

## SWINE HEALTH

**Title:** Prospective investigation of *Streptococcus suis* isolates from diseased pigs and healthy pen-matched controls in outbreaks of *Streptococcus*-associated neurologic disease using metagenomics, serotyping and sequencing. #17-117

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### Scientific Abstract:

The impact of *Streptococcus suis*-associated neurologic disease has substantially increased since first described and has emerged as a common and serious clinical challenge. The goals of this project were to identify the presence of virulence factors, genes or markers in isolates derived from diseased versus healthy pigs; determine if these 'markers' can be used to predict virulence; and to investigate the immunopathogenesis of *S. suis*-associated neurologic disease using a well-defined, aseptically collected set of samples. Over 60 crossbred, commercially raised pigs from multiple herds with a history of *S. suis*-associated neurologic disease were necropsied. Four to seven live pigs (affected and pen- or litter-matched controls) from each outbreak were necropsied with two to six pigs meeting the case definition. Aseptically collected samples submitted for bacterial culture included a meningeal swab, bronchial lavage, tonsil scrape, nasal swab, small intestine, large intestine, cerebrospinal fluid as well as swabs of any grossly visible serofibrinous exudates in joints or on serosal surfaces. Serotyping was performed per the Iowa State University Veterinary Diagnostic Laboratory protocol. The total number of *S. suis* isolates obtained and saved was 324 of which 155 isolates originated from pen-matched controls and 169 isolates were recovered from pigs with bacterial meningitis. These isolates were serotyped by PCR-based serotyping and traditional co-agglutination tests. The most common serotypes identified in descending order were 10, 21, 7, 1 and 4. The serotype that was identified as causing meningitis in a majority of the cases was serotype 1, followed by, in descending order, serotypes 2 or 1/2, 10, 4, 5, 11 and 14. Over 20% of the pigs had *S. suis* isolated from the alimentary tract and the enteric system of three animals was colonized by same strain causing disease in the herd and/or the individual. The majority of *S. suis* isolates originated from the tonsil and nasal cavity, making up 39% and 37% of the isolates, respectively. One hundred and twenty-five isolates were screened for 3 virulence-associated genes (*mrp*, *epf* and *sly*) and only serotypes 1 and 14 were positive for all three genes. Fifty-seven *S. suis* isolates (both from diseased and non-diseased pigs) obtained during this study as well as 13 *S. suis* isolates from pigs with histologic evidence of meningitis submitted to the ISU VDL were sequenced and compared to identify virulence factors, markers, and genes. Based on the results of this study, *S. suis* isolates of the same serotype originating from systemic sites with bacterial lesions and the nasal/tonsil of diseased pigs and healthy carriers in a single diagnostic case submission are consistently the same strain when

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characterized by whole genome sequence. Accordingly, herds can be screened by nasal and/or tonsil swab to identify carriage once a virulent strain has been identified from aseptically collected samples from either the joint or brain. Litter-matched healthy piglets were commonly colonized by the same *S. suis* strain causing disease further supporting the sow as an early source of *S. suis* colonization. These findings also further confirm that the site of isolation can be a confounding factor when cataloguing isolates and trying to discern virulence factors as commensal or pathogenic as virulent strains can be found in the tonsil and nasal cavity of healthy carriers. In the future, the use of genotyping tools in addition to serotyping could aid the control of *S. suis* disease in swine herds and expand the knowledge of critical factors involved in the epidemiology and pathogenesis of infections by *S. suis*. Given the large genome size and extensive genome variability between *S. suis* strains as well as the lack of circularized *S. suis* genomes and lack of critical clinicopathologic information of *S. suis* strains, identification of virulence 'markers' using genetic information of *S. suis*, if at all possible, is going to necessitate innovative approaches that may entail machine learning in combination with a large very well-characterized set of isolates.