ONTARIO PORK

Ontario Pork Research Final Report (19-005) Executive Summary

(Use the headings below to create an executive summary of the project with a maximum of 1 page double-spaced)

Reporting Date: July 08, 2022

Introduction

Streptococcus suis is a leading cause of meningitis in pigs and in rare cases, infects humans working closely with pigs. To date, the pathogenesis of *S. suis* is still poorly understood and although only a low percentage of infected pigs become clinically ill the disease poses an economical threat to the swine industry. There is no universal vaccine available to prevent *S. suis* infections and so the routine use of antimicrobials to control *S. suis* disease contributes to the global increase in the development of antimicrobial resistance (AMR) among bacterial communities. The actual involvement of virulence associated factors in *S. suis* disease remains controversial and there is still a gap in understanding which factors are involved in different steps of the infection process. The capsular polysaccharide (cps) is considered an important factor and is used as the basis for serotyping of S. suis isolates. However, untypable isolates have also been recovered from pigs with clinical signs of S. suis infection which may represent novel serotypes or mutants of known serotypes.

Objectives

The objectives of this study were to analyze the whole genome sequencing data of *S. suis* isolates recovered from systemic and non-systemic sites of sick and healthy pigs on several farms in Ontario in order to determine the serotype of the *S. suis* isolates using an *in-silico* method and identify the distribution of VAFs and AMR genes across the different serotypes, farms and disease status in those isolates. The interplays between *S. suis* virulence factors, tonsil microbiome, and pig genetics were also investigated.

Materials and Methods

In total, 273 *S. suis* isolates recovered from systemic and non-systemic sites of sick pigs or from non-systemic sites of healthy pigs on 17 Ontario farms were subjected to whole-genome sequencing (WGS). The WGS data were used to serotype the isolates by *in-silico* method, and to identify the virulence associated factors (VAFs) and antimicrobial resistance (AMR) genes as well as the mobile genetic elements in the isolates. In addition, the tonsil microbiome was analyzed, and the single nucleotide variants (SNVs) in pig genome associated with *S. suis* disease were determined. Finally, a multi-omics modelling method was used to explore the interplays between S. suis VAFs, tonsil microbiome, and pig genetics in association with *S. suis* disease.

Results and Discussion

Twenty-one serotypes were identified with serotypes 9 and 2 as the most prevalent. A large proportion of untypable isolates could be serotyped using *in-silico* method, while 20% of the isolate still remained untypable which may be the novel serotypes. Virulence-associated factor (VAF) genes were abundant in typable and untypable isolates but there was a higher frequency of VAF genes in the isolates recovered from systemic and non-systemic sites of sick pigs compared to isolates from healthy pigs. Additionally, 98% of the isolates were found to carry at least one antimicrobial resistance (AMR) gene with *tetO* and *ermB* as most the prevalent. Several AMR genes and VAF genes were found in mobile genetic elements such as integrative and conjugative elements, and phages. Several candidate genetic markers (SNVs) were found to be suggestively associated with the tonsillar microbiota or *S. suis* disease, suggesting that both tonsillar microbial community and *S. suis* susceptibility in pigs could partly be under host control.

Conclusions

The wide distribution of VAFs genes in *S. suis* isolates suggests that host factors such as tonsil microbiome, and pig genetic markers as well as different farm related factors may contribute to *S. suis* disease development. In addition, the diversity of MGEs carrying AMR genes in *S. Suis* isolates from healthy pigs indicates a potential reservoir of AMR genes to pathogenic *S. suis* in swine. The results of this study contribute to understanding the pathogenesis of *S. suis* and its control efforts.

ONTARIO PORK

Ontario Pork Research Final Report (19-005)

(maximum of 6 pages double-spaced)

