Microbiome of the Tonsils of the Soft Palate of Swine – Possible Roles in Health and Disease

Janet MacInnes1*, Shaun Kernaghan1, Durda Slavic2, Shu Chen1, and Zvonimir Poljak3
1Department of Pathobiology, 2Animal Health Laboratory, 3Department of Population Medicine, University of Guelph, Guelph, Ontario, N1G2W1 *macinnes@uoguelph.ca

Background
The last decade has seen a tremendous increase in the use of culture-independent techniques to characterize microbes present in humans and animals. Much of this work is concerned with the role that these microbial communities play in health and disease. For example, it has been shown that microbiome members can provide protection against infection through competitive exclusion or can contribute to disease by providing nutrients to pathogens. In pigs, the role of microbiomes in nutrition has been studied, but complex bacterial communities in the upper respiratory tract are only beginning to be investigated. In addition to harbouring a large number of poorly-characterized commensal organisms, the tonsil of the soft palate in swine is known to be the reservoir of many primary and opportunistic pathogens, but much remains to be learned about interactions at this site.

Materials and methods
Swabs and tonsillar samples were obtained from unfit animals in closeout groups and from healthy pigs at slaughter. Routine microbiological analysis was performed and both tissue and culture samples were characterized by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis using the Phusion® Bacterial Profiling kit from Finnzymes. The distribution of T-RFLP fragments in healthy and unfit pigs was compared and tentative identifications were made by searching the Finnzymes, RDP, and a custom “pig-specific” database. Cluster analysis of the OTU data of healthy and unfit pigs was done to see if there was an association with 13 clinical signs or with the presence of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type2 (PCV2), or Mycoplasma hyopneumoniae infection.

Results
When the data were analyzed as anonymous OTUs, greater diversity was seen in the microbiomes of unfit vs. healthy animals. Comparison of the T-RFLP results with clinical data revealed significant associations with the presence of anemia, abscess, PRRS virus, and Mycoplasma. For example, a statistically significant association was seen between abscess and membership in clusters II and IX while membership in clusters III, VI, and X was positively associated with the presence of PRRSV. Putative identifications of the T-RFLP bands were done using several databases. At the phylum level, the microbiomes of unfit pigs were more likely than those of healthy animals to contain Actinobacteria and Fusobacteria and less likely to have Firmicutes and Spirochaetes. At the genus level, Streptococcus sp., Flavobacteria sp., Clostridia sp., Lactobacillus sp., Bacillus sp., and Fusobacterium sp. were present in more than 50% of both healthy and unfit animals although Streptococcus sp. was more frequently found in healthy than unfit animals (94.4 vs. 69.2%, respectively).

Implications
This work provides information about the bacterial communities present in diseased and healthy pigs and allows us to begin to identify organisms that might promote pig health or exacerbate disease.

Acknowledgements
This work was supported by the OMAFRA Animal Health Strategic Investment program and the Natural Sciences and Engineering Research Council.