Suilysin (SLY) is an indicator of *Streptococcus suis* derived capsule 1 pathogenesis only

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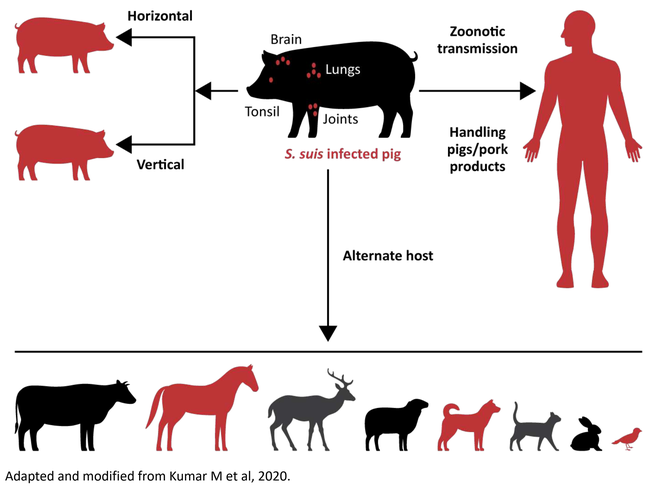
INTRODUCTION

Streptococcus suis is an important swine pathogen with zoonotic potential (Figure 1) causing severe economic hardship to the US swine industry. This pathogen possesses a protective outer coat composed of polysaccharides known as capsular polysaccharide (CPS). There are 35 CPS types based on serum agglutination test. *S. suis* can be found in the nasal and tonsillar cavities of clinically normal pigs as well. Among the systemic infection causing *S. suis* types in pigs, serotypes 1, 2 and 1/2 are the most common ones in the USA. These three serotypes account for at least 50% of the infections resulting in septicemia, arthritis, meningitis mostly in pigs, 2-6 weeks. There are hundreds of virulence and virulence-associated genes described in the literature. The most studied ones with known functions are suilysin (SLY), muramidase-released protein (MRP), and extracellular factor (EF). Capsular polysaccharides (*cps*) genes are critical for immune evasion; however, antibodies directed against CPS offer protective immunity. The correlation between other virulence genes and pathogenesis is sketchy at the best.

This study was done with the objective of understanding the degree of association of SLY, MRP and EF proteins among pathogenic and commensal serotypes 1, 2 and 1/2 isolated from clinically diseased pigs or obtained from nasal or lung swabs during necropsy. High throughput sequencing (HTS) was used to generate the whole genome nucleotide sequence data and amino acid sequence derived to obtain structural genes. The Derived Capsule (DC) locus was assigned based on curated genomic sequence data using specialized software.

Figure: 1 Transmission dynamics of *S. suis*



Adapted and modified from Kumar M et al, 2020.

RESULTS

Among the systemic DC1 isolates, 99% had functional SLY and 80-90% expressed MRP and EF (Table 1). However, within the commensal DC1 types only 42% expressed SLY followed by MRP and EF. In systemic DC2 types, 60-70% expressed all 3 proteins. Only 18-50% commensal DC2 expressed all 3 genes. Among the DC 1/2 types, commensal express (60%) SLY more than systemic isolates (12%).

Table:1 Distribution of MRP, EF and SLY genes among three commonly isolated *S. suis* serotypes in the USA

	SYSTEMIC			COMMENSAL		
DC	**MRP**	**EF**	**Suilysin**	**MRP**	**EF**	**Suilysin**
DC 1	87 %	91 %	99 %	58 %	29 %	43 %
DC 2	72 %	62 %	62 %	55 %	18 %	18 %
DC 1/2	67 %	9 %	13 %	30 %	20 %	60 %

MATERIALS AND METHODS

S. suis isolates were selected from the swine belt within the USA. The samples were carefully collected for systemic (brain, joints, meninges, heart surface) infection or commensal (nasal/lung swabs). The isolates were sequenced *via* MiSeq (illumina Inc), HTS system to generate at least 20x coverage for 150 bp pair reads per genome. The raw data was trimmed off adapters and scaffolds generated using SPADES. The Q value for bases selected were ≥ 30.
The generate FASTA sequence data for each genome was compared with curated *cps* gene locus and structural protein for SLY, MRP and EF, (https://www.ncbi.nlm.nih.gov/).

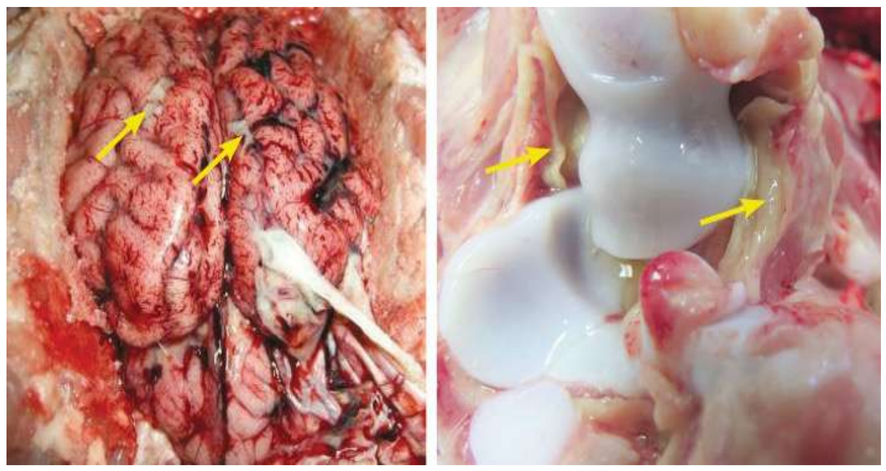


Figure: 2 Affected brain and joint swab for isolating systemic *S. suis*

Brain affected with meningitis due to *S suis* serotype 2or 1/2 infection showing prominent meningeal blood vessels and fibrin accumulations (arrowed); (ii) fibrinosuppurative arthritis due to *S suis* serotype 14 or 1 (fibrin deposits arrowed). Adapted from BMJ Publishing Group Limited Veterinary Record 2018;183:408-410 .

Figure: 3 Steps to identify *S. suis* DC and functional genes

Select pigs showing clinical *S.suis* infection and without infection

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Collect tissues/swabs from brain/joints /lungs or nasal cavities

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Isolate and verify *S. suis* by MALDI and 16s

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Sequence whole genome by illumina technology, trim raw data and generate scaffolds

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Use database to generate derived capsule (DC) locus, and structural genes

CONCLUSIONS

The current study indicates that SLY expression is tightly associated with systemic DC 1 followed by EF and MRP. The DC 2 pathogenic types had higher percentage expressing MRP, EF and SLY compared to commensal. The DC 1/2 commensal types had higher % expressing SLY than pathogenic isolates.

Irrespective of virulence or virulence-associated gene constellation CPS gene expression play a critical role in protective immunity and strain selection for autogenous vaccines (Lawrence 2019).

REFERENCES:
1. **Dinesh. M, et al., 2020. Understanding Streptococcus suis Infection in Pigs. International Journal of Animal Biotechnology and Applications. 6(2):14–21.**
2. **Lawrence, PK, 2019. 4th International Workshop on *Streptococcus suis*. June 3-5.**