomly assigned to the left or right hock. The technique resulting in the least contamination in each paired comparison was then compared to the next sampling method. This elimination procedure continued until the least contaminated technique was identified.

To collect the samples, Pigs were placed in dorsal recumbency with the front and rear legs reflected for collection. Sterile gloves and scalpels were used for all procedures. In techniques involving swab collection, the joint was flexed and extended while palpated to properly identify the joint space. With the joint flexed, an incision was made through the skin and just into the joint capsule. Torque was then used to open the joint enough to pass a sterile swab. In needle aspiration techniques, the joint space was again identified and a 16 gauge needle was advanced into the joint space. A 12 mL syringe was drawn back to collect the sample. In skinning procedures, a slit was made through the skin along midline, but not directly over the joint, and the leg was skinned past the hock.

**Laboratory Analysis:** Samples were refrigerated overnight for next day processing at the Iowa State University Veterinary Diagnostic Laboratory. In the lab, samples were vortexed on a setting of eight for 1 minute to homogenize the sample. After vortexing, sterile gloves were worn to wring out the swab by forcefully rotating it against the side of the test tube allowing more sample to leave the swab. Samples were analyzed by standard plate counts using serial dilutions of  $10^{-2}$  through  $10^{-6}$ . Plating was performed by the same individual who was blinded to the treatments in all evaluations.

**Image 1. Swab collection without skinning and corresponding standard plate count.**



**Image 2. Needle aspiration with skinning of the joint and corresponding standard plate count.**



**Statistical Analysis:** Due to the large range in bacterial contamination levels present a log transformation of the data was performed prior to statistical analysis. The data for each round of comparison was then analyzed using a paired T-test for two sample means.

### Results and Discussion

The preliminary evaluation suggested contamination of synovial fluid samples occurs with high variation and standard plate counts more precisely quantified differences than the semi-quantitative analysis.

The first round of the primary study evaluated needle aspiration preceded by skinning of the joint versus open joint swab collection without skinning of the joint. The mean bacterial count for needle aspirated samples was  $6.00 \times 10^1$  with a range of 0 to  $2.00 \times 10^2$ . The mean bacterial count for swab collection was  $5.58 \times 10^5$  with a range of  $4.37 \times 10^3$  to  $3.53 \times 10^6$ . Needle aspiration preceded by skinning of the joint resulted in statistically significant lower bacterial contamination levels ( $P < 0.001$ ). The second round of the primary study evaluated needle aspiration and swab collection both preceded by skinning of the joint. The mean bacterial count for needle aspirated samples was  $1.30 \times 10^3$  with a range from 0 to  $1.71 \times 10^4$ . The mean bacterial count for swab collection was  $2.25 \times 10^4$  with a range from 0 to  $2.83 \times 10^5$  in contamination levels. This difference was found to be statistically significant ( $P = 0.019$ ).

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