5th International Workshop on *Streptococcus suis*

Centre de Recherche en Infectiologie Porcine et Avicole
Swine and Poultry Infectious Diseases Research Center

CRIPA
Université de Montréal

AHS

ABSTRACT BOOK

5th International Workshop on Streptococcus suis (5th IWSs): Advanced Research in the Era of Antimicrobial Restriction

JUNE 6-7, 2023

Sapphire Room 201, IMPACT Forum, Muang Thong Thani, Thailand

This workshop has been possible thanks to the support of"Innovative Veterinary Solutions for Antimicrobial Resistance" program (InnoVet-AMR) of the International Development Research Centre

GAMRIF
Global AMR Innovation Fund

IDRC · CRDI
International Development Research Centre
Centre de recherches pour le développement international

UKaid
from the British people

CONTENTS

Keynote speakers 5
Oral presentations 16
Student presentations 28
Posters 34

5th International Workshop on *Streptococcus suis*

5th International Workshop on *Streptococcus suis* (5th IWSs): Advanced Research in the Era of Antimicrobial Restriction

Streptococcus suis is a swine re-emerging pathogen and an emerging zoonotic agent afflicting people in close contact with infected pigs or pork meat, especially in Asia, making this bacterium a primary health concern in this part of the globe. In pigs, *S. suis* disease results in decreased performance, increased mortality and extensive use of antimicrobials, having a significant economic impact on swine production worldwide. Facing the new regulations in preventive use of antimicrobials in livestock and lack of effective vaccines, control of *S. suis* infections became worrisome. Indeed, *S. suis* research attracted the attention of the scientific community worldwide in the last years. Therefore, in 2013, with the aim to unify the growing "*S. suis* research family" the "1st International Workshop on *Streptococcus suis* (IWSs)" was jointly organized by Canada and China and took place in Beijing. It successfully reunited for the first time more than 80 researchers and students working on *S. suis* in the same room. Following the success of this workshop, the 2nd IWSs was held in Argentina in 2014, whereas the 3rd and the 4th IWSs took place in Germany (2106) and Canada (2019), respectively.

As one of the milestones of a Canada-Thailand collaborative grant financed by the International Development Research Center under InnoVet-AMR: Innovative Veterinary Solutions for Antimicrobial Resistance Programs, the 5th International Workshop on *Streptococcus suis* (5th IWSs) is jointly organized with the 8th International Symposium on Emerging and Re-emerging Pig Diseases (ISERPD 2023) and is held in Bangkok, Thailand in June 2023. We expect over 100 researchers and trainees from all over the world to present and discuss recent advances on this challenging swine and zoonotic pathogen. An important aim of the workshop is building an opportunity for knowledge exchange involving stakeholders (veterinarians and swine producers) on the risk posed by *S. suis* infection, diagnostics, and preventive measures.

We are pleased to welcome you in Bangkok and we hope you will enjoy the exciting program of the workshop,

Prof. Mariela Segura

Prof. Marcelo Gottschalk

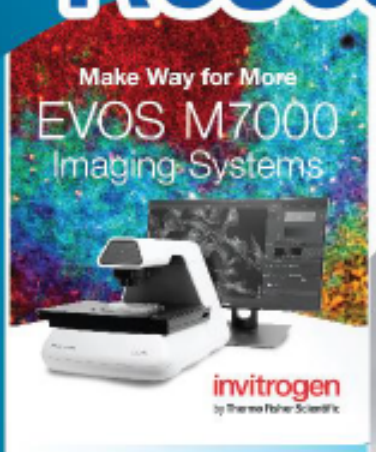
Associate Prof. Potjanee Srimanote

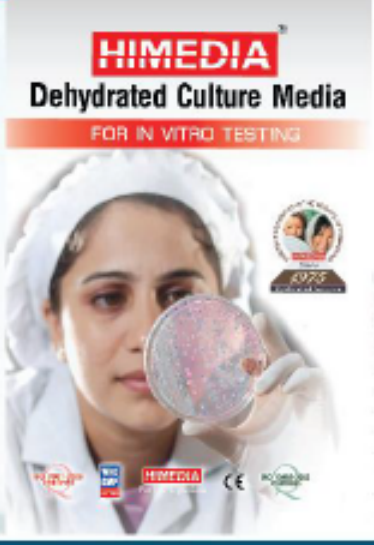
Chairs of the 5th International Workshop on *Streptococcus suis*

AS A TRUE PARTNER

Biotechnology Research

gibthai
A 3N HOLDING COMPANY





Gibthai Company Limited
3N HOLDING HOUSE, 44/6 Suthisarnvinitchai Road, Samsennok, Huay Kwang, Bangkok 10310
Tel : (66) 0 2274 8331 | Fax : (66) 0 2274 8336, (66) 0 2274 8580 | E-mail : info@gibthai.com
gibthaifan
@gibthai
gibthaicompany
www.gibthai.com

KEYNOTE SPEAKERS

The 8th International Symposium on Emerging And Re-Emerging Pig Diseases

ISERPD 2023

BANGKOK, THAILAND

5th International Workshop on *Streptococcus suis*

Hosted by :

TSVA

PATHOGENOMICS:

New insights into *Streptococcus suis* lifestyle and pathogenesis

PROF. JERRY WELLS
Host-Microbe Interactomics
Wageningen University and Research
Wageningen, The Netherlands

6th
June
2023

Funded by:

IDRC · CRDI
International Development Research Centre
Centre de recherches pour le développement international

GAMRIF

UKaid
from the British people

Perspectives on Strategies to Elucidate Mechanisms of Streptococcus suis Pathogenesis

Jerry M. Wells[1,2]

[1]Host-Microbe Interactomics, Wageningen University and Research, Wageningen, the Netherlands; [2]University of Cambridge, Department of Veterinary Medicine, Cambridge, U.K.

Virulence and *S. suis* population genetics

S. suis is a diverse species shown by comparison of sequenced genomes to consist of both highly pathogenic isolates with serotypes commonly associated with invasive disease as well as non-disease associated isolates considered to have a commensal-like association with the porcine host. Population genetic studies have shown that the highly pathogenic isolates from humans and pigs consist of five main clades [1]. A recent review highlighted several controversies concerning the role of virulence factors in *S. suis* of which at least 37 have been described as critical [2]. This can be due to redundancy of function, type of animal model used, infection route, but also inherent differences between sequence types and clades identified by bacterial population genetics. This complicates the characterization of virulence factors and emphasizes the need to compare mechanisms within and between clades not only serotypes. With this in mind I will present some recent research on important aspects of pathogenesis in the context of genetic conservation in the population of pathogenic and non-disease associated *S. suis* [2].

The initial steps in *S. suis* pathogenesis

There have been many studies on adhesion of *S. suis* to different cells including epithelial cells with the notion that invasion or translocation in the upper respiratory tract or intestine could be the mechanism leading to systemic infection. However, most studies with knockout strains of candidate adhesin genes do not often demonstrate substantial differences to the wild type strain, even when the difference is significant (summarized in [2]). Additionally, invasion or translocation are not often investigated.

The porcine palatine tonsils have been considered a plausible portal of entry owing to their role in naturally sampling bacteria and antigens entering the crypts to elicit mucosal immune responses. Pathogenic isolates can often be isolated from swabs of the tonsil surface [3, 4] but it is unclear how *S. suis* could avoid killing by innate phagocytes in the tonsil lymphoid compartment and reach the bloodstream. I will present new data supporting a working hypothesis to explain how pathogenic *S. suis* can travel through the lymphatic ducts which empty into the right subclavian vein and then enter the bloodstream and travel to other parts of the body.

The BBB and choroid plexus as a route for *S. suis* entry into the CNS

To cause meningitis, *S. suis* is proposed to traverse the blood-brain barrier (BBB), which mainly comprises brain microvascular endothelial cells, astrocytes, and pericytes. Numerous studies have been published using different cells, varied *in vitro* conditions and different strains of *S. suis* sometimes with contradictory results.

Another important anatomical site of importance in pathological interactions with meningitis causing bacteria is the Blood-Cerebral Spinal Fluid (BCSF) Barrier. This barrier is formed by the choroid plexus (ChP) epithelium which separates the blood from the CSF. The BCSF barrier has been postulated as the primary site of CNS invasion in natural and experimentally induced cases of *S. suis* meningitis [5, 6]. The ability of *S. suis* to translocate across the choroid plexus has been studied in primary porcine ChP cells and immortalized ChP epithelial cells. In this part of the lecture results using advanced stem-cell models of BCSF and BBB barriers will be presented and compared to previously reported studies on *S. suis* virulence factors involved in meningitis.

Phase-variable regulons in *S. suis* and implications for future research on pathogenesis

In the last part of the lecture, I will focus on restriction-modification systems in *S. suis* that exhibit phase-variable expression leading to global changes in DNA methylation. In several bacteria phase variation in Type I and Type III restriction-modification systems have been shown to occur at the level of the target recognition domains of the *hsdS* (methylase specificity) gene via the action of recombinases and reversible recombination processes [7]. This leads to changes in specificity of the methylase and alternative patterns of DNA methylation which affect gene expression. I will describe conservation of phase phase-variable DNA methylation systems in different pathogenic clades of *S. suis*, how this affects virulence phenotypes and the implications of these regulatory systems for research on virulence mechanisms *in vitro* and *in vitro*.

References

[1]. Weinert et al. (2015). Nat Commun 6, 6740.
[2]. Segura M. et al., (2017). Trends Microbiol. 25(7): 585-599.
[3]. Fredriksen S. et al., (2023). BioRxiv online doi.org/10.1101/2022.08.01.500980.
[4]. Fredriksen S et al., (2022). BMC Microbiol. 22(1):224.
[5]. Williams, A.E., and Blakemore, W.F. (1990). J Infect Dis 162: 74–481.
[6]. Madsen, L.W et al., (2002). J Comp Pathol 126: 57–65.3.
[7]. Sieb K.L. et al., (2020). Annu. Rev.Microbiol. 2020. 74:655–71

The 8th International Symposium on Emerging And Re-Emerging Pig Diseases
ISERPD 2023
BANGKOK, THAILAND

5th International Workshop on *Streptococcus suis*

Hosted by :
TSVA

HUMAN *STREPTOCOCCUS SUIS* INFECTIONS IN THAILAND:
Sociocultural factors, epidemiology, and genotypes

PROF. ANUSAK KERDSIN
Faculty of Public Health
Kasetsart University, Thailand

6th
June
2023

Funded by:
IDRC · CRDI
International Development Research Centre
Centre de recherches pour le développement international
GAMRIF
UKaid
from the British people

Human *Streptococcus suis* Infections in Thailand: Sociocultural Factors, Epidemiology, and Genotypes

A. Kerdsin

Faculty of Public Health, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus

Introduction

The number of reported human *Streptococcus suis* cases has substantially increased, with Southeast Asian countries leading the counts. In Thailand, human *S. suis* infection was first described in 1987 in Bangkok. Sporadic human cases had been reported in several provinces in Thailand, especially in the north. After the Sichuan outbreak in China in 2005, the importance of this disease has been increasingly recognized in Thailand, as well as in many countries worldwide.

In this review, we focus on the societal and cultural characteristics and behaviors, epidemiology, clinical manifestations, and genotypes of human *S. suis* in Thailand. This can provide information for policy implementation, active surveillance, and prevention of this disease.

Materials and Methods

This review collected all reported papers written in either Thai or English that documented human *S. suis* infections in Thailand from the available online databases including PubMed, ScienceDirect, Scopus, Google, Thai Index Medicus of Chulalongkorn University, and Siriraj Hospital, Bureau of Epidemiology. Search terms were *S. suis*, human, clinical, Thai, Thailand, outbreak, all years.

Results

In total, 1798 cases from 59 reports since 1987-2021 were identified. Most cases were male (n = 1287) and involved consumption or exposure to pig or raw pork products (n=1052). Septicemia and meningitis were predominant clinical manifestations and hearing loss was a major complication. One outbreak with 10 fatal cases due to septic shock was documented in 2000 in northern Thailand, well before the largest outbreak occurred in Sichuan, China in 2005. After the Sichuan outbreak, five large outbreaks of *S. suis* infections in humans have been documented in Thailand: four of these outbreaks were in the north, whereas a fifth outbreak occurred in the northeast.

Adult age, male sex, alcohol drinking, pig-related occupation or exposure, and raw pork consumption were common risk factors of *S. suis* infections in Thailand. Fatal risk factors included septic shock, rapid onset of illness, prolonged bacteremia ≥ 6 days, low serum albumin, high serum total bilirubin, low platelet count, and elevated alanine transaminase.

Consumption of the raw pork or blood dishes was major caused of sporadic and outbreak in Thailand (Figure 1). Most cases were related to Thai festivals and ritual ceremonies, such as Buddhist ordinations, weddings, housewarmings, and funerals. Some Thai people believe the raw pork/blood dish can promote health and insufficient knowledge of the health risks posed by the consumption of raw pork or partially cooked pork products was documented.

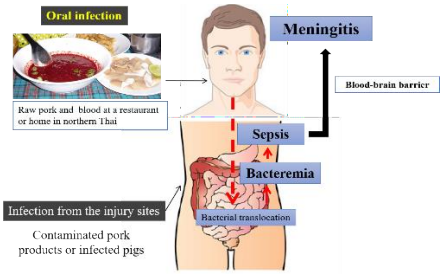


Figure 1. Causes of human *S. suis* infections in Thailand

S. suis serotype 2 (93.4%) was dominant from patients in Thailand, followed by serotypes 14 (5.2%), 24 (0.6%), 5 (0.4%), 4 (0.1%), 9 (0.1%), 31 (0.1%), and unencapsulated (0.1%), respectively. MLST classified serotype 2 into five CCs: CC1, CC25, CC28, CC104, and CC233/379. Of these, CC1 with ST1 and CC104 with ST104 are the predominant STs in Thai human infections.

Discussion

Thailand has a very high cumulative incidence during 1987–2021. Reducing human *S. suis* disease requires a multidimensional approach combining government and public health efforts through policy, regulations, and education, and active community involvement to effect behavioral changes that are evidence-based but culturally sensible and acceptable, along with the implementation of more rapid diagnostics and more relevant screening tools in health-care systems.

Acknowledgement

The Kasetsart University Research and Development Institute (KURDI), Thailand provided English-editing assistance

References

1. Kerdsin et al. (2022) Foods 11, 1190, 1-13.

2. Kerdsin (2022) Trop Med Infect Dis 7, 359, 1-12.

The 8th International Symposium on Emerging And Re-Emerging Pig Diseases

ISERPD 2023

BANGKOK, THAILAND

5th International Workshop on *Streptococcus suis*

Hosted by :

TSVA

DIAGNOSIS AND EPIDEMIOLOGY OF *STREPTOCOCCUS SUIS* INFECTIONS IN PIGS

PROF. MARIA CLAVIJO
College of Veterinary Medicine
Iowa State University, USA

6th
June
2023

Funded by:

IDRC · CRDI
International Development Research Centre
Centre de recherches pour le développement international

GAMRIF

UKaid
from the British people

Diagnosis and epidemiology of *Streptococcus suis* infections in pigs

Maria J. Clavijo[1,2]

[1] Veterinary Diagnostic and Population Animal Medicine, Iowa State University, Ames, IA, United States
[2] PIC®, Hendersonville, TN USA

Email: mclavijo@istate.edu **Phone:** 612-8684396

S. suis diagnostic trends

S. suis was the most frequently detected and diagnosed systemic bacterial agent at the ISU VDL between 2017-2022, e.g., 27% of all bacteriology cases included *S. suis* isolation and 22% of all cases with infectious etiology (bacteria or viruses) included a *S. suis* disease diagnosis. Furthermore, although a trend was not detected, it was the predominant pathogen in neurological cases (70.6%% of all cases). Within nervous cases, *S. suis* disease was more frequently diagnosed without other infectious etiologies. *S. suis* disease was regularly diagnosed in suckling and nursery phases of pig production, regardless of the lesion, as reported previously. Specifically, the number of cases of *S. suis* meningitis disease was higher in suckling and early-nursery (up to 6-week-old) than late-nursery pigs, correlating with previous reports. The anlaysis also highlighted the significant upward trend in *S. suis* endocarditis in growing and finisher pig cases. Taken together, these findings highlight the significant role *S. suis* plays in central nervous system in younger piglets as a primary pathogen and endocarditis in older pigs.

These data also showed constant upward trend in *S. suis* bronchopneumonia cases in the last 6 years; however, within bronchopneumonia cases, *S. suis* was co-detected with other agents, such as *G. parasuis*, *M. hyorhinis*, IAV, and PRRSV. Therefore, the significant increase of *S. suis* bronchopneumonia might also by explained by the increase of primary viruses (IAV and PRRSV). These observations align with previous reports on the role of *S. suis* as a secondary pathogen in respiratory disease cases. This study demonstrated that *S. suis* played a role within the multiple systemic cases along with other infectious pathogens, e.g., *S. suis* disease cases were often found as unique diagnosed etiology and interacting with primary viruses and other bacteria.

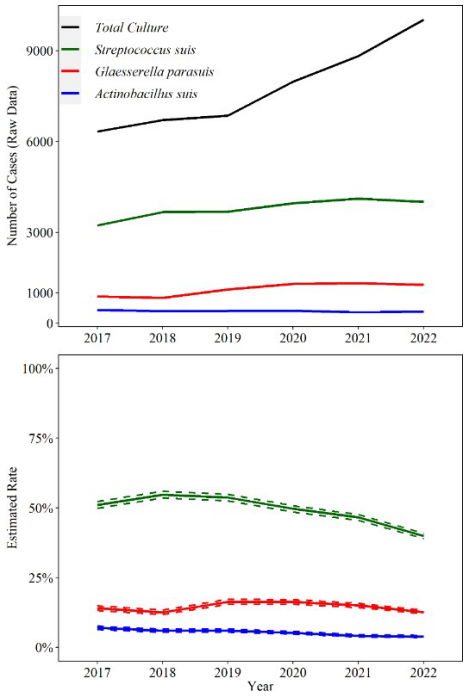


Figure 1. Trendlines (raw data and estimated rate) of overall detection of *Streptococcus suis*, *Glaesserella parasuis*, and *Actinobacillus suis* using bacteriologic culture. The annual estimated rate (solid line) and 95% confidence interval (dashed line) referred to the output of a Binomial regression model which modeled the total number of cases with detection of each agent (colored lines in raw data plot) divided by the total number of bacterial cultures for each year (black line in raw data plot). Black line is based on specimens of interest (see Figure 2).