Reporting Date: July 8, 2022

Introduction: *Streptococcus suis*, with 29 known serotypes, is a common bacterial inhabitant of the upper respiratory tract of healthy pigs but infections with some serotypes, under certain conditions, are the cause of many important diseases in pigs including arthritis, meningitis, pneumonia, septicemia, endocarditis, polyserositis, and abscesses (Gottschalk and Segura, 2019). There are numerous virulence associated factors (VAFs) characterized and reported to play important roles in *S. suis* virulence (Segura et al., 2017; Fittipaldi et al., 2012). However, the exact virulence mechanisms of *S. suis* are not well understood. The most studied VAFs include the capsular polysaccharide (CPS), muramidase-released protein (MRP), extracellular protein factor (EPF), and suilysin (SLY) (Segura, 2012; Li et al. 2017; Fittipaldi et al., 2012; Smith et al. 1999; Smith et al. 1992; Lun et al., 2003). Three key VAFs have been characterized as important for virulence in serotype 2 isolates (EPF, SLY and MRP); however, virulent *S. suis* strains which do not produce or carry genes encoding these proteins have been reported (Segura et al., 2017). One of the main drivers of evolution in microbial communities is horizontal gene transfer through mobile genetic elements (MGEs) and AMR genes associated with tetracyclines, chloramphenicol, aminoglycoside carrying by MGEs have been frequently described in *S. suis* (Libante et al., 2019; Zheng et al., 2018; Athey et al., 2016; Huang et al., 2016; Ambroset et al., 2016). Bacteriophages have also been reported to play the role of AMR gene vehicles in disseminating AMR in the environment (Calero-Cáceres & Muniesa, 2016; Calero-Cáceres et al., 2014; Marti et al., 2014). Understanding the potential roles of these MGEs in transferring virulence factor genes and the evolution of pathogenic *S. suis* strains is crucial in developing *S. suis* infection controls. Further, the presence of VAFs genes may not be sufficient to develop the disease and it needs to be accompanied by other factors such as farm management, diet, pig flow, pig density and presence of other diseases. Other factors such as host genetics and interactions with other members of the tonsil microbiota may also have important influences in the virulence mechanisms of each serotype, and each may have serotype specific genetic determinants (Zou et al., 2018). Therefore, understanding the interplay between S. suis virulence factors, composition of and the host genome is essential.

Objectives: (original objectives from project proposal)

1. Using whole-genome sequencing to type *S. suis* isolates by *in silico* method (Additional objective)
2. Explore the molecular similarity between *S. suis* isolates recovered from systemic sites (blood, meninges) of pigs with clinical signs of S. suis infection with the isolates present in non-systemic sites (tonsil, nasal cavities, and intestine) of the same pigs
3. Study the homogeneity between the *S. suis* isolates recovered from non-systemic sites in pigs with clinical signs of S. suis infection and in their healthy pen-mate
4. Determine the most important virulence associated factors and antimicrobial genes in *S. suis* isolates involved in disease outbreaks in Ontario nursery pigs, and to determine the molecular characteristics of untypable strains of *S. suis*
5. Identify the mobile genetic elements carrying virulence and antimicrobial resistance genes in S. suis isolates (Additional objective)
6. Identify pig genotypes associated with a strong immune response to *S. suis*
7. Determine the relationship between tonsil microbiome and *S. suis* disease (Additional objective)
8. Examine the interplay between *S. suis* whole genome sequencing data, tonsil microbiome data, and pig genetic variants in association with *S. suis* disease (Additional objective)

Note: Four objectives highlighted in yellow (obj 1, 5, 7, and 8) have been included as the project was progressing.

Materials and Methods

Whole Genome Sequencing of *Streptococcus suis* isolates (Objective 1 to 8): The isolates used in this study were recovered from nursery pigs with clinical signs of *S. suis* infection and from age-matched healthy animals on the same farm whenever possible on Ontario farms between 2013 and 2018 (Denich et al. 2020; Arndt et al. 2018). A total of 294 *S. suis* isolates were subjected to whole-genome sequencing (WGS) by HiSeqX PE150 (225 isolates) or NovaSeq6000 (36 isolates) by Genome Quebec (Montreal, QC, Canada) and 16 isolates were sequenced using MiSeq by the Laboratory Services at University of Guelph, Ontario. The isolates were recovered from systemic (blood, meninges, spleen and lymph node) and non-systemic (tonsil, nasal, ileum and rectal) sites of 112 pigs on 17 farms: 183 isolates from 65 sick pigs and 90 isolates from 47 healthy pigs. Sick pigs displayed one or more *S. suis* infection clinical signs such as ataxia, paralysis, shaking, paddling, opisthotonos, convulsions, nystagmus and/or incoordination. The isolates were grouped into four different categories: Systemic-confirmed (SC), Non-systemic-confirmed (NSC), Non-systemic-probable (NSP) and Non-systemic-healthy (NSH). The SC (n=47) and NSC (n=65) isolates were recovered from systemic and non-systemic sites of 32 pigs, respectively, that were both symptomatic and confirmed to have *S. suis* recovered from at least one systemic site. The NSP (n=71) isolates were obtained from 33 non-systemic sites of symptomatic pigs, for which *S. suis* was not recovered from any systemic site. The NSH isolates (n=90) were recovered from 47 healthy pigs.

