RESEARCH ABSTRACT
SWINE HEALTH

Title: Characterization of the genetic diversity of *Mycoplasma hyorhinis* field isolates by multiple locus variable number of tandem repeats analysis (MLVA) and multi-locus sequence typing (MLST) - **NPB #12-044**

Investigator: Albert Rovira

Institution: University of Minnesota

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Scientific Abstract:

M. hyorhinis associated disease has been one of the main concerns of the U.S pork industry. It appears that differences in virulence of the infecting *M. hyorhinis* strain, the host immune response, and concomitant infections may play a role on disease manifestation. There are currently no genotyping tools available for the characterization *M. hyorhinis* isolates circulating amongst swine populations. The molecular typing of *M. hyorhinis* would aid in better understanding transmission routes, in assessing sources of infection and also in evaluating interventions such as vaccination and use of antibiotics. The objective of this study was **to develop and validate a multi-locus sequence typing (MLST) protocol for the characterization of *M. hyorhinis* field isolates.** Thirty-nine *M. hyorhinis* field isolates together with one reference ATCC strain were utilized. The genome sequences of four *M. hyorhinis* isolates were utilized to identify potential target genes. Primers were designed with MEGA 5. PCR was carried out and agarose gel electrophoresis was performed on the amplified products. PCR products were bidirectionally sequenced by standard Sanger sequencing. Quality of the generated sequencing data was evaluated and sequences were aligned utilizing ClustalW and trimmed to equal sizes. Phylogenetic analysis was carried out using MEGA 5.2.1. A total of 25 genes were evaluated as potential target genes. Genes where discarded when the sequence of all 4 genomes where identical, when primers could not be designed due to high variability of the sequences, when no PCR amplification product was obtained or had a poor reproducibility. Finally, a total of 5 target genes were included in the MLST protocol: *ung*, *pdhB*, *mtlD*, *p3*, and *p95*. Within each gene the percent informative sites ranged from 0.5% to 20%. The number of alleles per gene varied from 3-11, giving rise to 27 sequence types (STs) within the 39 isolates. Two major lineages where observed. The concatenated tree showed clustering of isolates by system.