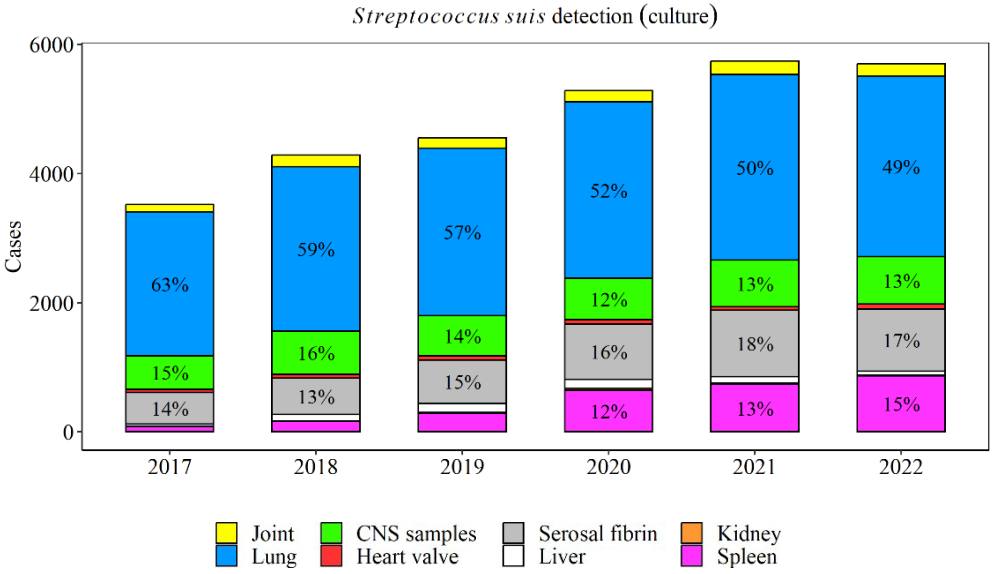


Figure 2. Distribution of specimens with isolation of *Streptococcus suis* over a 6-year period.

Dynamics of infection of disease-associated *Streptococcus suis* (DASS*)* in the lactation phase

Disease-associated *Streptococcus suis* (DASS) is one of the most impacting bacterial diseases in swine worldwide. A key aspect to the control and prevention of DASS in swine farms hinges upon accurate detection of the disease-causing strains within the herd. This can be challenging due to its commensal nature, variability of on farm sampling methodologies, and lack of feasible diagnostic methods that predict virulence with high sensitivity. Developing improved sampling and testing methodologies allow veterinarians and producers to improve *S. suis* surveillance programs, make timely adjustments to prevention programs. Therefore, novel diagnostic strategies are needed to understand the infection dynamics of virulent strains and the value of potential interventions. Understanding the infection dynamics of key agents that drive antimicrobial use in swine farms is paramount, and there is limited data on the infection dynamics of DASS strains in swine farms. The overall goal of this study was ***to describe the parity effect, optimal sampling site for DASS detection, and dynamics of infection of DASS in healthy dams, piglets, and the environment using a novel DASS qPCR assay.*** In this study, we implemented a novel way of tracking DASS in a prospective cohort study in two pig flows, using an informative virulence-associated marker. A sensitive and specific real time PCR was validated, optimized and used to test antemortem samples from a longitudinal study to track DASS dynamics (e.g., detects only disease associated strains), without the need to pursue bacteriological culture. In this study, 50 dams (25 P0 and 25 p4+) from the same farrowing week per farm were selected via stratified random for sampling by tonsil, nasal and vaginal swab collection at farrowing, and only tonsil swabs at weaning. In farm 2, additional samples were collected from sows, which included fecal swabs, farrowing crate swabs, and udder swabs. On the same day of farrowing, 14 piglets were randomly selected from each of the 50 litters, between 2-12 hours post birth. Piglets were ear-tagged, and one tonsil swab was collected from each piglet. A total of 631 and 629 piglets were sampled from farms 1 and 2, respectively. The piglets were sampled again at 7 and 21 days of age. All samples were also tested by a species-specific PCR (e.g. detects all strains from the species by targeting the *recN* gene). All dams and piglets were *recN* positive in tonsil swabs, which highlights the high frequency of this species within pig population. Tonsil scrapings and nasal swabs revealed to be the samples of choice for detection of DASS in healthy dams compared to vaginal swabs. In one farm, nasal swabs provided the same or a better level of detection of DASS, compared to tonsil scrapings, suggesting the use of a more convenient sample type for surveillance of DASS. In terms of DASS carriage in dams from farm 1, the odds of 1130 gene positivity was 82% lower in sows compared to gilts (odds ratio 0.17, 95% CI 0.4=35, 0.53, $P < 0.05$), a trend not seen in farm 2. The piglet DASS positivity followed a similar pattern between the two farms. In these herds between 20-70% of piglets were positive at birth, with a significant decrease in prevalence observed at day 7 (18-45%), followed by a significant increase in the prevalence of 1130 at day 21. The decrease at day 7 could reflect a transient detection at birth, or the effect of antibodies in colostrum. Furthermore, the DASS positivity was 83% and 63% lower in piglets from sows than piglets from gilts on farms 1 and 2, respectively ($p<0.05$), highlighting a potential parity effect on DASS piglet carriage. At the litter level, the average litter prevalence was 32.7% and 64.97%, for farm 1 and 2, respectively. DASS was detected in farrowing crates, fecal, and udder samples, suggesting other relevant sources of infection for piglets, that should be further explored. Determining the timing and prevalence of colonization with virulent *S. suis* strains allows for the refinement of control and/or elimination strategies, such as strategic medication and vaccination, early weaning or test and removal of individuals harboring DASS.

The 8th International Symposium on Emerging And Re-Emerging Pig Diseases

ISERPD 2023

BANGKOK, THAILAND

5th International Workshop on *Streptococcus suis*

Hosted by :

TSVA

UPDATE ON AUTOGENOUS VACCINES

PROFS. MARIELA SEGURA & MARCELO GOTTSCHALK
Faculty of Veterinary Medicine
University of Montreal, Canada

7th
June
2023

Funded by:

IDRC · CRDI
International Development Research Centre
Centre de recherches pour le développement international

GAMRIF
Global AMR Innovation Fund

UKaid
from the British people

Update on autogenous vaccines (bacterins)

M. Segura[1] and M. Gottschalk[1]

[1]Research Group on Infectious Diseases in Production Animals (GREMIP) & Swine and Poultry Infectious Diseases Research Center (CRIPA), Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, QC, Canada.

The usefulness of autogenous vaccines for *S. suis*

Restrictions in the use of antibiotics brought, among other consequences, an increase of clinical disease in post-weaned piglets. Among those pathogens affecting nursery piglets, *S. suis* is one of the most important concern. Controlling stress and predisposing factors (mainly concomitant infections, environmental and management factors) may significantly help to reduce disease[1]. However, as the complexity of *S. suis* epidemiology in swine increases (multiple strains, multiple serotypes), field reports describing difficulty in disease control are common[1,2]. A logical alternative is the use of vaccines. However, so far, there is no commercial vaccine able to protect against all serotypes/strains of *S. suis*. Since universal commercial vaccines are still not available, the only alternative practitioners have in hands is the use of autogenous vaccines (mostly bacterins). Indeed, autogenous vaccines are "simply" bacterins based on the predominant strain(s) recovered from diseased pigs in the affected farm and produced by accredited laboratories[3].

Defining a universal autogenous vaccine is not possible

In theory, producing a killed bacterin is not complicated. However, there are so many ways to produce such a vaccine that it is literally impossible to compare two autogenous vaccines produced by two different licensed laboratories. Some of the variables introduced during the production of these bacterins, that may affect their immunogenicity and their protective capacity, are presented in the table below:

Characteristic	Variables
Bacterial growth (a)	Exponential vs Stationary
Bacterial growth (b)	Solid medium vs Liquid medium
Bacterial growth (c)	If liquid medium: shaking flasks? biofermentor?
Bacterial growth (d)	Type of medium
Bacterial growth (e)	Aerobic, microaerophilic or anaerobic conditions
Body of the vaccine	Washed bacteria or bacteria + supernatant
Bacterial concentration	High vs Very high; keep concentration when several serotypes/bacterial species included?
Inactivation procedure	Formalin (concentration?) vs others
Adjuvants (a)	Type of adjuvant: type of immune response?
Adjuvants (b)	Concentration?

Some experimental data with bacterins produced under different conditions

a) Since adjuvants can dramatically influence the vaccine-induced antibody response, we will present data on the capacity of different adjuvants to induce antibody response and protection against an experimental infection with *S. suis* serotype 2.

b) The use of multiple serotypes within the same bacterin is a common practice in the field. We will also discuss results obtained with a polyvalent bacterin (including up to five different serotypes) when compared to a monovalent bacterin.

c) Some extracellular antigens, such the suilysin, have been suggested to be involved in protection[4]. We will be presenting results on the comparison of a bacterin produced with washed-killed bacteria *vs.* a product containing also concentrated supernatant produced during bacterial culture (including suilysin).

Are young piglets able to induce an antibody response when vaccinated with a bacterin?

Previous field studies carried out by us with an autogenous vaccine showed a complete lack of antibody response (and protection) when piglets are vaccinated at processing and weaning (around 1 and 3 weeks of age). These results could be attributed to the following reasons: a) lack of immunogenicity of the formulation used; b) interference with maternal antibodies and/or c) lack of maturity of the immune system of piglets. In the last part of the talk, we will present data on:

1. Comparison of the immune response of piglets vaccinated at 1 and 3 weeks of age *vs.* those vaccinated at 3 and 5 weeks of age with a same bacterin.
2. The influence of maternal antibodies when piglets are vaccinated at 1 and 3 weeks of age.

To study the second objective, we developed a model of colostrum-deprived conventional piglets, which were vaccinated with a *S. suis* bacterin at 1 and 3 weeks of age and their response compared to piglets vaccinated with the same protocol but in the presence of very high titer of maternal antibodies.

References

1. "Diseases of Swine".11th Edition. p.934-950, 2019.
2. Vet Res. 52:492021, 2021.
3. Porcine Health Manag. 6:12, 2020.
4. Vet Rec. 139:225-8, 1996.

ORAL PRESENTATIONS

A small RNA Promotes *Streptococcus suis* Survival in Host by Enhancing Oxidative Stress Resistance, Survival in Iron-limited Condition, and Glycerol Uptake

Zijing Liang, Zongfu Wu*

OIE Reference Lab for Swine Streptococcosis, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

Introduction

Streptococcus suis is a Gram-positive pathogen that can cause septicemia and meningitis in pigs and humans. However, the mechanisms of its pathogenesis are still not fully understood. Recent studies have suggested that small RNAs (sRNAs) play crucial roles in regulating the virulence of several bacteria [1]. In our previous study, we found that the sRNA rss03 promotes the survival of *S. suis* in pig blood and that deletion of rss03 reduces *S. suis* virulence [2]. Nonetheless, the targets of rss03 and the underlying pathogenic mechanisms remain unclear.

Materials and Methods

The location and sequence conservation of rss03 in the *Streptococcus* genome were determined using bioinformatics analysis. To identify rss03 targets, a combination of CopraRNA prediction, MS2-affinity purification coupled with RNA sequencing (MAPS), and quantitative differential proteomics were utilized, and the targets were validated by gel retardation assays. To investigate the regulatory networks of rss03, several *in vivo* and *in vitro* experiments were conducted, including gel retardation, measurement of mRNA stability, intracellular H_2O_2 concentration, oxidative stress survival, mice infection, and intracellular survival in macrophages.

Results

The small RNA, rss03, was found in *S. suis* various serotypes and its close relatives, *Streptococcus parasuis* and *Streptococcus ruminantium*, and was conserved in location and sequence in the genome of *S. suis* serotype 2 (SS2), suggesting a similar biological function in SS2. The targetome of rss03 was identified, and it was found to bind to multiple targets. The MAPS method was used to identify *S. suis* sRNA direct targets, which had significant advantages. rss03 promotes *S. suis* survival in the host by promoting the mRNA stability of its direct target, *glpF*, which encodes the glycerol channel protein and contributes to glycerol uptake and efflux of H_2O_2. Furthermore, rss03 enhances *S. suis* survival under iron-limited conditions and resistance to oxidative stress by positively regulating the protein levels of its indirect target, the iron-sulfur cluster synthesis gene cluster *sufCDSUB*. Additionally, rss03 further promotes *glpF* expression by inhibiting the protein level of the transcriptional regulator DeoR.

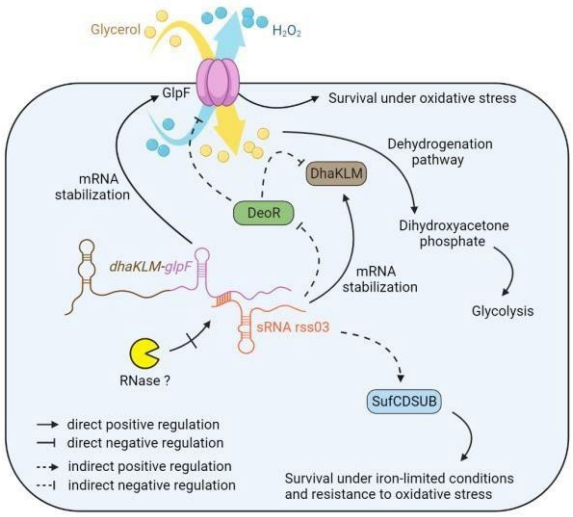


Figure 1. Schematic drawing of the regulatory mechanism of rss03 in *S. suis* pathogenesis.

Discussion

While there has been a lot of research on sRNA and its regulatory mechanisms in Gram-negative bacteria, less is known about Gram-positive bacteria. In this study, we employed various strategies to identify and validate the targetome of sRNA rss03 in *S. suis*. Our findings show that sRNA rss03 enhances *S. suis* survival in the host by improving resistance to oxidative stress, survival in iron-limited conditions, and glycerol uptake. This research provides a deeper understanding of the pathogenic mechanism of *S. suis* and enriches knowledge of the sRNA regulation of virulence for Gram-positive bacteria.

Acknowledgements

This work was supported by National Natural Science Foundation of China [31872469].

References

1. Quereda et al. (2017) Annu Rev Microbiol 71, 263-280.
2. Wu et al. (2014). RNA 20, 882-898.

***Correspondence:**
Zongfu Wu, Email: wuzongfu@njau.edu.cn

Coinfection with *Bordetella bronchiseptica* does not enhance disease with *Streptococcus suis*

S.J. Hau[1]; D.W. Nielsen[1,2]; S.L. Brockmeier[1]
[1]*National Animal Disease Center, USDA, Ames, IA;* [2]*ORISE, ORAU, Oak Ridge, TN*

Introduction

Bordetella bronchiseptica and *Streptococcus suis* are widely distributed swine pathogens that contribute to post-weaning morbidity and mortality. *B. bronchiseptica* is a primary pathogen that causes atrophic rhinitis and bronchopneumonia (1). *S. suis* is a contributing agent to porcine respiratory disease complex and causes systemic diseases including arthritis, meningitis, polyserositis, and septicemia (2). *B. bronchiseptica* can enhance colonization with pathogenic bacteria, such as *Pasteurella multocida*, *Glaesserella parasuis*, and *S. suis*. It also has been shown to increase severity of viral and bacterial infections by causing increased tissue damage and increased release of pro-inflammatory cytokines (3-5). When animals are commingled post-weaning, *B. bronchiseptica* can rapidly disseminate to naïve animals. This may enhance colonization with other bacterial pathogens and increase the prevalence of disease within the herd. To better understand the interaction between *B. bronchiseptica* and *S. suis* and determine if *B. bronchiseptica* is contributing to the development of *S. suis* disease post-weaning, we evaluated coinfection in conventional and cesarean derived, colostrum deprived (CDCD) pigs.

Materials and Methods

Two studies were performed: study one evaluated coinfection in conventional pigs and study two evaluated coinfection in CDCD pigs. For study one, 47 pigs were divided into four groups: *B. bronchiseptica* challenged (n=12), *S. suis* challenged (n=12), *B. bronchseptica* and *S. suis* coinfection (n=12), and non-infected (n=11). Conventional pigs in study one were challenged with a highly virulent *S. suis* isolate 7 days after *B. bronchiseptica* inoculation. Necropsies were performed on day 7 and 21 post-challenge with *S. suis* to evaluate systemic lesions and distribution of *S. suis*. For study two, 30 CDCD pigs were divided into three groups: *B. bronciseptica* challenged (n=6), *S. suis* challenged (n=12), and *B. bronchiseptica* and *S. suis* coinfection (n=12). CDCD pigs in study two were challenged with a less virulent *S. suis* isolate 7 days after *B. bronchiseptica* inoculation. For study two, all animals were necropsied 14 days post-challenge with *S. suis*. In both studies, colonization was assessed every other day for the first week post-challenge and weekly thereafter. Serum was collected on day 0 and at necropsy to evaluate antibody titers.

Results

Study one revealed increased nasal colonization with *S. suis* in animals inoculated with *B. bronchiseptica*. No conventional animals developed systemic disease with *S. suis* during the study period and no evidence of systemic disease or systemic distribution of *S. suis* was found at necropsy. Similarly, study two found increased *S. suis* colonization in coinfected animals. There was no statistical increase in *S. suis* disease; however, three animals developed clinical *S. suis*: one in the *S. suis* infected group and two in the coinfected group. Antibody titers to *B. bronchiseptica* were higher for animals inoculated with *B. bronchiseptica*, but *S. suis* titers were similar between groups.

Discussion

Infection with *B. bronchiseptica* caused increased colonization with *S. suis* but did not enhance systemic disease. However, increased colonization may contribute to disease when animals are stressed or immunocompromised, such as during commingling at weaning.

References

1. Brockmeier et al. (2019) Diseases of Swine 11 ed.
2. Gottschalk M. (2019) Diseases of Swine 11 ed.
3. Harris and Switzer (1968) Am J Vet Res 29, 777-85.
4. Vecht et al. (1989) Am J Vet Res 50, 1037-43.
5. Brockmeier et al. (2001) Vet Res 62, 521-5.