***In silico* serotyping (Objective 1):** The *S. suis* sequences were typed through the pipeline SsuisSerotyping_pipeline (Athey et al. 2016) (https://github.com/streplab/SsuisSerotyping_pipeline).

Virulence-associated factors (VAFs) (Objective 2 to 4): The VAFs of interest were putative proteins involved in *S. suis* pathogenesis reviewed by Fittipaldi et al., (2012). The isolates were also classified into eight different unique genotypes based on the presence of three classical virulence genes (*mrp*, *sly*, epf, *epf**) mainly associated with serotype 2.

Antimicrobial resistance (Objective 2 to 4): CARD's Resistance Gene Identifier (RGI, 5.1.1) software was used to determine the presence of antimicrobial resistance genes using CARD database version 3.1.0. The analysis used three models supported by RGI including CARD's protein homolog models, protein variant models and rRNA mutation models.

Mobile genetic elements (Objective 5): The screening of ICE-related elements involved a curation of a database containing protein sequences of integrases, DDE transposase superfamily and other conjugation modules (CP, relaxase and VirB4 proteins). The screening for phages with complete phage associated proteins were done manually and were confirmed through Head-Neck-Tail analysis of VIRFAM.

Antimicrobial resistance and virulence factor genes in MGEs (Objective 5): The extracted putative ICEs/IMEs and putative phage sequences were screened for the presence of antimicrobial resistance genes using CARD's Resistance Gene Identifier (RGI, 5.1.1) software. Annotations of each putative MGE and BLASTX analysis were used to detect the presence of virulence factor genes previously characterized in *S. suis* in ICEs and phages. The AMR and virulence factor genes were confirmed to be encoded within the elements if found between ICE-related modules in ICEs and phage-related modules in putative phages.

Pig genotyping (Objective 6): DNA samples from 23 confirmed, 19 probable, and 23 healthy pigs were sent to Eurofins Bio Diagnostics, Inc. (River Falls, Wisconsin, USA) to genotype using a custom 54K Affymetrix Axiom® myDesign™ custom chip-based DNA microarray designed by the Canadian Centre for Swine Improvement, Inc. (Ottawa, Ontario, Canada). The SNP chip also contained SNVs from other laboratories, which had lab exclusive labels. Two pigs were removed during data pruning. A genome-wide association study (GWAS) was conducted on three group of pigs.

Tonsil microbiome (Objective 7): The community bacterial DNA from tonsils of 63 pigs including 20 confirmed, 23 probable, and 20 healthy pigs were used to prepare the 16S rRNA gene (V3-V4 region) libraries at the Advanced Analysis Center at the University of Guelph, following Illumina's 16S metagenomic sequencing library preparation protocol. Sixty-five libraries were then sequenced using Illumina MiSeq technology (Illumina Inc) with Illumina MiSeq version 3 (paired-ends with 300 bp reads). Compositional microbiome profille analysis were performed using the DADA2 analysis pipeline and analysis modules available through the QIIME2 workflow.

The multi-omics model (Objective 8): To evaluate pig genomic, S. suis virulence factors, and the tonsillar microbiota prediction ability of *S. suis* disease, three statistical machine learning methods were employed. To develop the multi-omics predictive model, observations from 63 pigs with 670 SNVs, 79 genera in tonsil microbiome, and one virulence gene were used as predictors. A Random Forest model was then developed to combine the three datasets..

