RESEARCH ABSTRACT

SWINE HEALTH

Title: Culture-independent analysis of microbial communities in tonsils of healthy, carrier, and diseased pigs - **NPB #09-072**

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Date Submitted: January 31, 2012

Scientific Abstract:

Porcine tonsils are the colonization site for many pathogenic as well as commensal microorganisms and are the primary lymphoid tissue encountered by organisms entering through the mouth or nares. Pathogens such as *Actinobacillus pleuropneumoniae, A. suis, Haemophilus parasuis, Pasteurella multocida, Mycoplasma hyopneumoniae*, and *Streptococcus suis*, some of the most important causes of bacterial pneumonia, septicemia, polyarthritis, and meningitis, are carried asymptomatically in the tonsils and nasopharynx of pigs. While vaccines that protect against serious disease caused by some of these pathogens have been developed, these vaccines generally do not prevent acquisition of the organism and carriage in the upper respiratory tract. Carriage can result not only in perpetuating the reservoir of the pathogen, but also in reduced growth efficiency, which can in turn lead to the use of antibiotics in feed as growth promoters. To further reduce or eliminate disease caused by these pathogens, and to reduce the use of antibiotics as growth promoters, there is a critical need to understand how the carrier state is established and maintained and to determine effective management strategies to reduce carriage of pathogens in the upper respiratory tract.

The objective of this project was to analyze the microbial communities in tonsils from healthy pigs and compare these to the communities found in animals from herds experiencing disease problems due to specific pathogens. To accomplish this, we first characterized the composition and structure of the microbial communities in the tonsils of healthy pigs. Whole tonsils were collected at necropsy from twelve 16-week-old finisher pigs from two healthy herds. Tonsil brushes designed for this project were also used to collect samples from four of these animals. Bacterial DNA was isolated from each sample, amplified by PCR with universal primers specific for the bacterial 16S rRNA genes, and the PCR products sequenced using high throughput bar-coded 454-FLX pyrosequencing. An average of 13,000 sequences were generated from each sample. Microbial community members were identified by sequence comparison to known bacterial 16S rRNA gene sequences.

The microbiomes of these healthy herds showed very strong similarities in the major components as well as distinct differences in minor components. *Pasteurellaceae* dominated the tonsillar microbiome in all animals, comprising 60% of the total, although the relative proportions of the genera *Actinobacillus, Haemophilus,* and *Pasteurella* varied between the herds. Also found in all animals were the genera *Alkanindiges, Peptostreptococcus, Veillonella, Streptococcus* and *Fusobacterium*, as well as *Enterobacteriaceae* and *Neisseriaceae*, which comprise the “core microbiome” of porcine tonsils. Tonsil brushes yielded similar results to tissue specimens, validating the use of this non-invasive technique for future studies.

To compare the microbial communities found in healthy pigs to those found in pigs that are carriers of specific porcine pathogens, we collected tonsil brush specimens from 164 pigs from 12 herds, including two high health status herds and 10 herds with acute or chronic problems with *Actinobacillus pleuropneumoniae, Streptococcus suis, Salmonella, Haemophilus parasuis,* or mixed pathogens. Community bacterial DNA was isolated from 41 samples and controls and the 16S rRNA genes amplified and sequenced using Titanium pyrosequencing. An average of 2500 reads per sample and 15,000 reads per herd were generated and analyzed. Our working hypothesis, that the composition of the communities in the tonsils would differ between healthy and infected herds, was supported by the results. For example, there were increased numbers of *Streptococcus* in the tonsils of the pigs from a herd with chronic problems with *Streptococcus suis* (more than double the usual amount in healthy pigs) and increased *Enterobacteriaceae* in the tonsils of pigs known to shed *Salmonella*, but not in the tonsils of non-shedders from the same herd. However, in some herds, use of antibiotics also altered the tonsil microbiota, and this could not always be separated from effects of carriage of a pathogen. Further studies examining the effect of antibiotics on the tonsillar communities in healthy pigs, and on subsequent susceptibility to colonization by pathogens and development of disease, would likely clarify these results.