The streptococcal phase-variable Type I Restriction-Modification system SsuCC20p dictates *Streptococcus suis* methylome and impacts virulence

T.J. Roodsant[1,2], B. van der Putten[1,2], J. Brizuela[1,2], J.P.M. Coolen[3], T.J.H. Baltussen[3], K. Schipper[2], Y. Pannekoek[2], K. van der Ark[1,2] and C. Schultsz[1,2]

[1] *Amsterdam UMC, location University of Amsterdam, Department of Global Health, Amsterdam Institute for Global Health and Development, Meibergdreef 9, Amsterdam, The Netherlands*

[2] *Amsterdam UMC, location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Meibergdreef 9, Amsterdam, the Netherlands*

[3] *Radboud University Medical Centre, Department of Medical Microbiology, Nijmegen, The Netherlands*

Introduction

Phase-variable Type I Restriction Modification (RM) systems are epigenetic regulation systems that can impact gene expression and virulence of bacterial pathogens [1]. Type I RM systems consist of three host *hsd* genes encoding a specificity subunit (HsdS), a modification subunit (HsdM) and a restriction subunit (HsdR) [2]. A trimeric subunit complex (2HsdM,1HsdS) can methylate specific sites of the bacterial genome [2]. Phase-variable Type I RM systems can recombine the *hsdS* gene to form different functional *hsdS* alleles, which have unique methylation profiles [2]. The emerging zoonotic lineage of *Streptococcus suis* clonal complex (CC) 20 has acquired a Type I RM system named SsuCC20p [3]. SsuCC20p is suggested to be phase variable and hypothesized to impact virulence via epigenetic gene regulation [3].

Materials and Methods

SsuCC20p homologues were searched in the NCBI Refseq Genomes Bacterial Database using (t)blastn. The phase variability of SsuCC20p was assessed using a FAM-labelled PCR with subsequent endonuclease digestion and fragment analysis, named FAM assay. The methylome of *S. suis* wildtype, locked mutants (LM) that expressed only a single *hsdS* allele and a D*hsdS* mutant were characterized by PacBio SMRT and HiFi sequencing. LM gene expression was determined by RT-qPCR and virulence was compared in a zebrafish larvae infection model.

Results

The SsuCC20p locus was identified in 22 *S. suis*, 17 *S. agalactiae* and 5 other streptococcal isolates, which were all associated with disease. Three SsuCC20p alleles were identified within a single isolate using the FAM assay, demonstrating SsuCC20p phasevariability. Knocking out the site-specific recombinase encoded in SsuCC20p halted phase variation. While multiple methylation profiles could be detected in a WT strain, the D*hsdS* lacked genome methylation demonstrating that SsuCC20p methylates the *S. suis* genome. In LMs, we characterized the methylation profile of the three *hsdS* alleles identified in the WT strain (Fig 1A). The LMs that were genetically identical except the *hsdS* allele and genome methylation differed in gene expression levels and virulence in a zebrafish larvae infection model (Fig 1B).

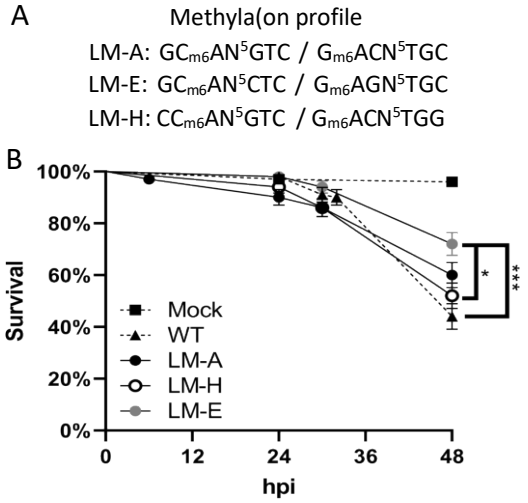


Figure 1. m6A methylation profiles identified in SsuCC20p LM (A). Zebrafish larvae survival after Yolk injection by LM or WT *S. suis* (B).

Discussion

The Type I RM system SsuCC20p is phase-variable, present in multiple streptococcal species and methylates the *S. suis* genome. Phase variability depends on the site-specific recombinase encoded in the SsuCC20p locus. Different *hsdS* alleles result in unique genome methylation profiles, which impacts gene expression and virulence of zoonotic CC20 *S. suis*.

Acknowledgements

We thank T.Bradley (Amsterdam UMC) I. Schouten (Amsterdam UMC) E. Johnson (SNPsaurus) M.P. Kwint (Radboudumc) and R. Derks (Radboudumc) John Atack (Griffith University) for their support and collaboration. Our work was funded by the European Union Horizon2020 grant 727966 (PIGSs).

References

1. Seib et al. (2020) Clin Infect Dis 48:617–625.
2. De Ste Croix et al. (2017) FEMS Microbiol Rev 41:S3-S15.
3. Willemse & Schultsz (2016) Pathogens 5:62.

Burden of disease and productivity impact of *Streptococcus suis* infection in Thailand

A. Rayanakorn[1,2], Z. Ademi[3], D. Liew[3], L-H. Lee[1]

1 Novel Bacteria and Drug Discovery Research Group (NBDD), Microbiome and Bioresource Research Strength, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway, Malaysia; 2 Faculty of Public Health, Chiang Mai University, Chiang Mai, Thailand; 3 School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia

Introduction

Streptoccocus suis (S.suis) infection is a neglected zoonosis disease in humans mainly affecting men of working age. It can cause serious infection in humans through contact with pigs or pig meat, especially via the ingestion of uncooked pork. Meningitis (68%), sepsis (25%) and infective endocarditis (12.4%) are major clinical manifestations of *S.suis* infection, with a mortality rate of 13% [1]. Long-term complications among survivors comprise sensorineural hearing loss (SNHL) with or without vestibular dysfunction and valvular heart disease. SNHL is the most common complication among *S.suis* meningitis patients which is typically bilateral and permanent [1, 2]. The highest prevalence is in Thailand (0.487 per 100,000) [3], followed by Vietnam (0.249 per 100,000) [4]. In western countries, the disease predominantly affects farmers and abattoir workers whereas risk behaviors including raw pork consumption are an important risk factor in Asia. The previous study in Vietnam estimated that disability-adjusted life years (DALYs) last ranged 1,437-1,866 from 2011-2014 with the mean direct cost of US$1,635 (95%CI 1,352-1923) reflecting substantial economic impact [4]. To our knowledge, no previous study estimating economic burden related to *S.suis* infection in Thailand. Therefore, we sought to quantify the health and economic burden of *S.suis* infection in Thailand in terms of years of life lost, quality-adjusted life years (QALYs) lost and productivity-adjusted life years (PALYs) lost.

Materials and Methods

Model subjects were Thai people infected with *S.suis* in 2019, with a starting age of 51 years. Transition probabilities, and inputs pertaining to costs, utilities and productivity impairment associated with long-term complications were derived from published sources. A lifetime time horizon was adopted, with follow-up until death or age 100 years. A decision-analytic Markov model was developed to simulate the impact of *S.suis* infection and its major complications among Thai people (Fig. 1).

Results

In 2019, it was estimated that the infection incurred 769 years of life lost (14% of predicted years of life lived if infection had not occurred), 826 QALYs lost (21%) and 793 PALYs (15%) lost. These equated to an average of 2.5 years of life, 2.6 QALYs and 2.5 PALYs lost per person. The loss in PALYs was associated with a loss of 346 million Thai baht (US$11.3 million) in GDP, which equated to 1.1 million Thai baht (US$ 36,033) lost per person.

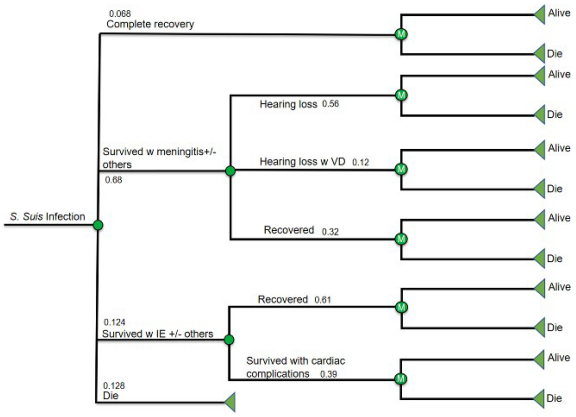


Figure 1. A decision tree and Markov models of *S.suis* infection involving acute and post-infection phase of which there were different health states: complete recovery, partial recovery (hearing loss, hearing loss with vestibular dysfunction, not recovered with infective endocarditis), and death

Discussion

S.suis imposes significant economic burden on quality of life and productivity in Thai population. The findings call for increased public health awareness and comprehensive efforts to control and prevent disease. Further research to investigate the effectiveness of public health and disease control interventions should be performed to provide a clearer picture for decision making in public health strategies and resource allocations.

References

1. Huong et al. (2014) Emerg Infect Dis. 20(7):1105-14.
2. Tan et al. (2010) Singapore Medical Journal. 51(2):e30-e3.
3. Bureau of Epidemiology DoDC, Ministry of Public Health, Thailand. (2019) [cited 2019 25 November]. Available from: https://pr.moph.go.th/?url=pr/detail/2/02/133931/.
4. Huong VTL et al. (2019) Trans. R. Soc. Trop. 113(6):341-50.

Streptococcus suis is an emerging underreported zoonotic pathogen in Europe: a systematic review and molecular epidemiology study

J. Brizuela[a, b] , , T. Roodsant[a, b], Q. Hasnoe[a, b], B.C.L. van der Putten[a, b], J. Kozakova[c], H.C. Slotved[d], M. van der Linden[e], I.G.A. de Beer-Schuurman[f], E. Sadowy[g], J.A. Sáez-Nieto[h], V. Chalker[i], K.C.H. van der Ark[a, b], C. Schultsz[a, b]

a Amsterdam UMC, location University of Amsterdam, Department of Global Health, Amsterdam Institute for Global Health and Development, Meibergdreef 9, Amsterdam, The Netherlands;b Amsterdam UMC, location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Meibergdreef 9, Amsterdam, the Netherlands; c National Reference Laboratory for Streptococcal Infections, Centre of Epidemiology and Microbiology, National Institute for Public Health, 100 42 Prague, Czech Republic; d NSRlab, Department of Bacteria, Parasites and Fungi, Statens serum Institut, DK-2300 Copenhagen, Denmark; e German National Reference Center for Streptococci, Department of Medical Microbiology, University Hospital RWTH Aachen, D-52074 Aachen, Germany; f Dutch Reference Laboratory for Bacterial Meningitis, Department of Medical Microbiology, Amsterdam UMC, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands; g National Medicines Institute, 00-725 Warsaw, Poland; h Department of Bacteriology. National Center for Microbiology. Int. Health Carlos, Majadahona, Madrid, Spain.; i Respiratory and Vaccine Preventable Bacteria Reference Unit, National Infection Service, Public Health England, NW9 5EQ London, United Kingdom

Introduction

Streptococcus suis is an opportunistic swine pathogen which can cause severe infections in humans leading to meningitis and sepsis [1]. Zoonotic infections in Europe account for ~10% of the total reported infections worldwide [2]. Because *S. suis* infections are not notifiable in Europe, these infections are likely under-reported. We aimed to assess reporting of European zoonotic *S. suis* infections across the past three decades and to describe the molecular epidemiology of European zoonotic *S. suis*.

Materials and Methods

We performed a retrospective survey of seven reference laboratories in the top pork producing European countries, and a systematic review of the scientific literature. We also scanned the grey literature for signs of under-reporting. We used Illumina whole genome sequencing (WGS) to analyse the genomes of 46 human *S. suis* isolates, supplemented with 28 publicly available human *S. suis* genomes, by reconstructing a core-genome phylogeny of European zoonotic *S. suis*.

Results

A total of 236 unique cases of *S. suis* infection were identified through systematic review and survey. We detected 87 additional unique cases in the grey literature. Meningitis was the most common clinical syndrome followed by sepsis. Clonal complex (CC) 1 serotype 2 isolates were reported in all countries and accounted for 72% of sequenced isolates. We identified the first human infections caused by clades CC25, CC87 and CC94 in Europe. There were signs of clonal expansion within the zoonotic lineages, as well as capsular switching.

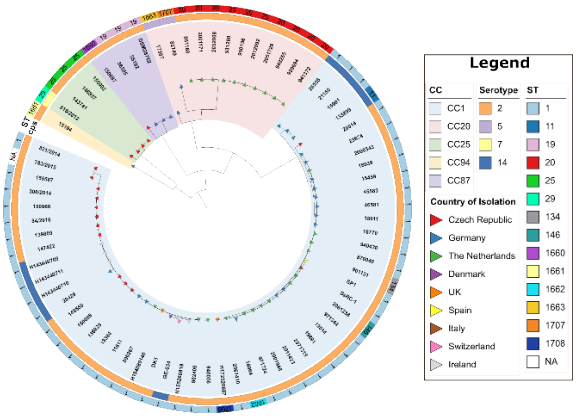


Figure. Genomic population structure of European zoonotic *S. suis*. The Maximum likelihood tree was reconstructed using IQ-TREE using a core genome alignment produced with Panaroo. The country from which each isolate was collected is indicated by the coloured triangles at the tip of the branches. Each lineage (CC) is marked by the coloured ranges. The inner coloured ring indicates sequence type (ST) and is labelled accordingly. The outer coloured ring marks the serotype as determined by the antigenic properties of the capsular polysaccharide (cps). iTOL was used to visualize the tree.

Discussion

S. suis infections are emerging and under-reported in Europe. The emergence of zoonotic clades and the severity of zoonotic *S. suis* infections warrants their notification.

References

1. Huong et al. (2014) Emerg Infect Dis 20(7):1105–14.
2. Segura et al. (2020) Pathogens 9(5):374.

The emergence and spread of pathogenic lineages of *Streptococcus suis*

G. G. R. Murray[1,2], A. S. Md. M. Hossain[2], M. Matuszewska[2], H2020 PIGSs Consortium, ZELS MPP Consortium, M. Gottschalk[3], N. T. Hoa[4], M. Clavijo[5], J. M. Wells[2,6], A. D. Tucker[2], L. A. Weinert[2]

[1]Department of Genetics, Evolution and Environment, UCL, UK; [2]Department of Veterinary Medicine, University of Cambridge, UK; [3]Département de pathologie et microbiologie, Université de Montreal, Canada; [4]Oxford University Clinical Research Unit, Ho Chi Minh city, Vietnam; [5]College of Veterinary Medicine, Iowa State University, USA; [6]Animal Sciences Department, University of Wageningen, The Netherlands

Introduction

The intensification of farming systems has led to increases in the size, density and mobility of livestock populations. This is predicted to promote pathogen emergence and spread [1]. *Streptococcus suis* is a common cause of disease in modern pig farms and an important human zoonotic pathogen. It is also a ubiquitous component of the microbiota of the upper respiratory tract of healthy pigs [2]. Here we use population-genomic analyses to investigate how and why more pathogenic lineages of *S. suis* have emerged and diversified within farmed pig populations.

Materials and Methods

We sequenced the genomes of 1,484 isolates of *S. suis* and assembled the genomes of an additional 1,587 isolates from short-read data from published collections and collaborators. These isolates were sampled from Europe, North America, Asia and Australia, and date from 1960 to 2020. They include samples from the tonsils of healthy pigs, sites of infection in pigs with *S. suis* disease, and human infections. We analysed variation within core genes and in the presence of accessory genes to established the global population structure of *S. suis* and its association with pathogenicity. We constructed dated phylogenies of pathogenic lineages and fit a discrete trait model to estimate rates of between-country transmission. We identified genomic islands associated with pathogenic lineages, and analysed their genetic diversity to determine when and from where they were acquired.

Results

We identified ten pathogenic lineages of *S. suis* that account for 80% of disease-associated isolates in our collection and 90% of disease-associated serotypes. They fall within a subpopulation that is distinguished by variation in core genes and accessory genome content. The six lineages that are most common in our collection originated between 1838 and 1951 (Fig. 1a). All show evidence of repeated between-country transmission, broadly reflecting patterns of international trade in live pigs (Fig. 1b).

We identified three genomic islands associated with these pathogenic lineages. They have distinct evolutionary histories. One was vertically inherited from a common ancestor of all pathogenic lineages, another was horizontally acquired several times from outside of *S. suis*, and another has been repeatedly horizontally transferred between pathogenic lineages. We also find evidence of the adaptive diversification of these lineages through further horizontal gene transfer, including the transfer of the serotype 2 capsular locus.

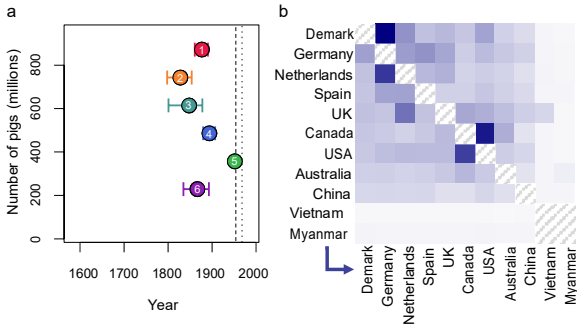


Figure 1. (a) Estimates of the dates of origin of the six most common pathogenic lineages with 95% confidence intervals. The solid line is an estimate of the global number of pigs, the vertical lines the first reported cases of *S. suis*-disease in pigs (dashed) and humans (dotted). (b) A heat plot showing rates of between-country transmission averaged over the six lineages. Deeper colours represent higher rates.

Discussion

Our results reveal how global shifts in farming practices have promoted the emergence and spread of pathogenic lineages of *S. suis*. We find that all pathogenic lineages emerged from one sub-population via a process of gene acquisition both from other species and from existing pathogenic lineages. This means that measures that control the spread of existing pathogenic lineages should also impede the emergence of novel pathogenic lineages. Our results also provide a new whole-genome based typing schema that will support new insights into the epidemiology of *S. suis*.

Acknowledgements

This study was supported by a Horizon 2020 grant "PIGSs", a BBSRC ZELS grant "MPP", a Sir Henry Dale Fellowship to L. A. Weinert, and a Newnham College Research Fellowship to G. G. R. Murray.