Results and Discussion:

***In silico* serotyping (Objective 1):** Whole genome sequencing data were used to serotype *S. suis* isolates using *in silico* method. Twenty-one different serotypes identified with the most common serotypes recovered were serotype 9, serotype 2, serotype 29, and serotype 16. The isolates from systemic sites of sick pigs belonged to 14 different serotypes with serotypes 2 and 9 as the most prevalent, while the isolates from non-systemic sites of healthy pigs consisted of 18 different serotypes with serotypes 2 and 9 accounting only for less than 10% of isolates. Overall, 20% of isolates remained untypable, however, a larger proportion (27%) of isolates from healthy pigs were untypable compared to only 8.5% untypable isolates from systemic sites of sick pigs. The PCR and *in silico* serotyping methods were compared and no agreement was found between the two methods. The observed disagreement was mostly attributed to the differentiation of serotypes 1/2 and 2 by *in silico* method, as well as serotyping of more than 40% of PCR-untypable isolates. In comprehensive studies, *in silico* serotyping may be able to provide more information by typing the strains which could not be serotyped by PCR or slide co-agglutination method. It is important to keep in mind however that the *in silico* pipeline by Athey et al. (2016) may not identify all novel isolates recovered since 2015.

Virulence associated factors (VAFs) and Antimicrobial Resistance (AMR) (Objective 2 to 4): Overall, the isolates recovered from sick pigs deemed to carry a higher number of VAF genes, which is in accordance with previous analysis of frequencies of the same set of VAFs in *S. suis* isolates (Weinert et. Al, 2015). There was no significant difference between the carriage of VAFs by isolates recovered from systemic and non-systemic sites of sick pigs. However, the relative frequency of VAF genes in the isolates recovered from non-systemic sites of sick pigs was higher than that in the isolates recovered from non-systemic sites of healthy pigs. It is possible that some carrier piglets develop clinical signs due to dissemination of *S. suis* from non-systemic sites such as tonsils and other mucosal surfaces to systemic sites (Gottschalk & Segura, 2019; Cloutier et al., 2003). This could also indicate that there are environmental or host-associated factors that benefit isolates carrying these genes and therefore could lead to higher likelihood of disease development. However, as the NSH isolates in the current study were obtained from the same farms as the sick pigs, farm-level environmental or management factors may be less of a factor than animal-level variations for pathogen success. For instance, we detected ST25 strains of serotype 2 from healthy pigs on the same farm where ST25 strains detected from systemic sites of sick pigs indicating that host- and environment-associated factors are likely involved in disease development.

There are numerous VAFs characterized and reported to play important roles in S. suis virulence (Segura et al., 2017; Fittipaldi et al., 2012). However, in this study only three VAF genes including *dltA*, *luxS*, and *troA* had higher frequency in isolates from systemic sites of sick pigs relative to the isolates from healthy pigs. In fact, most of the individual VAFs did not have a significant correlation with disease status despite the higher relative frequency of VAFs in sick animals. It is possible that VAFs generally increase the fitness of *S. suis* strains and are favoured for colonization but are not necessarily causing the illness, or it could indicate that combinations of different VAFs can contribute to disease development.

The three classic virulence markers including MRP, EPF, and SLY have previously been used as indicators of high virulence in serotype 2 strains (Segura et al., 2017). In this study, the *mrp* gene could be detected in all serotype 2 isolates from systemic sites of sick pigs suggesting that it may play a role in adherence of serotype 2 strains to host cells and survival in blood (Li et al. 2017). However, more than half of the isolates with other serotypes recovered from systemic sites of sick pigs and all serotype 9 strains from healthy and sick pigs were *mrp* negative. Fittipaldi et al. (2011) have also shown the absence of *mrp* gene in serotype 2 isolates recovered from pigs with clinical disease in Canada and United States. The *mrp* gene was also absence in most serotype 9 strains in sick pigs in other studies (Oh, et al., 2017; Blume et al., 2009). The absence of *mrp* gene in *S. suis* isolates recovered from sick pigs supports the explanation that VAFs may be favored for their roles in colonization but not necessarily in causing disease (Peterson, 1996).

Notably, none of the serotype 2 and 9 isolates in this study carried either *sly* or *epf* gene, even though these genes are involved in important virulence mechanisms in *S. suis*. Although not essential, SLY is very important in the invasion and survival of *S. suis* in its host (Tenenbaum et al., 2016). The absence of classical markers in most isolates suggest that the virulence potential of the *S. suis* isolates in Canada and the United States (Estrada et al., 2021) are likely lower than isolates from EU countries such as Spain (Zheng et al., 2018). The presence of all three markers in isolates recovered from healthy pigs in this study may suggest that important VAFs are not exclusive to pathogenesis but likely play other essentials roles in the protection and propagation of *S. suis*. Alternatively, it might also be possible that these genes in healthy isolates are not expressed.

In this study, serotype 9 and serotype 2 isolates from systemic sites carried additional VAFs that were distinct to each serotype. This suggests that there might also be differences in the genetic bases of their virulence. It has previously been

shown that serotype 9 isolates were generally less virulent compared to serotype 2 isolates (Greeff et al., 2011; Beineke et al., 2008). Further, in our analysis, isolates from the most prevalent serotypes formed clusters within a bigger cluster of typable isolates indicating similarities in VAF genes carried among the well-characterized serotypes despite differences in gene carriage by individual serotypes. This supports the possibility that virulence mechanisms of each serotype may slightly be different, but there are not enough representative isolates within each serotype in the current study to determine distinct sets of VAFs per serotype. The recent increase in prevalence of disease caused by serotype 9 isolates requires more attention to understanding the pathogenesis mechanisms that may be unique to each serotype. Overall, 38 different genes encoding resistance to 15 antibiotic drug classes detected in 273 *S. suis* isolates, with 98% of isolates carried at least one AMR gene and 79% of isolates carried genes associated with resistance to at least four drug classes. The four major drug classes to which resistance genes were found in more than 90% of isolates included tetracycline, lincosamides, macrolide, and streptogramin. A high rate of carriage of AMR genes in *S. suis* isolates has reported in previous studies globally (Tan et al., 2021; Nicholson et al., 2021; Segura et al., 2020), with most of the isolates carried genes associated with resistance to tetracycline, lincosamides and macrolides. Consistent with those studies, tet(O) was the most prevalent tetracycline resistance gene detected in the *S. suis* isolates in our study. Further, AMR genes were found in higher diversity and frequency in healthy pigs compared to isolates from sick pigs which may in part be attributed to the higher diversity of serotypes detected in healthy pigs. The SC isolates consisted mostly of genotypically related isolates, particularly those of serotypes 2 and 9, with each group of related isolates carrying a similar set of AMR genes. The presence of some AMR genes was statistically associated with specific serotype, but it was more likely seen in a group of isolates recovered from pigs on the same farm. Previous findings suggests that resistance patterns in *S. suis* can vary with pig health, geographic location, serotypes and different farm practices such as use of different antibiotics (Varela et al., 2013; Hendriksen et al., 2008) which likely contributed to presence of the same AMR genes on a specific farm.

Mobile genetic elements <u>(Objective 5):</u> The isolates in most serotypes, in particular serotype 2, from systemic sites of sick pigs had a smaller genome size compared to the isolates from non-systemic sites isolates (p V 0.05). The smaller genome sizes in *S. suis* isolates recovered from systemic sites of sick pigs has previously been reported (Weinert et al., 2015; Merhej et al., 2013). However, serotype 9 isolates from systemic sites had larger genome size than the isolates recovered from non-systemic sites ($p < 0.05$). Further, most of the genes prevalent only in serotype 9 systemic isolates were associated with MGEs including phage-related and integrative conjugative elements (ICEs) and the putative virulence factor (purA). In total, 345 putative ICE-related elements and 646 putative phages were detected. Fifty-five ICE-related elements and 17 putative phages encoded AMR genes, with genes *tet*O and *erm*B as the most prevalent. Many of the genes found in serotype 9 systemic isolates and not in serotype 9 isolates from non-systemic sites of healthy pigs were related to ICEs and phages. It is widely acknowledged that streptococcal genomes are susceptible to extensive genetic recombination events and *S. suis* is no exception (Nicholson et al., 2021; Greeff et al., 2011; Bisno et al., 2003). The serotype 9 isolates from systemic sites in this collection had a stronger association with MGEs compared to serotype 2 which could result from more genetic acquisitions or a higher likelihood of maintaining genes after they have been acquired. One plausible explanation may be that these genomic elements may have been retained due to their contributions to the fitness of the strains, such as the ICEs and phages encoding AMR genes and putative phages encoding a virulence factor found in serotype 9 isolates in systemic sites. It is also possible that serotype 9 isolates from systemic sites may have acquired AMR or virulence genes more recently and thus these genes are contained within more intact MGEs.