References

1. Jones et al. (2013) Proc Natl Acad Sci 110, 8399-8404.
2. Vötsch et al. (2018) Front Microbiol 9, 480.

European *Streptococcus suis*: Genomic Insights into Diversity and Resistance

Type text here

K. Li[1,2], S. Lacouture[1], H. Gantelet[3], E. Lewandowski[3], E. Thibault[3], M. Gottschalk[1], N. Fittipaldi[1].

[1] *Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada;* [2] *Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada;* [3] *Ceva Biovac, Beaucouzé, France.*

Introduction

Streptococcus suis is a swine pathogen responsible for a variety of diseases, leading to substantial economic losses in the porcine industry [1]. To date, 29 distinct serotypes have been identified based, originally, on a serological reaction against the capsular polysaccharide [2, 3]. Multi-locus sequence typing (MLST) is employed to determine the sequence type (ST) of a strain, with over 2,100 *S. suis* STs currently defined [4]. Among European isolates, ST1 serotype 2 and ST16 serotype 9 are the most common groups, although other serotypes and genetic groups can also cause infections [1]. Concerns regarding antimicrobial resistance (AMR) in *S. suis* are emerging [5]. In this study, we used genomic-level approaches to analyze the population structure, AMR profiles, and virulence-associated gene (VAG) content of *S. suis* strains from seven European countries.

Materials and Methods

Using convenience sampling, 251 isolates were obtained from diseased swine in Belgium, France, Germany, Hungary, the Netherlands, Spain, and the United Kingdom between 2012 and 2020. These isolates were previously considered for use in autogenous vaccine formulations. Genome sequencing was performed using Illumina short-read technology, and bioinformatic methods were applied to determine serotype, ST, AMR genes, and VAG content from the short-read data [6]. Phylogenetic analysis and population structure were determined through single-nucleotide polymorphism (SNP) at core-genome resolution relative to a set of reference genomes for each major MLST group.

Results

Serotypes 1, 1/2, 2, 3, 4, 5, 7, 8, 9, 10, 16, 18, and 23 were identified in our collection (Figure 1), along with 33 total STs, of which 14 were novel. Serotypes 2 and 9 comprised 72.9% of the isolates, with the majority belonging to ST1 and ST16, respectively, consistent with previous findings on European *S. suis* strains. Isolates within these groups exhibited differences in AMR gene content; however, no observable differences were noted in the classical VAG content (*epf*, *mrp*, *sly*) among strains in each of the major groups. Among all strains, 85.3% contained at least one AMR gene, with tetracycline and macrolide resistance-associated genes being the most prevalent.

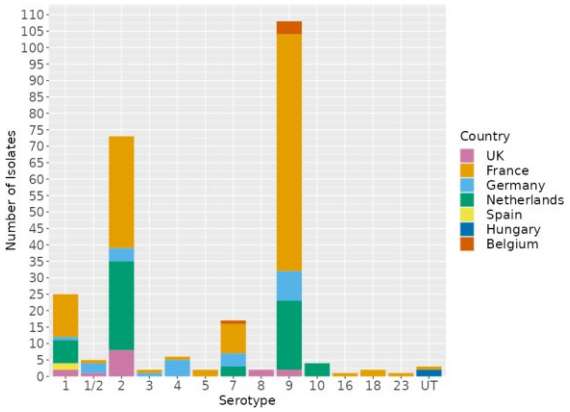


Figure 1. Serotype distribution of *S. suis* strains in 7 different European countries. UT: untypable.

Discussion

S. suis isolates from European clinical cases demonstrate considerable genetic diversity. Strains belonging to major serotype 2 and 9 genetic clades display conserved classical VAG profiles but differ in their AMR profiles. Genomic-level population characterization provides essential, accurate information for appropriate antimicrobial treatment and autogenous vaccine formulations using relevant strains.

Acknowledgements

This study was funded in part by the Natural Sciences and Engineering Research Council of Canada (Grant # 2022-04223 to NF and grant #-2022–03730 to MG).

References

1. Goyette-Desjardins et al. (2014) Emerg Microbes Infec, 3(6), e45.
2. Feng et al. (2014) Trends Microbiol, 22(3), 105-108.
3. Kerdsin et al. (2016) J Med Microbiol, 65(6), 546-552.
4. Jolley et al, (2018). Wellcome Open Res, 3, 124.
5. Huang et al. (2016) Front Cell Infect Microbiol, 6, 118.
6. Zhou et al. (2018) Genome Res, 28(9), 1395-1404.

A new generation glycoconjugate vaccine against *Streptococcus suis*

Todd L. Lowary[1], Pei-Jhen Li[1], Manas Jana[1], Marcos Lo Fiego[1], Ryan P. Sweeney[1], Potjanee Srimanote[2], Mélanie Lehoux[3], Marcelo Gottschalk[3], and Mariela Segura[3]

[1] *Department of Chemistry, University of Alberta, Edmonton, AB, Canada;* [2] *Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand;* [3] *Swine and Poultry Infectious Diseases Research Center (CRIPA), Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada*

Introduction

Streptococcus suis causes significant economic losses to the swine industry and raises concerns about animal welfare. This organism is also an emerging zoonotic pathogen. In the absence of effective commercial vaccines, the incidence of disease in pigs is controlled by extensive antimicrobial prophylaxis [1]. *S. suis* is covered by a capsular polysaccharide (CPS), which is the only essential virulence factor. Of the 29 described serotypes based on the CPS antigenicity, serotype 2 is the most clinically prevalent. Studies have proven the protective capacity of CPS-specific antibodies. CPS is thus an attractive antigen but its poor immunogenicity limits its use as vaccine. However, when conjugated to protein carriers to produce glycoconjugate vaccines, carbohydrates acquire the required immunochemical ability, as shown by their successful application in human medicine. We established a proof-of-concept vaccine that protects pigs against *S. suis* serotype 2 challenge using native CPS conjugated to tetanus toxoid [2]. Unfortunately, glycoconjugate standard production methods are complex, resulting in high-cost vaccines. Recent advances in chemical synthesis and formulation design have spawned a new generation of carbohydrate-based vaccines. These developments overcome many of the limitations associated with traditional glycoconjugate vaccines. In this study, we designed the first chemically-synthesized glycoconjugate vaccine against *S. suis* and provide proof-of-concept of its protective capacity.

Materials and Methods

Eight fragments of the CPS from *S. suis* serotype 2, ranging in size from a monosaccharide to a heptasaccharide (1, 4, 7 and 10–14, **Fig. 1A**) were selected for synthesis. These compounds were prepared bearing an 8-azidooctyl (or 8-aminooctyl) linker to facilitate their conjugation to the carrier protein CRM197 (a non-toxic mutant of diphtheria toxin). To prepare conjugates of the antigens and CRM197, a maleimide–thiol coupling reaction was used. Loadings on the CRM-197 were determined by MALDI mass spectrometry. Immunogenicity and protective capacity of glycoconjugate candidates were evaluated in mouse and swine models.

Results

Mouse immunization pre-trials were performed for a preliminary target selection based on the capacity of conjugated-CPS fragments to induce a high anti-CPS

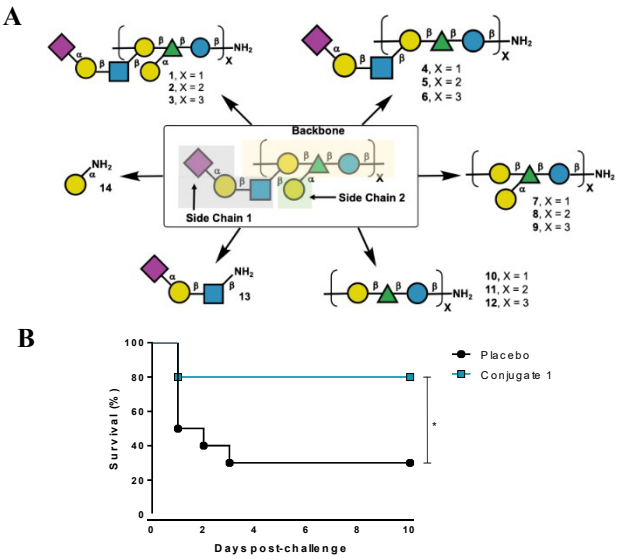


Figure 1. A) Schematic representation of CPS fragments (epitopes) targets. **B)** Survival rate of piglets vaccinated with glycoconjugate #1 formulated with Montanide ISA 61 VG.

antibody response, a diversity of IgG sub-classes and functional ability of antibodies to eliminate *S. suis* by opsonophagocytosis. Results of mouse immunization studies revealed that fragments #13 and #14 are unable to induce an optimal immunological response. In the swine immunization and challenge model, the levels of antibodies induced by the different pre-selected conjugated-CPS fragments (#1, #4, #7, and #10) vary but the IgG isotype pattern was similar. However, not all conjugated-CPS fragments were efficient in inducing protection. Conjugated-CPS fragments #1 (**Fig. 1B**) and #10 showed strong to partial protection and are thus promising targets for a swine vaccine.

Discussion

Our findings demonstrate the importance of not only chemically design the right epitope but also that clinical evaluation in pigs is required to predict the real value of a chemically-synthesized fragment as a vaccine candidate.

Acknowledgements

The present study was financially afforded by International Development Research Centre (IDRC) and GlycoNet, Canada.

References

1. Gottschalk and Segura (2019) ISBN: 978-1-119-35092-7.
2. Goyette-Desjardins et al. (2016) Infect Immun 84:2059.

Comparative virulence and antimicrobial resistance distribution of *Streptococcus suis* isolates obtained from the United States

P01

T.L. Nicholson, D.O. Bayles

National Animal Disease Center, Agricultural Research Service (USDA), Ames, IA, United States

Introduction

Streptococcus suis is a zoonotic bacterial swine pathogen causing substantial economic and health burdens to the pork industry worldwide. Most *S. suis* genome sequences available in public databases are from isolates obtained outside the United States. The goals of the current study were to fill this gap by utilizing whole-genome sequencing (WGS) analysis to evaluate the sequence type (ST) and serotype distribution of *S. suis* isolates obtained within the U.S., screen genomes for genomic elements encoding antimicrobial resistance (AMR), determine whether or not identified AMR genes were located within mobile genetic elements (MGE), and screen for genomic elements encoding factors known to increase or contribute to the capacity of *S. suis* to transmit, colonize, and/or cause disease in humans.

Materials and Methods

WGS and draft assemblies were obtained for 106 *S. suis* isolates acquired from swine samples collected within the U.S. and submitted to the University of Minnesota Veterinary Diagnostic Laboratory between 2015 and 2017. MLST and serotype determination was performed *in silico* [1-3]. Clonal complexes were identified by goeBURST analysis [4]. Known *S. suis* virulence-associated genes [5], were identified using BLASTN searches. The Virulence Factor Data Base [6] was additionally employed to search for genes encoding virulence-associated factors. Phenotypic antibiotic resistance was determined using the broth microdilution method by National Veterinary Services Laboratories (Ames, IA). Abricate was used to identify AMR genes from the Comprehensive Antibiotic Resistance Database (CARD) [7], ResFinder [8], and the NCBI Bacterial Antimicrobial Resistance Reference Gene Database. Identification of MGEs was conducted using MGEfinder v1.0.6 [9].

Results

Forty-six STs were identified with ST28 observed as the most prevalent, followed by ST87. Of the 23 different serotypes identified, serotype 2 was the most prevalent, followed by serotype 8 and 3. Of the virulence genes analyzed, the highest nucleotide diversity was observed in *sadP*, *mrp*, and *ofs*. Hierarchical clustering of the nucleotide percent identity for the virulence-associated genes revealed a correlation between clonal complex and the presence of genes encoding known *S. suis* virulence factors. Tetracycline resistance was the most prevalent phenotypic antimicrobial resistance observed followed by macrolide-lincosamide-streptogramin B (MLSB) resistance. Numerous AMR elements were identified, many located within MGE sequences, with the highest frequency observed for *ble*, *tetO*, and *ermB*. No genes encoding factors known to contribute to the transmission, colonization, and/or causation of disease in humans were identified in any of the *S. suis* genomes in this study, including the 89 K pathogenicity island carried by the virulent *S. suis* isolates responsible for human infections.

Discussion

Collectively, the data reported here provide a comprehensive evaluation of the genetic diversity among U.S. *S. suis* isolates. This study also serves as a baseline for determining any potential risks associated with occupational exposure to these bacteria, while also providing data needed to address public health concerns.

Acknowledgements

The authors are grateful for exceptional technical support provided by Sarah M. Shore. Funding was provided by the USDA, ARS project number 5030-32000-119-00-D. This research used resources provided by the SCINet project of the USDA, ARS project number 0500-00093-001-00-D.

References

1. Athey et al. (2016) BMC Microbiol 16, 162-170.
2. Liu et al. (2013) PLoS One 8, e72070-e72081.
3. Qiu et al. (2016) Appl Environ Microbiol 82, 7102-7112.
4. Francisco et al. (2009) BMC Bioinform 10, 152-167.
5. Fittipaldi et al. (2012) Future Microbiol 7, 259-279.
6. Liu et al. (2022) Nucleic Acids Res 50, D912-D917.
7. Jia et al. (2017) Nucleic Acids Res 45, D566-D573.
8. Zankari et al. (2012) J Antimicrob Chemother 67, 2640-2644.
9. Durrant et al. (2020) Cell Host Microbe 27, 140-153.

Immunoglobulin M-degrading enzyme of *Streptococcus suis* (Ide$_{Ssuis}$) impairs porcine B cell signaling

A.K. Breitfelder[1], W. Schrödl[1], V. Rungelrath[1], C.G. Baums[1], G. Alber[2], N. Schütze[2], U. Müller[2]

[1] *Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Germany*

[2] *Institute of Immunology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Germany*

Introduction

Streptococcus suis (*S. suis*) is one of the most important porcine pathogens, causing severe pathologies like meningitis, polyarthritis, and septicemia (1). Disease mostly occurs in piglets after weaning, but *S. suis* is also a very successful colonizer of mucosal surfaces in pigs of various ages without causing disease.

The cysteine-protease Immunoglobulin M-degrading enzyme of *S. suis* (Ide$_{Ssuis}$) is host and isotype-specific as it specifically cleaves porcine IgM between constant domain C2 and C3 (2). Cleavage of soluble IgM by Ide$_{Ssuis}$ has been shown to be a novel complement evasion mechanism as it reduces labelling of bacteria with C3b and subsequent opsonophagocytosis (3, 4). We hypothesized that Ide$_{Ssuis}$ also cleaves the IgM B cell receptor (BCR). Therefore, this study investigated the cleavage activity of Ide$_{Ssuis}$ on porcine IgM$^+$ B cells and tested the working hypothesis that cleavage of the IgM BCR interferes with IgM BCR-mediated signaling.

Materials and Methods

Cleavage of the IgM BCR by recombinant Ide$_{Ssuis}$ and respective variants as well as BCR signaling was analyzed by flow cytometry of porcine B cells. Furthermore, loss-of-function experiments with supernatants of specific *S. suis* mutants were conducted.

The proportion of B cells positive for tyrosine-phosphorylated phospholipase C-γ2 (PLC-γ2) after *in vitro* stimulation was analyzed as a parameter of early B cell activation after treatment with different variants of Ide$_{Ssuis}$.

Results

Flow cytometry analysis revealed cleavage of the IgM BCR by recombinant (r) Ide$_{Ssuis}$_homologue as well as Ide$_{Ssuis}$ derived from culture supernatants of *S. suis* serotype 2 on porcine PBMCs and mandibular lymph node cells. Point-mutated rIde$_{Ssuis}$_homologue_C195S did not cleave the IgM BCR. After receptor cleavage by rIde$_{Ssuis}$_homologue, it takes at least 20 h for mandibular lymph node cells to restore the IgM BCR to levels comparable to cells previously treated with rIde$_{Ssuis}$_homologue_C195S. pPLC-γ2$^+$ cells as parameter for BCR-mediated signaling after specific stimulation via the IgM F(ab')$_2$ portion were significantly reduced by rIde$_{Ssuis}$_homologue receptor cleavage in IgM$^+$ B cells, but not in IgG$^+$ B cells. Within IgM$^+$ cells, CD21$^+$ B2 cells and CD21$^-$ B1-like cells were equally impaired in their signaling capacity upon rIde$_{Ssuis}$_homologue BCR cleavage. To check the ability of B cells to phosphorylate PLC-γ2 after BCR-independent, intracellular stimulation, tyrosine phosphatase inhibitor pervanadate was used. This stimulation increased signaling in all investigated B cell types, including IgM$^+$ B cells pretreated with rIde$_{Ssuis}$_homologue (5).

Discussion

This study demonstrates Ide$_{Ssuis}$ cleavage efficacy on the IgM B cell receptor and its consequences for B cell signaling. These findings suggest modulation of antigen-dependent B-cell responses by *S. suis* through Ide$_{Ssuis}$ expression and a putative advantage in colonization and early infection. We plan to investigate this hypothesis *in vivo* in future experiments.

Acknowledgements

The present study was funded by the German Research Foundation (DFG).

References

1. Gottschalk and Segura (2019) Streptococcosis. In: Zimmerman JJ, editor. Diseases of swine. 11th edition. Hoboken, NJ: Wiley-Blackwell; p. 934–50.

2. Seele et al. (2013) Identification of a novel host-specific IgM protease in Streptococcus suis. J Bacteriol; 195(5):930–40.

3. Seele et al. (2015) The immunoglobulin M-degrading enzyme of Streptococcus suis, IdeSsuis, is involved in complement evasion. Vet Res; 46:45.

4. Rungelrath et al. (2018) IgM cleavage by Streptococcus suis reduces IgM bound to the bacterial surface and is a novel complement evasion mechanism. Virulence; 9(1):1314–37.

5. Breitfelder et al. (2023) Immunoglobulin M-degrading enzyme of Streptococcus suis (IdeSsuis) impairs porcine B cell signaling. Front. Immunol; 14

Assessment of immunogenicity of different autogenous Streptococcus suis bacterins in piglets

H. Gantelet[1], A. Gaudreau[2], E. Thibault[1], E. Lewandowski[1], M. Segura[2], M. Gottschalk[2]
[1] Ceva Biovac, Beaucouzé, France
[2] Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada

Introduction

Streptococcus suis (S. suis) is a major porcine pathogen causing high morbidity and mortality worldwide, mostly in weaned piglets [1]. Both the lack of suitable vaccines and the pressure to reduce the use of antibiotics led in the recent years to a strong rise of use of autogenous vaccines in Europe [2]. These are inactivated bacteria (bacterins) which are often adjuvanted in different ways. However, their composition is highly variable, and very few studies report the immunological responses induced by the autogenous S. suis bacterins. Our study was performed to evaluate the humoral response.