<u>Pig genotyping (Objective 6):</u> Among confirmed and probable *S. suis* cases, there were no SNVs that reached the conservative significance threshold but one intergenic SNV was approaching suggestive association near ARL4C ($p = 1.9 \times 10^{-5}$). One intronic SNV in each PLEKHM1, LRRC37A2, and WNT3 ($p = 1.7 \times 10^{-6}$, $p = 1.74 \times 10^{-6}$, $p = 5.8 \times 10^{-6}$, respectively) was suggestively associated with confirmed *S. suis* cases. Among probable *S. suis* cases, one intronic SNV within NSL1 ($p = 5.5 \times 10^{-6}$) had a suggestive association. The variants identified in this study that were associated with *S. suis* disease were related to host immunity, cytoskeletal rearrangement, and chromosomal rearrangements.

<u>Tonsil microbiome (Objective 7):</u> The bacterial population in tonsil of sick pigs had a higher diversity than that in tonsil of healthy pigs. However, *Streptococcus* abundance was not different between the sick and healthy pigs. The tonsil microbiota could be partitioned into two distinct bacterial community types. The community type 1 was dominated by *Streptococcus* while the community type 2 was dominated by *Escherichia-shigella*. None of those two bacterial community types was associated with *S. suis* disease, but there was a significant association between recovery of *S. suis* serotype 2 isolates and community type 1 ($p < 0.05$). Further, the healthy pigs were found to have more *Bacteroides* and *Lachnospiraceae* which are considered beneficial commensals and have been associated with healthy piglets in previous

research (Correa-Fiz et al., 2016). The tonsil provides an important niche for commensals and pathogens (Lowe et al., 2012). The colonization of tonsils with these beneficial commensal bacteria may play a role in preventing S. suis disease and should be investigated further.

The multi-omics model (Objective 8): One intergenic variant in each of KCNE4, UNC5C, BTD10, SUSD6, and LRFN5 gene was associated with the tonsillar microbiota composition. In addition, one intron variant in each of TMEM117, DKK3, SP3, KCNK1, BMPER, CROT and LRFN5 gene, and one upstream variant gene in GABRB1 was suggestively associated with the tonsillar microbiota composition. Genetic region in KCNE4 gene in chromosome 15 was found to be associated with the *Moraxella* abundance. *Moraxella* was a key member in bacterial community type 1 that was dominated with *Streptococcus*. In our recent study, in addition to identifying swine genetic variations that associated with the structure of tonsil microbial communities, we found that these genes are highly enriched in functional pathways and immunity genes. Further studies are needed to investigate the interactions between *Moraxella* and *S. suis* disease. Three SNVs (2 in LRFN5 and 1 in SUSD6 gene) were associated with *Mycoplasma* abundance. *Mycoplasma* was the key member in the bacterial community type 2 which was dominated by *Escherichia-shigella*. One intron variant in TMEM117 gene in chromosome 5 was also associated with *Streptococcus* abundance in the tonsillar microbiota. One intron variant in chromosome 2 (rs81356987) nearest to CCND1 was suggestively associated with *S. suis* disease and the presence of the virulence factor *dltA* gene ($p = 1.35\times 10^{-5}$). The predictive ability increased when the tonsillar microbiome data were included. Random forest model could identify *Bacteroides*, unculture_*Erysipelotrichaceae* bacterium, *Porphyromonas*, and *Streptococcus* as the high predictors in the model. Notably, *Streptococcus*, *Actinobacillus indolicus*, *Pasteurella*, *Veillonella*, *Glaesserella parasuis* and *Bergeyella*- which were identified by the DMM model as key different taxa between the two community types- were determined as *S. suis* predictors by either model, suggesting that tonsillar microbiota may improve the prediction of *S. suis* disease. This suggests that while microbial communities are affected by environmental factors, genetic influence of the host on the microbiome communities is also expected (Camarinha-Silva et al., 2017). The multi-omics analysis provided a better understanding of the contribution of each component to *S. suis* disease.

Conclusions

The findings indicate that *in silico* serotyping using WGS data can improve the classification of *S. suis* and its contributions in comprehensive genomic *S. suis* studies. Serotype 2 and serotype 9 as the most prevalent serotypes from sick pigs on studied farms in Ontario. This study also highlighted that the presence of virulence associated factors are not the sole indicators of disease development and other factors related to environment and pig could contribute to *S. suis* disease. Further, the high frequency of AMR genes and the high number of MGEs carrying AMR and virulence factors genes in this study suggests that a consistent surveillance of AMR in S. suis is crucial. The introduction of AMR genes to *S. suis* bacteria in pigs not only may have a great impact on the swine industry but also on public health. In this study, the composition of the tonsillar microbiota of healthy pigs and pigs with *S. suis* disease was also determined. Two different bacterial community types were found and several bacterial members were shown to be significantly altered in pigs depending on their health status. The results also highlighted the role of host genetic variations in shaping the composition of the tonsillar microbiota. The interaction between host and *S. suis* virulence factors provide a starting point toward understanding the complex interaction between host genetics, the tonsil microbiome, and *S. suis* virulence factors in the context of *S. suis* disease in nursery pigs.

Knowledge Transfer

Peer-reviewed journal articles

1. Maysa Niazy, Sarah Hill, Khurram Nadeem, Nicole Ricker, Abdolvahab Farzan. (2022). Compositional analysis of the tonsil microbiota in relationship to *Streptococcus suis* disease in nursery pigs in Ontario. Animal Microbiome. 4(1) http://dx.doi.org/10.1186/s42523-022-00162-3.
2. Aradanas, M; Poljak, Z; Fittipaldi, N; Ricker, N; Farzan, A. (2021). Serotypes, virulence associated factors, and antimicrobial resistance of *Streptococcus suis* isolates recovered from sick and healthy pigs determined by whole genome sequencing. Front. Vet. Sci.8(742345) http://dx.doi.org/https://doi.org/10.3389/fvets.2021.74234
3. Denich, LC; Farzan, A.; Friendship, R; Arndt, E; Gottschalk, M; Poljak, Z. (2021). Study of the relationship between untypable and typable isolates of *Streptococcus suis* recovered from clinically ill and healthy nursery pigs.. Vet Microbiol. http://dx.doi.org/https://doi.org/10.1016/j.vetmic.2021.109064

4. Denich LC, Farzan A, Friendship R, Arndt E, Gottschalk M, Poljak Z. (2020). A Case-Control Study to Investigate the Serotypes of *S. suis* Isolates by Multiplex PCR in Nursery Pigs in Ontario, Canada. Pathogens. 9(1) http://dx.doi.org/10.3390/pathogens9010044

Thesis

1. Niazy, Maysa. A Multi-omics model to identify host-microbiome interactions and pathogen dynamic impacts on Streptococcus suis disease development in pigs. (2021). University of Guelph. Master's Thesis. https://atrium.lib.uoguelph.ca/xmlui/handle/10214/26343
2. Aradanas, Maverick. Investigation of virulence associated genes in *Streptococcus suis* Isolates recovered from sick and healthy nursery pigs. (2021). University of Guelph. Master's Thesis. https://atrium.lib.uoguelph.ca/xmlui/handle/10214/26563
3. Denich, Leann. A case-control study to investigate the serotypes and untypable *Streptococcus suis* strains recovered from nursery pigs on 12 farms in Ontario, Canada between 2017 and 2018. (2020). University of Guelph. Master's Thesis. https://atrium.lib.uoguelph.ca/xmlui/handle/10214/17734
4. Investigating single-nucleotide variants in swine associated with common infectious pathogens and diseases, with a focus on *Streptococcus suis* infection, using a genome-wide association study approach. (2020). University of Guelph. Master's Thesis. https://atrium.lib.uoguelph.ca/xmlui/handle/10214/18037