Materials and Methods

Three different formulations of S. suis serotype 2 bacterins were included in this study. All three formulations were bivalent since a serotype 9 isolate was also included (data not shown for serotype 9). Two formulations (groups A and B) were prepared with the same water-in-oil emulsion but at two different bacterial concentrations while the third one used an oil-in-water emulsion (group C).

Ten piglets were assigned to each vaccine group (A, B and C) and the group D was included as an unvaccinated negative control. Three-week-old piglets (from a farm free of Porcine Reproductive and Respiratory Syndrome virus and without endemic clinical diseased caused by S. suis) were vaccinated twice with a 1 mL dose intramuscularly at a 14 day-interval (groups A, B and C) in the animal facilities of the University of Montreal.

The magnitude (total Igs) and profile (antibody classes: IgG1, IgG2, IgM) of the vaccine-induced antibody response were evaluated by ELISA test against serotype 2 as coating antigen.

An opsonophagocytosis assay (OPA) was performed to determine the capacity of serum antibodies to induce a serotype 2 killing [3].

Results

The groups A (Figure 1) and B resulted in a very strong increase of anti-S. suis 2 total antibodies. Moreover, in these two cases, the immunological vaccine response was composed of high levels in IgG1 and IgG2 subclasses. In addition, the induction of potentially protective antibodies (as shown by OPA results) has been successfully explored for groups A and B against serotype 2 by the opsonophagocytosis assay.

Group C presented a weak but significant increase in total Igs 10 days after the second immunization. Moreover, the OPA activity of these antibodies was not detected (compared to the negative control group D).

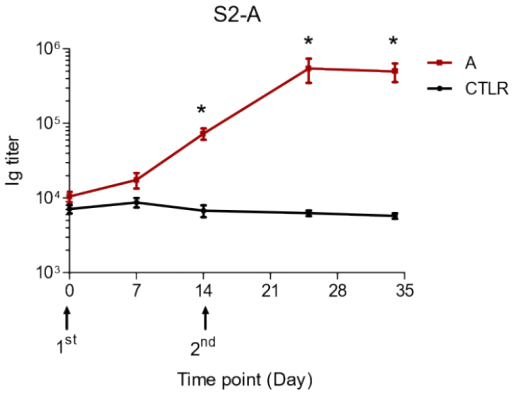


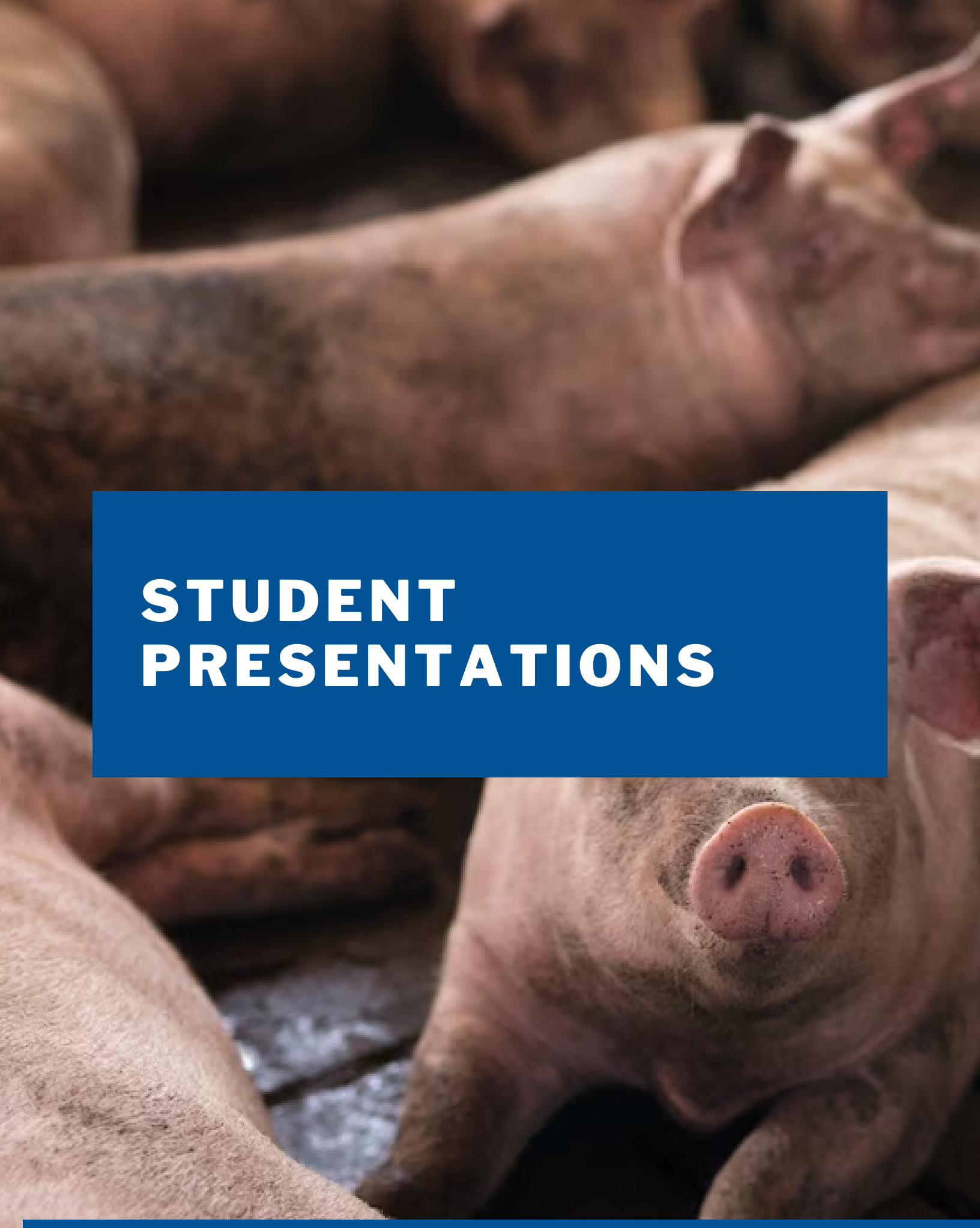
Figure 1. Kinetics of antibody production (total Igs: IgG + IgM) against serotype 2 (S2) with vaccine A, versus the control group (CTLR).
*, indicates a statistically difference ($P < 0.05$) vs control group at respective time point.
1st, first immunization; 2nd, second immunization.

Discussion

Our results showed that the methods used to produce autogenous vaccines are a major element to induce an effective immune response against S. suis. Autogenous vaccine bacterins using relevant adjuvants are key for the efficacy and the adjuvant used in the water-in-oil emulsion gave an excellent result which confirms previous studies done with this type of emulsion [4, 5].

References

1. Gottschalk and Segura (2019) Streptococcosis. Diseases of swine. 11th ed. West Sussex: WileyBlackwell, 934-951.
2. Rieckmann et al. (2020) Porc Health Manag 6:12.
3. Corsaut et al. (2020). Vaccines. 8:384.
4. Wisselink et al. (2001) Vet Microbiol 148:473-477.
5. Obradovic et al. (2021) Vet Res 52:133.



STUDENT PRESENTATIONS

Mobile genetic elements that carry macrolide-lincosamide and tetracycline resistance genes in *Streptococcus suis* strains isolated in France

Manon Dechêne-Tempier[1,2], Eric Jouy[1], Virginie Libante[2], Stéphanie Bougard[3], Sophie Payot-Lacroix[2], Corinne Marois-Créhan[1]

Manon Dechêne-Tempier, (manon.dechene-tempier@univ-lorraine.fr),
[1] Anses Laboratoire de Ploufragan-Plouzané-Niort, Unité Mycoplasmologie, Bactériologie et Antibiorésistance, BP53 22440 Ploufragan, France; [2]Université de Lorraine, INRAE, DynAMic, F-54000 Nancy, France ; [3] Anses Laboratoire de Ploufragan-Plouzané-Niort, Unité Épidémiologie, santé et bien-être, BP53 22440 Ploufragan, France.

Introduction

Streptococcus suis is a zoonotic bacterial pathogen whose serotype distribution varies between countries [1]. Tetracyclines and macrolides belong to antibiotics largely used in swine industry [2]. High resistance rates have been described for those antibiotics in *S. suis*. In 2012, a first plan (Ecoantibio) was launched in France with the objective of a 25% decrease of antibiotic use in the veterinary field. This plan was successful and led to a 37% reduction of antibiotic use in 5 years. In *S. suis*, all acquired antibiotic resistance (AbR) genes are localized on mobile genetic elements (MGEs) [3]. The aim of this study was to (i) quantify the resistance to macrolides-lincosamides and tetracycline of different pathotypes and serotypes of *S. suis* isolated in different French regions from different hosts, and at different time periods (ii) identify the genes conferring those resistances and the MGEs that carry them.

Material and Methods

A total of 200 strains isolated before 2010 and after 2015 in several French regions and belonging to 3 pathotypes (non-clinical, respiratory, systemic) and 21 serotypes (serotypes 2, 9, 7, 3, 1, 1/2, and others) isolated in swine, wild-boars and humans, were included in the analysis. Tetracycline, erythromycin, tilmicosin, tylosin, lincomycin and clindamicin, were tested using the disk diffusion method according the Clinical & Laboratory Standards Institute (CLSI). Results were interpreted with the criteria specified in CLSI performance standard VET01-S3.

Whole genome sequencing was done for 105 strains were using the Illumina® Technology. AbR genes were identified using CARD and Resfinder databases and MGEs were identified using ICEscreen [4]. Sequences types were determined using reference genes listed in pubMLST. Statistical analyses (Chi square tests with fisher correction) were used to explore the link between the presence of resistance genes and other strain characteristics.

Results

High resistance rates were observed for macrolides-lincosamides and tetracyclines (70-80% of resistance), as already described [5]. AbR genes were mainly carried by EGMs.

In total, 67 *tet*(O) and 76 *erm*(B) genes were identified in the 105 strains analyzed by WGS. Most of these genes were located on IMEs embedded in an ICE of the Tn*5252* family (in the *SNF2* or *PPI* gene of the ICE). Other ribosomal protection determinants were found (*tet*(W) or *tet*(M) in two strains), carried by ICEs (ICE_*lysS* and Tn*916,* respectively). The mosaic *tet*(O/W/32/O) gene was detected, in association with a *tet*(40) gene, in one IME_*SNF2* embedded in one ICE of the Tn*5252* family in three strains. Statistical analysis highlighted a link between the presence or absence of particular AbR genes and strain characteristics listed previously.

Discussion

High resistance rates against tetracyclines and macrolides were detected in the French *S. suis* isolates studied in this work. This correlates with the presence of various resistance genes, which are carried by MGEs. Dissemination of AbR genes by these MGEs likely explains why the reduction of antibiotic use in the veterinary field does not seem to have an impact of these resistance rates. More AbR genes were found in strains of the respiratory pathotype group, indicating a higher potential of resistance spreading of these strains.

References

1. Segura 2020; Pathogens 9(9):707.
2. Weinert et al 2015; Nat Commun 6(1):6740.
3. Lekagul et al 2019; Veterinary and Animal Science 7:100058.
4. Lopez de Egea et al 2023 ; Antibiotics;12(3):579.
5. Libante et al 2020; Pathogens 9(1):1-23.

Contact-dependent growth inhibition of serotype 2 *Streptococcus suis* by commensal non-serotype 2 *S. suis*

P. Prasopthum[1], J. Thanongsaksrikul[1], O. Khantisitthiporn[1], B. Arechanajan[1], P. Srimanote[1]

[1]*Graduate Program in Biomedical Sciences, Faculty of Allied health sciences, Thammasat University, Pathum Thani 12120, Thailand*

Introduction

S. suis is a zoonotic pathogen that can cause a wide range of diseases in young pigs, including meningitis, arthritis, septicemia, and pneumonia. In pigs, *S. suis* is considered a pathobiont. Under normal circumstances, they inhabit as a commensal when the host health is compromised, the same strain to be pathogenic [1]. Serotype 2 *S. suis* (*SS2*) is the most critical serotype isolated from disease pigs worldwide [2]. However, *SS2* is isolated from healthy pigs at a very low frequency compared to the other serotypes.

Most of the commensal bacteria interact and co-exist in a high-density community, leading to the competition to regulate the population number of each strain to utilize the limited nutrients and occupy space. It is possible that the rare isolation of *SS2* from the respiratory tract of healthy pigs may be a consequence of growth competition by the commensal streptococci or non-*SS2* strains. However, the growth competition of *S. suis* mediated by the intra- or inter-species has not yet been reported. Therefore, this study aims to determine the growth inhibition and interaction phenotype of *SS2* mediated by commensal non-*SS2*.

Materials and Methods

A total of 67 *SS2* and 180 non-*SS2* were use in this study. The *SS2* reference strain P1/7 was used as the control in all experiment. Firstly, the growth inhibition of *SS2* by non-*SS2* strains were screened using agar direct challenge assay. The agar proximity and transwell assays were used to differentiate whether the growth inhibition occurred by contact-dependent mechanism or soluble substances. Finally, the kinetic of growth inhibition and consequence of *SS2* growth inhibition by non-*SS2* strains were elucidated using bacterial broth co-culture assay. Whole genome sequencing was used to determine the factor involving growth inhibition

Results

Agar direct challenge assay revealed that only 14 non- *SS2* strains could inhibit the growth of *SS2* strains. These strains were analyzed further. Only three of these inhibit the growth of *SS2* by a contact-dependent mechanism. The two non-*SS2* strains and 10 *SS2* strains were selected and assigned into 22 competitive pairs to characterize the interaction phenotype and consequence of growth competition by broth co-culture assay. It was found that the growth of *SS2* strains can be contact-dependently inhibited by non-*SS2* with two interaction phenotypes, competition and amensalism. Competition phenotype resulted in the co-exist of *SS2* among non-*SS2* at a low density, while the amensalism phenotype yielded the complete elimination of *SS2* from the niche. The genetic determinant contributing to this contact-dependent mechanism may involve T7SS.

Discussion

Commensal non-*SS2* can inhibit the growth of SS2 by a contact-dependent mechanism, influencing the maintenance of a low density or absence of the virulent potential *SS2* strain in the upper respiratory tract of healthy pigs.

Acknowledgments

This work was supported by (i) Thammasat University Fundamental Fund Fiscal year 2566, (ii) Thailand Science Research and Innovation (TSRI), and (iii) National Science, Research and Innovation Fund (NSRF) (Project number180178)

References

1. Votsch et al. (2018) Front Microbiol 9, 480.
2. Goyette et al. (2014) Emerg Microbes Infect 3(6), e45.
3. Abdallah et al. (2007) Nat Rev Microbiol 5(11), 883-91.
4. Liang et al. (2022) Virulence 13(1), 781-7

Molecular Characterization of Spanish Streptococcus suis clinical isolates belonging to serotypes 1-14 and 2-1/2

Type text here

J. Arnal[1], S. Lacouture[2], A. Fernández[1], M. Ubieto[1], J. Arenas[3], C. Uruen[3], M. Gottschalk[2]

[1]Exopol, Zaragoza, Spain. [2]Université de Montreal, St. Hyacinthe, Canada. [3]Universidad de Zaragoza, Zaragoza, Spain.

Introduction

Laboratorial diagnosis of *Streptococcus suis* usually determines the serotype and certain virulence markers (VM) to try to foresee its virulent potential. Serotypes 1-14 and 2-1/2 are estimated to be involved at least in the 30% of swine streptococcal outbreaks in Spain [1].

Nevertheless, the serological cross reactivity and the high homology at cps gene level hinder a complete differentiation. Thus, we decided to develop a set of qPCR assays to detect specifically the abovementioned serotypes. The study of serotype, VM and Sequece Type (ST) through MLST [2] allowed us to generate useful epidemiological data of *S. suis* in Spain. We were also interested in the genetic diversity of those *S. suis* isolates which cause recurrent issues within a productive system during long periods of time.

Materials and Methods

Novel qPCR assays based on high affinity hydrolysis probes which target cpsK gene were validated using reference strains and clinical isolates previously characterized by serological method and mamaPCR [3]. Moreover, other 98 clinical strains coming from 49 different Spanish farms already characterized either as serotypes 1-14 (n=45) or 2-1/2 (n=53) were analyzed by qPCR and mamaPCR. Inconsistent results were solved through Sanger sequencing. In addition, virulence markers (VM: sly, epf and mrp) were studied. Then some isolates all coming from different farms were selected to perform MLST studies: serotypes 1-14 (n=21) and serotypes 2-1/2 (30). Furthermore, 18 isolates from 3 farms which had been affected by a particular serotype of *S. suis* during the last two years were studied also by MLST as follows: farm A (ser1, n=6); farm B (ser1/2, n=7) and farm C (ser2, n=5).

Results

The qPCR was capable to determine the serotype of every isolate and both techniques presented an almost perfect concordance (Kappa=0.94). Fortyfour samples (98%) of the 1-14 isolates were confirmed as serotype 1 meanwhile 1 isolate (2%) was determined as serotype 14. Thirty-one strains (58%) of the 2-1/2 isolates were confirmed as serotype 2 whereas 22 isolates (42%) were characterized as ½. All the isolates belonging to serotypes 1, 2 and 14 presented the same VM pattern: sly+/epf+/mrp+. Serotype 1/2 isolates showed 3 different VM combinations: sly+/epf+/mrp+(68%); sly-/epf-/mrp+(13%); sly+/epf-/mrp+ (18%).

Different ST`s were detected in this study, nevertheless, ST1 was the most frequently found in those isolates from serotype 1 (n=14, 70%), serotype 14 (n=1, 100%), serotype 2 (n=15, 94%) and serotype 1/2 (n=8, 57%). Two new alleles were described: 439 for *aroA* and 384 for *thrA*. Clonal complex 1 (CC1) were predominant in all the serotypes studied but significative differences ($p<0.01$) was found between serotype 2 and serotype ½ isolates.

All the isolates but one from the 3 selected farms resulted ST1. Two farms, those with serotypes 2 and ½, did not present any change in the ST of their isolates meanwhile the other with serotype 1 isolates obtained minor variability because all its strains belonged to CC1.