Presentations

1. Niazy M, Nadeem K, Lillie B, Farzan A, Ricker N. A multi-omics approach to identify the "host-pathogen-environment" factors associated with *Streptococcus suis* disease development in swine. One Health and Development for a World Under Pressure Symposium. Hybrid. Guelph, ON Canada. May 9-11, 2022. POSTER.
2. Niazy M, Nadeem K, Lillie B, Farzan A, Ricker N. A multi-omics approach to identify the "host-pathogen-environment" factors associated with *Streptococcus suis* disease development in swine. 71st Annual Conference of the Canadian Society of Microbiologists (CSM 2022). University of Guelph, Guelph, Ontario, Canada. June 26-29, 2022.
3. Niazy M, Ricker N, Nadeem K, Farzan A. Modeling the dynamics of the tonsil microbiota in relationship to *Streptococcus suis* disease in pigs. Canadian Society of Microbiologists (CSM), June 14-17, 2021. VIRTUAL. POSTER
4. Aradanas M, Poljak P, Fittipaldi N, Farzan V, Ricker N. Study of mobile genetic elements in *Streptococcus suis* isolated from pigs in Ontario, Canada. International Society for Plasmid Biology (ISPB). VIRTUAL. May 20, 2021. ORAL.
5. Aradanas M, Poljak P, Fittipaldi N, Ricker N, Farzan V. *Streptococcus suis* serotypes, virulence factors, and associated antimicrobial resistance in Ontario pig determined by whole genome sequencing: is it a public health concern? Centre for Public Health and Zoonoses (CPHAZ) Symposium. VIRTUAL. May 20, 2021. ORAL
6. Niazy M, Nadeem K, Lillie B, Farzan A, Ricker N. A multi-omics model to identify host-microbiome interactions and pathogen dynamic impacts on S. suis disease. Conference of Research Workers in Animal Diseases (CRWAD). VIRTUAL. 2021, Dec 3-7. ORAL
7. Aradanas M, Poljak P, Fittipaldi N, Ricker N, Farzan V. Study of virulence factors in *S. suis* isolates recovered from Ontario nursery pigs using Whole Genome Sequencing. Conference of Research Workers in Animal Diseases (CRWAD). VIRTUAL. 2021, Dec 3-7. POSTER.
8. Maverick Aradanas, Nicole Ricker, Vahab Farzan. Study of virulence factors in *S. suis* isolates recovered from Ontario nursery pigs using Whole Genome Sequencing. Conference of Research Workers in Animal Diseases (CRWAD). VIRTUAL. 2020, Dec 4-8. ORAL.
9. Maveric Aradanas. Distribution of virulence factors and AMR genes in *S. suis* isolates from nursery pigs in Ontario. Ontario Veterinary College Graduate Students Symposium. VIRTUAL. 2020. Aug 25. ORAL.
10. Jeremy Wong. Investigating single-nucleotide variants in the pig genome associated to *Streptococcus suis* disease using a genome-wide association study approach. Ontario Veterinary College Graduate Students Symposium. VIRTUAL. 2020. Aug 25. POSTER.
11. Denich, L; Farzan, A; Friendship, R; Arndt, E; Gottschalk, M; Poljak, Z. Investigation into the serotypes of Streptococcus suis isolates in nursery pigs in Ontario, Canada. The 4th International Workshop on *Streptococcus suis*, Montreal QC, Canada. June 03, 2019. POSTER.

12. Wong J, Fraser R, Farzan A, Lillie B. Investigating of single-nucleotide variants related to *Streptococcus suis* resistance using a genome-wide association study approach. University of Guelph Centre for Public Health and Zoonoses (CPHAZ) Scientific Symposium, Guelph Ontario, Canada. May 30, 2019. POSTER.
13. Wong J, Fraser R, Farzan A, Lillie B. Investigating single-nucleotide variants in the pig genome associated to *Streptococcus suis* disease using a genome-wide association study approach. The University of Guelph Swine Research Day (UGSRD). University of Guelph, May 9, 2019. POSTER
14. Denich, L; Farzan, A; Friendship, R; Arndt, E; Gottschalk, M; Poljak, Z. Investigation into the serotypes of *Streptococcus suis* isolates in nursery pigs in Ontario, Canada. The University of Guelph Swine Research Day (UGSRD). University of Guelph, May 9, 2019. POSTER.

Invited/Podcast

1. Nicole Ricker. *S. suis* sequencing. Ontario Association of Swine Veterinarians (OASV) Fall Conference. Nov 19, 2021. INVITED SPEAKER.
2. Vahab Farzan. OVC Swine Research Update. Ontario Association of Swine Veterinarians (OASV) Fall Conference. Nov 19, 2021. INVITED SPEAKER.
3. Vahab Farzan. Tonsil microbiome, Identifying biomarkers, and Microbiota analytics process. The Pig Microbiome Podcast, Phileo by Lesaffre. 2021.
4. Vahab Farzan. The 4th International Workshop on *Streptococcus suis*, Montreal QC, Canada. June 03, 2019. SEASION CHAIR.

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