Discussion

Our results highlight the importance of discerning between the serotypes 1-14 and 2-1/2, however, few laboratories offer this service. Vast majority of isolates from 1-14 collection was actually determined as serotype 1, that fact would make this individual serotype as one of most prevalent serotype involved in clinical cases in Spain.

Moreover, this research shows an epidemiological situation for serotype 2 very similar than the one from Europe; their isolates barely present genetic variability under the studied terms. Nonetheless, the situation differs in the case of serotype ½ which presented genetic and VM diversity.

References

1. Arnal et al. (2019) 4th International Workshop on Streptococcus suis.
2. King et al. (2002) J Clin Microbiol 40:3671–3680
3. Lacouture et al. (2020) J Vet Diagn Invest. 2020 May;32(3):490-494.

Do lipoteichoic acids play a role in the pathogenesis of *Streptococcus suis*?

S. Payen[1], N. Fittipaldi[1], N. Gisch[2], M. Segura[1] and M. Gottschalk[1].

[1]Faculty of Veterinary Medicine, University of Montreal, Quebec, Canada.
[2] Division of Bioanalytical Chemistry, Priority Area Infections, Research Center Borstel, Borstel, Germany.

Introduction

The pathogenesis of infection and the role of bacterial cell wall components in *Streptococcus suis* virulence have not been fully elucidated. Lipoproteins (LPs), peptidoglycan, as well as wall teichoic acids and lipoteichoic acids (LTA) have all been proposed to contribute to virulence. LTA D-alanylation has been associated with increased resistance to the action of cationic antimicrobial peptides (CAMPs) and phagocytosis [1,2]. *S. suis* LTAs might possess immunostimulatory properties, although it appears that co-purified LPs are the main activators of the innate immune system [3]. Our aim was to better understand the role of LTAs in pathogenesis of the *S. suis* infection.

Materials and Methods

We generated an isogenic mutant of the virulent *S. suis* serotype 2 P1/7 strain that is impaired in LTA production (Δ*ltaS* mutant). To characterize it, we performed hydrophobicity and self-aggregation assays, and evaluated its capacity to form biofilms. We have also analyzed the Δ*ltaS* mutant using different in vitro tests, including susceptibility to antimicrobial peptides, adhesion and invasion of swine respiratory epithelial cells, resistance to phagocytosis by murine macrophages and activation of dendritic cells (cytokine levels by ELISA). Virulence comparisons were also carried out using a mouse model of systemic infection.

Results

Using techniques previously described we confirmed that the isogenic Δ*ltaS* mutant is devoid of LTAs in its cell wall [3]. The growth of the Δ*ltaS* mutant was similar to that of the wild-type parental strain, and absence of LTA did not influence bacterial hydrophobicity. However, absence of LTA was associated with increased self-aggregation (Fig.1) and to increased biofilm production and led to reduced resistance to CAMPs. Importantly, absence of LTAs in the bacterial cell wall did not influence adhesion and invasion of epithelial cells nor resistance to phagocytosis. Impairment in LTA production did not have a noticeable impact on dendritic cell activation. When tested in an in vivo systemic murine model of infection, the Δ*ltaS* mutant behaved essentially as the parental virulent strain.

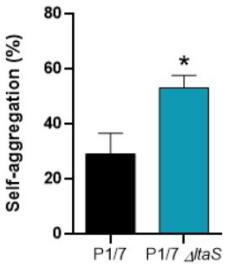


Figure 1. Self-aggregation of *S. suis* serotype 2 wild-type strain P1/7 and its *ltaS* mutant strain. Data represent the mean ± SEM (n = 4). * ($p < 0.05$) indicates a significant difference between wild-type and mutant strain.

Discussion

At present, we cannot determine the exact role of LTAs in the pathogenesis of *S. suis* serotype 2 infection. We are conducting electron microscopy studies to examine the bacterial morphology of the Δ*ltaS* mutant. While the absence of LTA led to changes in some phenotypic characteristics of the mutant, such as self-aggregation and biofilm formation, and increased sensitivity to bactericidal peptides, other characteristics and phenotypes were unaffected by absence of LTAs in the bacterial cell wall, with both mutant and parental strain showing similar results in adhesion and invasion of epithelial cells, resistance to phagocytosis, cytokine-mediated cell activation, as well as in virulence in a mouse model of systemic infection. Notably, previous research has shown that mutants lacking LTA D-alanylation exhibit some of these same characteristics, including a reduction in virulence [1,2] . Therefore, further studies comparing both the LTA D-alanylation and complete LTA-deficient mutants are warranted.

Acknowledgements

This study was funded by the Natural Sciences and Engineering Research Council of Canada to MG (grant #-2022–03730), MS (Grant # 2021–03020) and NF (Grant # 2022-04223).

References

1. Fittipaldi and al (2008) Infect Immun 76, 3587-3594.
2. Ohlmann and al (2022) Front Microbiol 13, 822369.
3. Gisch (2018) J Biol Chem 293, 12011-12025.

Precision genome engineering in *Streptococcus suis* based on a broad-host-range vector and CRISPR-Cas9 technology

A. Gussak[1], M.L. Ferrando[1#], M. Schrama[1], P. van Baarlen[1], J.M. Wells[1]

[1] *Host-Microbe Interactomics, Animal Sciences, Wageningen University, 6708 WD Wageningen, The Netherlands;* [#] current address: Emerging Bacterial Pathogen Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

Introduction

To investigate *S. suis* virulence mechanisms and enhance our understanding of its pathobiology via mutant analysis, it is key that its genome can be efficiently edited. Current methods for genome engineering of *S. suis* rely on the insertion of antibiotic resistance markers [1], which is time-consuming, labour-intensive and does not allow the precise and specific introduction of small genomic mutations. Here we report a system for CRISPR-mediated editing of *S. suis* genomes, utilizing linear DNA fragments for homologous recombination and a plasmid-based negative selection system.

Materials and Methods

The necessary DNA fragments were PCR-amplified and assembled by SOEing PCR or HiFi assembly for the linear and circular constructs, respectively. The sgRNA spacers were ordered as oligonucleotides, annealed to form a dsDNA fragment and cloned into plasmid pSStarget in a one-pot restriction-ligation reaction, thereby replacing the *ccdB* selection marker. For genome editing, the pSStarget plasmid and the repair template were simultaneously transformed into *S. suis* P1/7 as described [2].

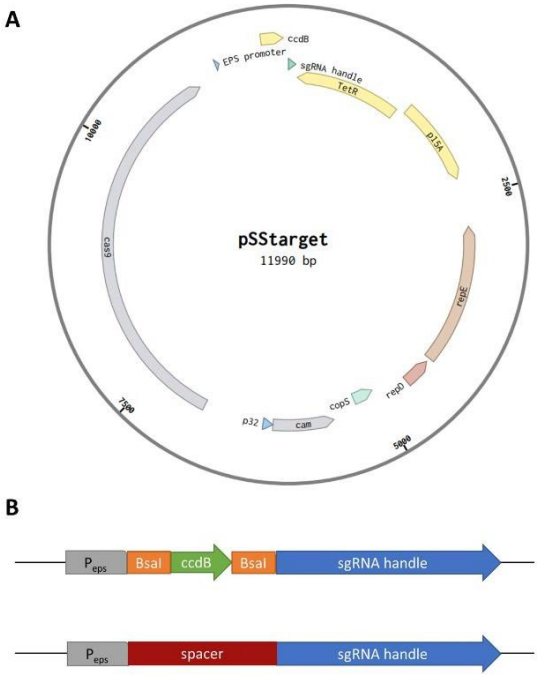


Figure 1. (A) Schematic representation of the pSStarget plasmid showing the complete plasmid map (B) Detailed view of the sgRNA cloning site. Insertion of the target-specific spacer sequence replaces the *ccdB* selection marker.

Results

The design of the pSStarget plasmid (Figure 1) allows cloning of the spacer sequence in a single reaction, simultaneously removing the *ccdB* selection marker from the plasmid. This streamlined procedure eliminates intermediate clean-up steps and colony screening by PCR.

To demonstrate the utility of this system for genome engineering in *S. suis*, we chose to construct KO mutants of three previously characterized virulence factors (*cpsEF, sly, lgt*) with known deletion phenotypes. Indeed, the phenotypes observed in the CRISPR-Cas-generated mutants corresponded to the previously published phenotypes. Absence of off-target effects has been confirmed by whole-genome sequencing of the mutants and the parental strain. The mutant strains were cultured for one night in absence of antibiotics to cure the plasmid, after which another plasmid could be introduced to make additional genomic edits.

To show the use of this system to study essential genes, we also constructed a strain with a single amino acid change in the essential enolase (*eno*) gene.

Discussion

This CRISPR-Cas9 genome editing system is a useful tool to quickly introduce markerless genetic modifications and allows to make precise nucleotide changes in essential genes. The streamlined cloning procedure enables rapid mutant construction. Due to the broad host range of this vector, this system is likely to be functional in a variety of bacterial hosts. Summarizing, we believe that this editing system is a valuable addition to the genetic toolbox for engineering of *S. suis* and possibly other bacteria.

Acknowledgements

We gratefully acknowledge the financial support from European Union's Horizon 2020 research and innovation programme (agreement ID 727966), funded under H2020-EU.3.2.1.1. This study was partially funded by the WIAS Graduate Programme (file number: 022.004.005), which is financed by the Netherlands Organization for Scientific Research (NWO).

References

1. Zhu et al. (2019) Fut. Microbiol 14, 207–222
2. Zaccaria et al. (2014) PLoS One 9, e99394

POSTERS

Factors contributing to the prevalence of Integrative and Conjugative or Mobilizable Elements carrying antimicrobial resistance genes in *Streptococcus suis*

S. Bougeard[1], M. Dechêne-Tempier[1,2], V. Loux[3,4], H. Chiapello[3], V. Libante[2], C. Marois[1], N. Leblond-Bourget[2], S. Payot[2]

[1] Anses Laboratoire de Ploufragan-Plouzané-Niort, Unité Mycoplasmologie, Bactériologie et Antibiorésistance, F-22440 Ploufragan, France; [2] Université de Lorraine, INRAE, DynAMic, F-54000 Nancy, France; [3] Université Paris-Saclay, INRAE, MaIAGE, F-78350, Jouy-en-Josas, France;[4] Université Paris-Saclay, INRAE, BioinfOmics, MIGALE bioinformatics facility, F-78350, Jouy-en-Josas, France

Introduction

Streptococcus suis – a normal inhabitant of the upper respiratory tract of pigs – can lead to severe infections in post-weaning pigs. It can also infect people in close contact with infected animals or through consumption of pork products. Cases are usually sporadic but large outbreaks with fatal cases have been reported in Asia (1). Among the 29 already described serotypes, only a few are responsible for most of the diseases caused by *S. suis.* This zoonotic pathogen carries many antimicrobial resistance (AMR) genes, most of them being located on Mobile Genetic Elements (MGEs) in particular Integrative and Conjugative Elements (ICEs) and Integrative and Mobilizable Elements (IMEs) (2). The purpose of this work was to study the prevalence of these MGEs carrying AMR genes in *S. suis* and to identify factors that can have an impact on their dissemination.

Material and Methods

Using the dRep bioinformatic tool, 2588 genomes (2429 genomes publicly available in RefSeq and 159 newly sequenced genomes) were quality filtered and clustered in order to eliminate quasi-identical genomes and select the best-quality representative genomes. Dereplicated genomes were then scaffolded with Medusa and annotated with Prokka. Core genes were computed using Roary and aligned to build a phylogenetic tree. Metadata (country, year of isolation, host and isolation source of the strains) were collected and several data were extracted from the genomes: (i) serotype, (ii) MLST group, (iii) AMR genes (search in ResFinder and CARD databases and of recently described AMR genes), (iv) virulence genes (72 genes as described by Wileman et al (3)), (v) ICE and IME content using the recently published ICEscreen tool (4), (vi) competence genes (full set of 34 genes) and (vii) restriction-modification (RM) systems (types I, I, III, IV and orphan methylases). Multi-dimensional statistical analyses (using the FactoMineR package) were applied on the dataset to study the relationships between the various variables as well as the similarities between the genomes.

Results

A dataset of 412 high-quality genomes was created that covers the diversity of the *S. suis* species. A total of 584 ICEs and 1069 IMEs belonging to seven families of ICEs and twelve families of IMEs were detected in these genomes. Among the 67 virulence/colonization factors searched, 54 were present in at least 5 strains. Only 12 strains were devoid of AMR genes (1943 AMR genes detected in total) and all the strains carry at least 2 RM systems (3683 systems detected in total). Only 32 strains carry a full set of competence genes. Multi-dimensional statistical analyses revealed: (i) specific MGE co-occurrences, (ii) statistical links between MGE, RM, AMR genes and virulence gene contents and (iii) particular distributions of these genes according to the serotype and isolation site (upper or lower respiratory tract or systemic).

Discussion

This study provides useful indicators in order to characterize the various pathotypes of *S. suis*. It confirms the huge diversity of ICEs and IMEs carrying AMR genes in *S. suis* despite the existence of a plethora of RM defense systems. It also provides interesting information on the interplay between the various families of ICEs and IMEs leading to hypotheses that will be tested experimentally. This dataset of representative high-quality genomes of *S. suis* will also be useful for genomic analysis of other traits of this zoonotic pathogen.

References

1. Dutkiewicz et al. (2018) Ann Agric Environ Med. 24, 683-695.
2. Dechêne-Tempier et al. (2021) Microorganisms 9,1765.
3. Wileman et al. (2019) J Clin Microbiol 57,15.
4. Lao et al. (2022) NAR Genom Bioinform 4, lqac079.

Combination therapy against multidrug-resistant *Streptococcus suis* strains isolated from diseased pigs in vitro

W. Chumpol[1], K. Lunha[1], S. Jiemsup[1], S. Samngamnim[2], P. Assavacheep[2] and S. Yongkiettrakul[1]

[1]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani 12120, THAILAND; [2]Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, THAILAND

Introduction

Prophylactic and therapeutic treatment of zoonotic *Streptococcus suis* infection in humans and pigs mainly rely on antibiotics. Meanwhile, the imprudent use of antibiotics in both agriculture and public health has steadily introduced the emergence of multidrug-resistant *S. suis*, resulting in failure of antibiotic treatment. As antibiotic-resistant *S. suis* strains spreading worldwide, antibiotic combination-based therapy becomes gaining increased attention. The multidrug therapies could allow rejuvenating the effectiveness of old antibiotics, expanding the scope of clinical treatment, and improving drug resistance [1]. This study attempted to discover a synergistic combination of available antibiotics, as an alternative solution to effective treatment of multidrug-resistant *S. suis* infection.

Materials and Methods

S. suis isolated from diseased pigs were kindly obtained from Dr. Pornchalit Assavacheep, Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University. Multidrug-resistant *S. suis* strains were identified and pairwise analysis of the correlation between the antimicrobial susceptibility status to the different antibiotics was investigated using Pearson's correlation analysis [2]. The *S. suis* strains exhibiting distinct multidrug resistance patterns were selected for checkerboard microdilution assay with cell wall synthesis inhibitors (ampicillin, penicillin G, vancomycin, and amoxicillin/ clavulanic acid) and protein synthesis inhibitors (gentamicin, neomycin, tylosin, and tilmicosin).

Results

The checkerboard data identified two possible combination regimens, PEN-GEN and AMP-NEO, exhibiting the greatest level of synergism against multidrug- resistant *S. suis* strains. The synergistic effect of NEO was measurable when combined with the cell wall synthesis inhibitors. No synergistic effect was observed for VAN in combination with GEN, TYL, and TMS. Among the β-lactam antibiotics, AMP only exerted a slight synergistic effect with TMS.

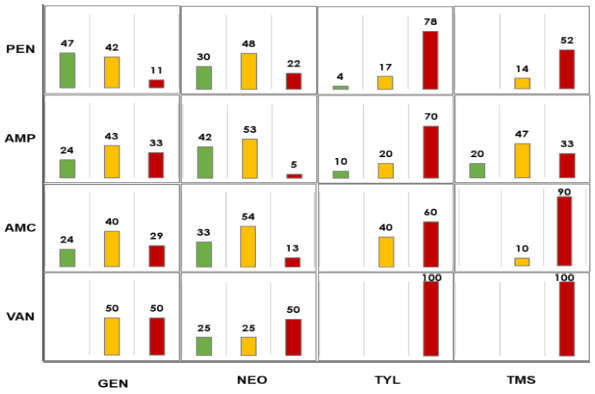


FIGURE 1 Panels of antibiotic combinations. Percentage of synergistic, partial synergistic and indifference are shown in each panel. Synergism (FICI≤ 0.5), partial synergistic (0.5 < FICI ≤ 0.75), and indifference (0.75 < FICI ≤ 2) effects are labeled with ■, ■, and ■, respectively in the corresponding panels. AMP: ampicillin, AMC: amoxicillin/ clavulanic acid, PEN: penicillin G, GEN: gentamicin, NEO: neomycin, VAN: vancomycin, TYL: tylosin, TMS: tilmicosin.

Discussion

The data demonstrated a significant decrease in the minimum inhibition concentration (MIC) value of β-lactams, when combined with aminoglycosides. The synergism of PEN-GEN and AMP-NEO are probably due to the increased permeation of aminoglycosides into cytoplasm after β-lactams inhibiting the cell-wall synthesize by binding with penicillin binding proteins (PBPs) [3]. Our study suggested that the synergistic combination could reduce the dosage of antibiotics which might slow down the advance of antibiotic resistance. This finding could shed light on alternative antibiotic combination therapies for treatment of multidrug-resistant *S. suis* in human and animal infection. However, side effects of multidrug therapy should be concerned in the clinical applications.

Acknowledgements

The present study was financially afforded by NSTDA RDI Non-Research Grant, grant number P21-51-753.

References

1 Coates et al. (2020) Expert Rev Anti Infect Ther, 18.
2 Lunha et al. (2022) Antibiotics (Basel), 410.
3 Wallace et al. (1985) Antimicrob Agents

Streptococcus suis surface-antigen recognition by antibodies and bacterial elimination is influenced by capsular polysaccharide structure

D. Dolbec[1], M. Lehoux[1], M. Okura[2], D. Takamatsu[3,4,5], M. Gottschalk[1], and M. Segura[1]

[1]*Research Group on Infectious Diseases in Production Animals (GREMIP) and Swine and Poultry Infectious Diseases Research Center (CRIPA), Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada*
[2]*Division of Transboundary Animal Disease Research, National Institute of Animal Health, National Agriculture and Food Research Organization, Kagoshima, Kagoshima, Japan*
[3]*Division of Infectious Animal Disease Research, National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan*
[4]*The United Graduate School of Veterinary Sciences, Gifu University, Gifu, Gifu, Japan*
[5]*Joint Graduate School of Veterinary Sciences, Gifu University, Gifu, Gifu, Japan*

Introduction

Streptococcus suis is an encapsulated bacterium that can cause severe invasive diseases in pigs. The thick capsular polysaccharide (CPS) surrounding *S. suis* is a critical virulence factor that provides resistance against host phagocytic cells, allowing the bacteria to survive in the blood and disseminate in the host. The antigenicity of the CPS defines 29 distinct serotypes of *S. suis*, of which the serotype 2 is the most clinically prevalent worldwide. Analysis of the biochemical structure of the CPS revealed a great diversity amongst the serotypes of *S. suis* (1). Knowing that the CPS is critical for virulence and that some serotypes are more commonly associated with the disease than others, our hypothesis was that the structure of the CPS influences survival in the host and resistance against antibodies targeting subcapsular antigens (such as proteins) at the bacterial surface.

Materials and Methods

Serotype-switched mutants (2) of *S. suis* serotype 2 strain P1/7 were employed to compare the role played by the CPS structures of serotypes 2, 3, 4, 7, 8, 9 and 14, since the only difference between these strains is the CPS expressed. Primary and secondary infections were done in a mouse model, and blood and serum samples were taken post-infection to monitor bacterial blood burden and production of antigen-specific antibodies. Binding of antibodies to the subcapsular bacterial antigens of live bacteria was examined by flow cytometry and antibody function was measured via opsonophagocytosis killing assay (OPA).

Results

CPS structure influenced survival in the host. Strains expressing the CPS of the serotypes 3 and 4 were the most susceptible to host defenses during a primary infection. During the secondary infection, strains expressing the CPS of serotypes 3, 4 and 14 were the most eliminated. CPS structure was found to influence antigen recognition by antibodies. The CPS of serotypes 3, 4 and 14 allowed more IgG binding to subcapsular antigens than the CPS of serotypes 2, 7, 8 and 9. CPS structure also affected antibody-induced killing. Mutants expressing the CPS of serotypes 3 and 14 had equivalent killing percentages that were significantly higher than those of the CPS 9 mutant.

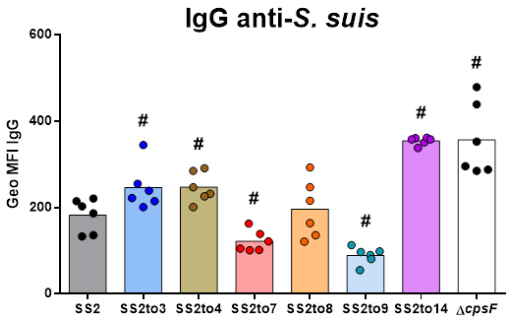


Figure 1. Binding of IgG to *S. suis* serotype 2 (SS2) or its isogenic mutants (SS2to3, SS2to4, SS2to7, SS2to8, SS2to9, SS2to14, Δ*cpsF*) was evaluated by flow cytometry. Data presented as geometric mean fluorescence intensity (Geo MFI) reads and mean.

Discussion

Results suggest that the different CPS structures of *S. suis* provide varying levels of protection by influencing antigen availability and elimination by the host immune system. This finding is of importance for vaccine development and highlights the need to closely monitor cross-protection when designing *S. suis* vaccines since CPS structure might eventually affect the efficacy of vaccines targeting subcapsular antigens at the bacterial surface.

Acknowledgements

This work was mainly supported by NSERC grants to M.S. (no. 342150) and to M.G. (no. 04435). D.D. is the recipient of FRQNT Doctoral Research Scholarship (#268339) and CRIPA scholarship supported by the FRQNT (#RS-170946). M.S. is a holder of a Canada Research Chair – Tier 1.

References

1. Goyette *et al.* (2020) Infect. Immun. 88, 10.
2. Okura *et al.* (2021) Sci. Rep. 11, 6513.

IgM antibodies play a major role in the elimination of *Streptococcus suis* serotype 2

D. Dolbec[1], M. Lehoux[1], A. Zahn[2], J. M. Di Noia[2], and M. Segura[1]

[1]*Research Group on Infectious Diseases in Production Animals (GREMIP) and Swine and Poultry Infectious Diseases Research Center (CRIPA), Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada*
[2]*Montreal Clinical Research Institute, Center for immunity, inflammation and infectious diseases, Montreal, Quebec, Canada*

Introduction

The adaptive humoral response is the result of a communication network between antigen presenting cells (APC), T cells and B cells. Activated B cells undergo antibody class-switch recombination (CSR) and can form germinal centers (GC) where they undergo somatic hypermutation (SHM), that result in the production of antibodies with high affinity towards the antigen of interest. Antibodies play a useful role in the elimination of *Streptococcus suis* (1), an encapsulated bacterium that can cause severe invasive disease in pigs. However, no universal effective commercial vaccine is available to prevent infections. Reports indicate that *S. suis* can interfere with APCs (2) and downstream primary and memory responses of T cells (3). However, the interactions between *S. suis* and B cells and the subsequent development of the adaptive humoral response have not been studied in detail. Thus, the aim of this study was to characterize the development of the adaptive immune response by evaluating GC B cell dynamics as well as the production and role of antibodies induced following *S. suis* infections in a mouse model.

Materials and Methods

C57BL/6 wild-type (WT) mice along with knock-out (KO) lines for factors required for CSR and SHM in GC B cells (*Aidca*$^{-/-}$ and *Ung*$^{-/-}$) were infected with *S. suis* serotype 2. To evaluate the impact of T cell help to B cells, KO lines for *SAP* and *Tcrb* were also employed. The muMT KO line was also used to assess the role played by mature B cells. The spleens and sera of animals were collected at various time points post-infection. Splenic GC B cell population dynamics were determined by FACS. Pathogen-specific antibodies were detected by ELISA against whole bacteria or purified capsular polysaccharide (CPS). Antibody functionality was evaluated by opsonophagocytosis killing assay (OPA).

Results

Anti-*S. suis* IgM and IgG were produced following infection and helped reduce bacteremia *in vivo* and induce partial OPA killing. However, the affinity of antigen-specific IgGs did not improve following infections and it was observed that GC formation was delayed. Depletion of total IgGs from sera did not influence the observed OPA capacity. Anti-CPS antibodies were mainly IgMs and did not require SHM. SAP and Tcrb KO mice had lower anti-CPS titers and hampered bacterial elimination, indicating that the production of anti-CPS IgMs is at least partially T cell-dependent, but did not require CSR or SHM, as shown by *Aicda*$^{-/-}$ and *Ung*$^{-/-}$ mice.

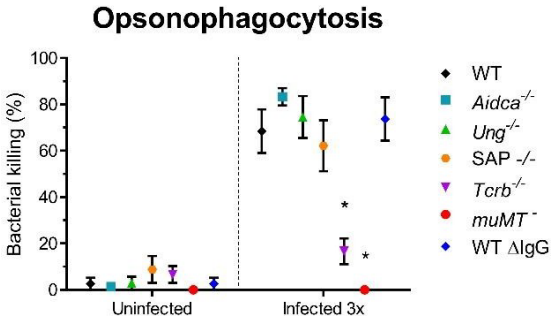


Figure 1. Mouse serum-induced killing of *S. suis* evaluated by opsonophagocytosis killing assay using sera collected from uninfected mice and mice infected three times. IgG antibodies were experimentally depleted of WT sera to obtain WT ΔIgG. Data ($n \geq 5$) presented as mean ± SEM. * indicates significant differences with WT mice.

Discussion

Contrary to what has been expected, *S. suis* does induce a GC reaction. However, GCs did not seem to be required for the optimal elimination of *S. suis* serotype 2, since unmutated IgM were the main antibodies responsible for bacterial elimination. Our findings that identify IgM antibodies as important for *S. suis* elimination, are supported by recent reports that the swine-specific IgM protease of *S. suis*, IdeSsuis, is important for bacterial survival in porcine blood (4).

Acknowledgements

This work was mainly supported by NSERC grants to M.S. (no. 342150). D.D. is the recipient of FRQNT Doctoral Research Scholarship (#268339) and CRIPA scholarship supported by the FRQNT (#RS-170946). M.S. is a holder of a Canada Research Chair – Tier 1.

References

1. Goyette-Desjardins *et al.* (2019) Pathogens 8(3).
2. Letendre *et al.* (2018) Front Immunol 9:p.1199-1199.
3. Lecours *et al.* (2016) Sci Rep 6(1):p.38061.
4. Seele *et al.* (2015) Vet Res 46(1):45.

Identification of an antibiotic targeting *Streptococcus suis* through mechanisms related to oxidative stress and metal homeostasis

Blanca Fernandez-Ciruelos[1], Marco Albanese[2], Paul Finn[2], Jerry M. Wells[1]

[1]Host-Microbes Interactomics, Wageningen University and Research, Wageningen, the Netherlands [2]Oxford-Drug Design, Oxford, UK

Introduction

Streptococcus suis is a commensal of the upper respiratory tract of pigs and pathogenic strains can cause invasive disease, mainly in nursery piglets, leading to substantial losses to the pig industry [1]. *S. suis* is also an emerging zoonotic pathogen causing sepsis and meningitis in humans [2]. *S. suis* disease outbreaks in piglets are mainly controlled by the metaphylactic use of antibiotics contributing to the selection and spread of antimicrobial resistance. Autogenous killed bacterin vaccines are used to control *S. suis* disease but they are not cross-protective and their effectiveness in preventing post-weaning disease in piglets is controversial [3]. An alternative approach to prevent *S. suis* disease around weaning would be to administer narrow-spectrum antibiotics targeting *S. suis*. In this study, we screened a collection of commercially available compounds for inhibition of growth of *S. suis* and identified compound **B97** which specifically inhibits growth of *S. suis* via a novel mechanism of action.

Materials and Methods

A set of 500 commercially available compounds was screened for inhibition of growth of *S. suis* P1/7 and *Staphylococcus aureus*, the minimal inhibitory concentration (MIC) was calculated using the broth microdilution method. Cytotoxicity in HEK293, HepG2 and pig ileum organoids was measured using Alamar Blue and WST-1 assays. Gene expression analysis was performed using qPCR, and emergence of resistance was assayed by growing *S. suis* for several days in subinhibitory concentrations of the compound and variants were analyzed using whole genome sequencing.

Results

A set of commercially available compounds was screened for inhibition of growth of *S. suis* P1/7 and *S. aureus*. Compound **B97** was selected due to tits lack of cytotoxicity to eukaryotic cells and selective inhibition of *S. suis* growth. Its inhibitory activity was conditionally dependent on the growth medium used. Synergistic effects on bacterial growth inhibition were observed with compound **B97** and H_2O_2 or low pH, suggesting that **B97** causes an increase in intracellular oxidative stress. Further mechanistic studies using checkerboard assays and qPCR of relevant genes suggested **B97** may be dysregulating iron homeostasis which would lead to an increase in oxidative stress. Exposure of *S. suis* to subinhibitory concentrations of **B97** led to resistance mutant containing SNPs in the repressor of the peroxide response PerR, supporting the theory that oxidative stress is involved in the mechanism of action of **B97**. Finally, compound **B97** showed bactericidal activity against *S. suis* in pig saliva, the natural habitat of *S. suis* in pigs.

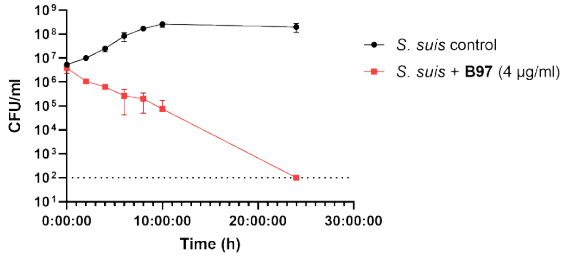


Figure 1. Compound **B97** is bactericidal for *S. suis* in pig saliva.

Discussion

Compound **B97** has been proved to lead to *S. suis* killing in pig saliva, which is an important vehicle for transmission. Compound **B97** has a novel mechanism of action related to oxidative stress and iron homeostasis dysregulation. We hypothesize that **B97** could be used as prophylactic for *S. suis* invasive infection by reducing *S. suis* in the oral cavity, leading to a decrease in invasive disease in piglets. This technique does not rely in the weak immune system of piglets, being an attractive alternative to traditional antimicrobials.

Acknowledgements

This publication is part of the EU Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) financed by ZonMW.

References

1. Neila-Ibáñez et al. (2021) Front Vet Sci 8.
2. Lun et al. (2007) Lancet Infect Dis 7, 201-209.
3. Weinert et al. (2015) Nat Commun 6, 6740.

Streptococcus suis infection on European farms is associated with an altered tonsil microbiome and resistome

Simen Fredriksen[1], Carlos Neila-Ibáñez[2,3,4], Isabel Hennig-Pauka[5], Xiaonan Guan[1,66], Jenelle Dunkelberger[7], Isabela Fernandes de Oliveira[1], Maria Laura Ferrando[1], Florencia Correa-Fiz[2,3,4], Virginia Aragon[2,3,4], Jos Boekhorst[1], Peter van Baarlen[1], Jerry M. Wells[1]

[1]Host-Microbe Interactomics Group, Animal Sciences Department, Wageningen University, Wageningen, The Netherlands, [2]Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193, Catalonia, Spain, [3]IRTA. Programa de Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA) Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193, Catalonia, Spain, [4]OIE Collaborating Centre for the Research and Control of Emerging and Re-emerging Swine Diseases in Europe (IRTA-CReSA), Bellaterra, 08193 Barcelona, Spain, [5]Field Station for Epidemiology, University of Veterinary Medicine Hannover, 49456, Bakum, Germany, [6]Schothorst Feed Research B.V., Lelystad, The Netherlands, [7]. Topigs Norsvin USA, Burnsville, MN, United States

Introduction

Streptoccocus suis is a Gram-positive opportunistic pathogen causing systemic disease in piglets around weaning age. Outbreaks of *S. suis* disease are controlled by metaphylactic use of antibiotics, leading to high levels of antimicrobial resistance in *S. suis* isolates. This is an issue for both animal and human health due to the zoonotic disease potential of *S. suis*. The mechanisms facilitating invasive disease are not known but may involve host and environmental factors. The palatine tonsils are considered a portal of entry for pathogenic strains to cause systemic disease. We hypothesized that tonsil colonization by pathogenic and commensal bacteria may impact on disease risk via colonization resistance and co-infections. We conducted a case-control study on 9 European farms, comparing the tonsil microbiome of piglets with *S. suis* systemic disease with asymptomatic controls. We also compared these to piglets on control farms and piglets reared naturally in a forest.

Results

We found a small but significant difference in the tonsil microbiota composition of case and control piglets. Case-control associations varied between amplicon sequence variants (ASVs) and metagenome assembled genomes (MAGs) within the same species. Variants of putatively commensal taxa including *Rothia nasimurium* were reduced in abundance in case piglets compared to asymptomatic controls. Case piglets had higher relative abundance of *Fusobacterium gastrosuis, Bacteroides heparinolyticus,* and uncultured *Prevotella* and *Alloprevotella* species. There was, however, no higher abundance of *S. suis* itself at the species-level or of clinical strain marker genes in case piglets. Piglets sampled prospectively weeks prior to developing clinical signs had reduced microbiota alpha diversity. Despite case-control pairs receiving equal antimicrobial treatment, case piglets had higher abundance of antimicrobial resistance genes (ARGs) conferring resistance to antimicrobial classes used to treat *S. suis*.

Conclusions

The tonsillar microbiota of *S. suis* case piglets had increased abundance of taxa not previously linked to *S. suis* disease. This coincided with increased ARG abundance in case piglets, possibly due to adaptation of the disease-associated microbiota to frequent antimicrobial treatment.

Acknowledgements

We thank the farmers and veterinarians that participated in the study. Most farms are 568 anonymized. Farm NL1 is Schothorst Feed Research B.V. (www.schothorst.nl) and the 569 forest piglets (FST) were collected from Boeren in het Bos (www.boereninhetbos.nl).

References

1. Aarestrup et al. (1998) Vet Microbiol 63, 71–80.
2. Mathieu-Denoncourt et al. (2018) Pathogens 7:7.
3. Obradovic et al. (2021) Vet Res 52:49.
4. Thanawongnuwech et al. (2000) Vet Pathol 37, 143–52.
5. Segura et al. (2016) FEBS Lett 590, 3772–3799.
6. Roodsant et al. (2021) Virulence 12, 2787–2797
7. Weinert et al. (2015) Nat Commun 6:1.

Sequence type distribution of *Streptococcus suis* isolates recovered from diseased pigs in western Canada

R. Gamage[1], J. Christensen[2], M. O. Costa[1,3]

[1] Large Animal Clinical Sciences; [2] Canada West Swine Health Surveillance Network; [3] Population Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht.

Introduction

Streptococcus suis is the leading cause of meningitis in humans in south east Asia, as well as one of the main infectious challenges commercially reared pigs face around the globe. Disease in pigs is particularly problematic following weaning due to the stress of separation from the dam, mixing with new pigs, change in diet, change in the environment, and reduction in passive immunity antibody levels. Currently there is no efficient swine vaccine for *S. suis*, resulting in the injudicious use of antimicrobials to control post-weaning disease and outbreaks. The goal of this work was to profile the sequence type (ST) distribution of *S. suis* isolated from diseased swine from commercial barns in Western Canada.

Material and Methods

Clinical samples (n=128) from swine with clinical signs of meningitis, pneumonia, arthritis, sudden death, and valvular endocarditis submitted for routine diagnostics were included in this trial. Aerobic bacterial culture that resulted haemolytic colonies were further identified by MALDI- TOF. Isolates characterized as *S. suis* were submitted for whole genome sequencing using Nanopore (GridION). Minimum 20x genome coverage was used for each isolate. Raw sequences were quality-controlled using in-rig software. High-quality sequences were assembled using SPAdes (v3.15) and annotated using Prokka (0.92). Finally, MLST typing was performed *in silico* using PubMLST.

Results

The most frequently identified was ST28 (18/128), followed by ST839 (15/128). New STs accounted for 66/128 isolates.

Discussion

The proportion of isolates associated with ST28 not surprising, as it is part of the ST1 complex, which includes most virulent serotypes and sequence types of *S. suis*. ST distribution in western Canada is markedly different from other regions of the globe, as well as eastern Canada. Further epidemiological data will be included in future analyses.

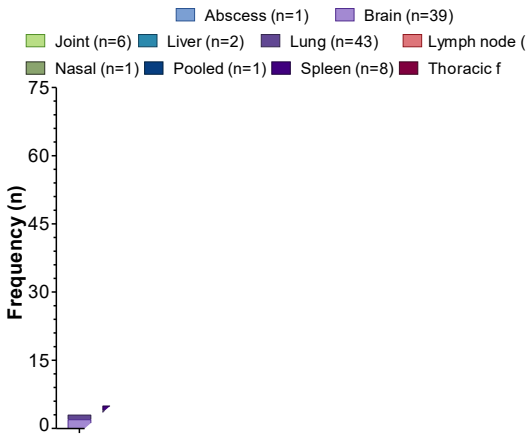


Figure 1 – *Streptococcus suis* isolate sequecen type distribution from diseased pigs.

A novel and efficient mechanism for the conjugative transfer of Streptococcal prophages harboring antibiotic resistance and virulence genes

Jinhu Huang[1]

[1] *MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China*

Introduction

Prophages are temperate phages that are either integrated into the bacterial chromosome or maintained extra-chromosomally as episomes [1, 2]. They play crucial roles in mediating bacterial pathogenicity by secreting virulence factors (VFs), such as toxins, adhesins, and effector proteins. Prophages are reported to harbor antimicrobial resistance genes (ARGs); however, several recent bioinformatic studies demonstrated that identifying phage-sourced ARGs is sometimes "artificial" by relaxing the detection limits, and in fact, phages rarely encode clinically relevant ARGs. ARGs residing on the bacterial genomes can also be transferred by phage-mediated general transduction, but the probability of such movement remains relatively low, due to the fact that bacterial DNA is randomly encapsulated by phage particles and the encapsulated DNA, if any, typically lysogenizes at low rates. Together, the role of prophages in harboring and transmitting ARGs in natural ecosystems remains debatable.

Materials and Methods

The genomes from both GenBank and clinical isolates were analyzed for the presence of ARGs, VFs and prophages. The prophage integration sites and excision events were further detected. Horizontal transfer of ARG-carrying prophages was performed. The lethal effects of VF-carrying isolates were extensively validated in cell and animal models.

Results

we characterize a prevalent family of prophages in Streptococcus, designated SARphages, which harbor twenty-five ARGs that collectively confer resistance to ten antimicrobial classes, including *vanG*-type vancomycin resistance locus and oxazolidinone resistance gene *optrA*. SARphages integrate into four chromosome attachment sites by utilizing three types of integration modules and undergo excision in response to phage induction. Moreover, we characterize four subtypes of Alp-related surface proteins within SARphages, the lethal effects of which are extensively validated in cell and animal models. Importantly, SARphages transfer via high-frequency conjugation that is facilitated by integrative and conjugative elements from either donors or recipients.

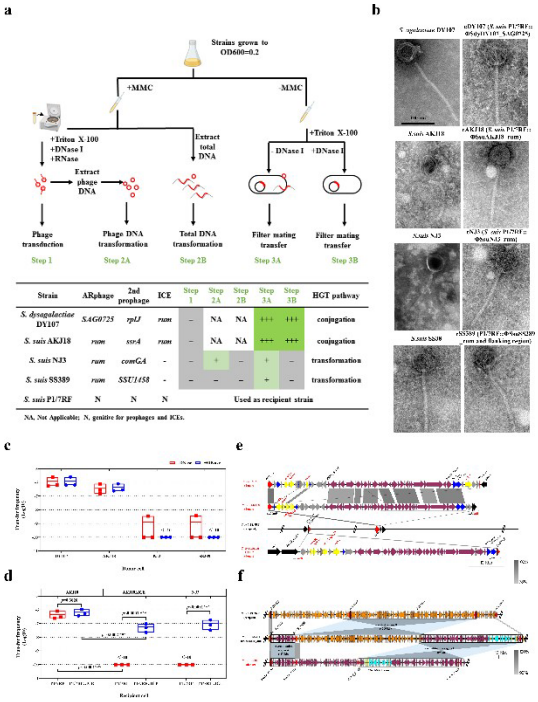


Figure 1. HGT mechanisms of SARphages.

Discussion

we show that prophages are reservoirs of clinically relevant ARGs and VFs and are distributed in many species of Streptococcus. Integration of SARphages into the bacterial chromosome occurs via either site-specific or homologous recombination. Furthermore, SARphages employ multiple non-transduction HGT mechanisms in the dissemination of ARGs and VFs, including transformation at low frequency, and a previously undescribed conjugation mechanism at high frequency that is facilitated by ICEs from either donor or recipient cells. This novel HGT mechanism may explain the increasing prevalence of ICE-associated SARphages in Streptococcus and provides a basis for future studies of prophage mobilization in other bacterial taxa.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 32172917).

References

1. Feiner R, et al. (2015) Nat Rev Microbiol. 13, 641-50.
2. Howard-Varona C et al. (2017) ISME J. 11:1511-20.

Antimicrobial Susceptibility and Normalized Resistance Interpretation Analysis of *Streptococcus suis* Isolates from Diseased Pigs in Taiwan

F.Y. Hsu[1], M.T. Chiou[1,2], C.N. Lin[1,2], W.H. Lin[1,2]

[1] *Animal Disease Diagnostic Center;* [2] *Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung 912301, Taiwan*

Introduction

Streptococcus suis (*S. suis*) is one of the most common causes of swine systemic disease worldwide. Over decades, antimicrobials were usually supplemented in feeds and drinking for disease prevention and control. An increasing level of antimicrobial resistance has been noted in *S. suis* recently and several antimicrobial susceptibility monitoring programs were conducted in different regions including Europe and Asia (1, 2). Additionally, the concept of epidemiological cut-off (ECOFF) values has been proposed to describe the susceptibility distribution and to categorize the examined isolates into wild-type and non-wild-type (NWT) populations. To our knowledge, the related antimicrobial susceptibility survey in Taiwan is scarce. The aims of this study were (i) to determine the minimum inhibitory concentration (MIC) values of *S. suis* isolates deriving from post-mortem samples and (ii) to analyze ECOFF values for each antimicrobial.

Materials and Methods

S. suis isolates were obtained from diseased pigs sent to Animal Disease Diagnostic Center from 2015 to 2019. MIC assays of in total 17 antimicrobials were assessed by broth microdilution according to the Clinical and Laboratory Standard Institute (CLSI) document, VET01 (3). MIC_{50} and MIC_{90} values were determined and MICs were interpreted as susceptible, intermediate, and resistant using European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI breakpoints (Table 1). The MIC distributions were also analyzed to acquire the ECOFF values by normalized resistance interpretation (NRI) (4).

Results

In total, MIC results of 369 *S. suis* isolates were included in this study. Amongst all 17 antimicrobials, *S. suis* isolates shared the lowest MIC_{50} values at 0.0625 μg/ml to both amoxicillin and cefquinome, on the contrary, the highest at ≥256μg/ml to tilmicosin, tylosin, and lincomycin/spectinomycin. The resistance rates of beta-lactams ranged from 6.2% to 44.4%; whereas, those of tetracyclines were virtually 100%. ECOFF values of each antimicrobial were successfully acquired except for tilmicosin and tylosin, due to the truncated MIC distributions. The proportions of the NWT isolates against beta-lactams amounted to 54.5%; on the other hand, those against tetracyclines were 0%.

Discussion

The resistance rates of amoxicillin, ampicillin, and ceftiofur in this study were similar to other research (1, 2). Nonetheless, by comparing the results of resistant and NWT populations, it indicated that some of these isolates may have acquired potential resistant factors and provided an early warning that the emergence of an *S. suis* population with reduced susceptibility. The differences between the proportions of resistant and NWT isolates in tetracyclines may be on account of MIC distributions at high concentrations. The present study provides valuable phenotypic antimicrobial resistance information in *S. suis* isolates from Taiwan. Acquired resistant genes screening and searching should be further investigated.

References

1. Oh et al. (2017) J Vet Med Sci, 79, 780-787
2. van Hout, et al. (2016) Vet Microbiol, 194, 5-10
3. CLSI standard VET01 (2018) Clinical and Laboratory Standards Institute, 5th ed
4. Kronvall (2010) J Clin Microbiol, 48, 4445-4452

Table 1. Dilution ranges and breakpoints of antimicrobials utilized in this study.

Antimicrobials	Dilution Ranges (μg/ml)	Breakpoints (μg/ml)		
		S ≤	R ≥	References
β-lactams				
Penicillins				
amoxicillin	0.031-16	0.5	2	EUCAST [a]
ampicillin	0.008-4	0.5	2	CLSI VET01S [b]
penicillin G	0.031-16	0.25	1	CLSI VET01S [b]
Cephalosporins				
cephalothin	0.125-64	–	–	n/a
cephalexin	0.125-64	–	–	n/a
cefotaxime	0.125-64	1	4	CLSI M100 [c]
ceftiofur	0.031-16	2	8	CLSI VET01S [b]
cefquinome	0.002-1	–	–	n/a
Fluoroquinolones				
enrofloxacin	0.063-32	0.5	2	CLSI VET01S [b]
Tetracyclines				
oxytetracycline	0.125-64	0.5	2	CLSI VET01S [b]
doxycycline	0.125-64	0.25	1	CLSI M100 [d]
Chloramphenicols				
florfenicol	0.063-32	2	8	CLSI VET01S [b]
Macrolides				
tilmicosin	0.5-256	–	–	n/a
tylosin	0.5-256	–	–	n/a
Pleuromutilins				
tiamulin	0.25-128	–	–	n/a
Lincosamides/Aminocyclitols				
lincomycin/spectinomycin [e]	0.5-256	–	–	n/a

n/a, non-available

[a] EUCAST Breakpoint tables for interpretation of MICs and zone diameters v. 11.0, 2021 – Viridans group *Streptococci*

[b] CLSI VET01S Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 5th edition, 2020 – *Streptococcus suis*

[c] CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing, 30th edition, 2020 – Viridans group *Streptococci*

[d] CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing, 30th edition, 2020 – *Streptococcus pneumoniae*

[e] Lincomycin and spectinomycin mixture in a ratio of 1:2

Using TurboID proximity labeling system to reveal the interacting proteome between suilysin of *Streptococcus suis* and HBMEC cells

C. Jiang[1], P. Zhou[1], Y. Hu[1], W. Li[1,2], Q. He[1*]

[1]*State Key Laboratory of Agricultural Microbiology, College of Animal Sciences and Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China;* [2]*Hubei Hongshan Laboratory, Wuhan 430070, China*

Introduction

Streptococcus suis (*S. suis*) is an important zoonotic pathogen associated with a wide range of diseases in pigs, including meningitis, septicaemia, pneumonia, endocarditis, and arthritis, it could also cause severe systemic infection in humans [1]. Suilysin (SLY), one of the secreted virulence factors of *S. suis*, is a pore-forming toxin that belongs to the cholesterol-dependent cytolysin which plays an important role in the invasion and pathogenesis of *S. suis* [2]. However, the role of suilysin in *S. suis* host-cell interaction is still unclear. TurboID is an enzyme that mediates the biotinylation of endogenous proteins that can fuse with proteins of interest to label protein interactors and local proteomes [3]. The purpose of this research was to reveal the host membrane proteins that interact with SLY of serotype 2 *S. suis* by TurboID-mediated proximity labeling in Human Brain Microvascular Endothelial Cells (HBMEC).

Materials and Methods

The HBMEC were seeded into 6-well tissue culture plates and cultured in RPMI 1640 medium containing 10% FBS for 24 h. After the cells had grown to 100% confluence, they were washed three times with sterile PBS, and 3 μg His-TurboID-SLY or His-TurboID recombinant protein was added to fresh RPMI 1640 medium and incubated on ice for 1 h to allow the proteins to bind cells. Then, 50 mM biotin was added and incubated at 37°C for 20 min to label the interacting proteins. The cells were washed three times with ice-cold PBS and then lysed in 500 μL lysis buffer. Supernatants were incubated with streptavidin magnetic beads on a rotator at 4°C overnight. Then, the beads were washed three times with cell lysis buffer. Biotinylated proteins were then eluted by boiling the beads in 100 μL SDT buffer, and identified by mass spectrometry.

Results

A total of 1,358 and 1,269 biotinylated host cell proteins interacting with His-TurboID-SLY and His-TurboID were identified, respectively. Among them, 251 interacting proteins were unique in the His-TurboID-SLY group (Fig. 1). Membrane proteins are often involved in the recognition of pathogens by HBMEC. So we were especially interested in membrane proteins. Of these 251 proteins, eight membrane proteins were identified by UniProt website.

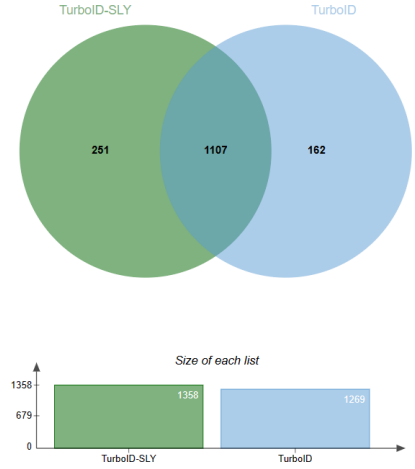


Figure 1. Venn diagram of TurboID-SLY-interacting host proteins and TurboID-interacting host proteins.

Discussion

S. suis requires a series of virulence factors to successfully infect and cause host disease. SLY plays a crucial role in this process and may be related to meningitis. Therefore, SLY could be a potential new target for the treatment of *S. suis* infection. Our study provides new insight into host-pathogenic microorganism interactions. The TurboID proximity labeling system could be applied to study the interactions of other zoonotic agents such as *Salmonella* and *Brucella* with host proteins to find the receptors or key proteins that mediate infection. These findings will lay a theoretical foundation for preventing, controlling, and treating disease, breeding disease-resistant animals, and developing vaccines.

Acknowledgements

This research was supported by the China Agriculture Research System of MOF and MARA (No. CARS-35).

References

[1] Lu et al. (2021) J Microbiol 59, 949-957
[2] Seitz et al. (2013) Vet Microbiol 167, 584-591
[3] Roux et al. (2012) J Cell Biol 196, 801-810.

(Unpublished data)

Rapid genome-wide identification of conditionally essential genes in *Streptococcus suis*, using Tn-seq and high accuracy Nanopore sequencing

M. Juanpere-Borras[1], J. Boekhorst[1], B. Fernandez-Ciruelos[1], P. van Baarlen[1] and J. Wells[1].

[1] *Host-Microbe Interactomics, Animal Sciences, Wageningen University, 6708 WD Wageningen, The Netherlands.*

Introduction

Streptococcus suis is a Gram-positive coccus and the main cause of bacterial disease in the swine industry, resulting in high economic losses (1). The identification of *S. suis* genes involved in survival or growth in different host niches and the farm environment is needed for a better understanding of mechanisms of pathogenesis and transmission and can underpin the development of intervention strategies. Transposon-insertion sequencing (Tn-seq) is a high throughput method for the genome-wide identification of essential and conditionally essential genes (CEG). Tn-seq is based on the construction of a saturated transposon mutant library, where every mutant contains a transposon randomly inserted in the genome. The Tn library is grown under *in vivo* mimicking conditions and the conditionally essential genes identified by comparing the expected vs observed number of tn-insertions per gene. In this study, a *S. suis* tn-library was grown in activated porcine serum, and we identified tn-insertion sites by nanopore long-read sequencing with highest-accuracy base-calling.

Materials and methods

A transposon library containing about 15,000 mutants was grown in activated porcine serum until late-exponential phase. The genomic DNA of the control and test conditions was purified and treated as described in Camilli *et al.* (2). PCR amplicons containing flanking regions of tn-insertions were barcoded and sequenced using the MinION™ Mk1C device using the Guppy pipeline set to highest-precision base-calling (Oxford Nanopore Technologies). The reads were filtered by size and trimmed to select reads with adapter sequences. Finally, the flanking regions of the tn-insertions were mapped to the genome sequence, generated in-house by nanopore sequencing, of our laboratory *S. suis* P1/7 strain. *Transit* software (3) was used to calculate the $\log_2$Fold-Change and corresponding adjusted p-value representing the abundance of every mutant in test and control conditions.

Results

We identified a total 88 CEG with a fold change greater than 2. These 88 CEG included genes involved in biosynthesis of purine and pyrimidines, sugar metabolism, and membrane or cell wall synthesis.

Three hypothetical proteins, encoded by genes organized in tandem in the genome, shared high identity with an operon encoding a tryptophan uptake system (4). 198 genes were associated with higher growth in serum, including genes encoding a diacylglycerol kinase protein and a tyrosine-protein kinase that had a fold change greater than 8. We hypothesized that higher growth of *S. suis* P1/7 in serum is associated with less tightly regulated membrane/cell wall synthesis.

Discussion

This study has shown that implementation of in-house long-read sequencing as part of a high-throughput mutagenesis method, enabled us to rapidly identify genes that supported growth of *S. suis* in porcine serum. We will present results from ongoing studies on the characterization of selected conditionally essential genes by CRISPR/Cas9 mutagenesis and phenotypic assays.

Acknowledgments

We thank the members of Tim van Opijnen lab, for kindly providing us with tn-seq materials and protocols. This project has received funding from the EU Horizon 2020 Research and Innovation Program under the Marie Sklodowska-Curie grant agreement nº 956154.

References

(1) Grenier et al. (2018) Elsevier Masson SAS, pp. 159–166.

(2) Camilli et al. (2013) Nat Rev Microbiol 11, 435–442.

(3) De Jesus et a. (2015) PLoS Comput Biol 11.

(4) Nerlich et al. (2022) Sci Rep 12.

Development of MassARRAY-based method for species and serotype identification of zoonotic *Streptococcus suis*

S. Jiemsup, W. Chumpol, K. Lunha, and S. Yongkiettrakul

National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani 12120, Thailand.

Introduction

Streptococcus suis is an important porcine pathogen, causing a zoonotic disease in human worldwide. It is classified into 29 serotypes that are associated with pathogenicity of *S. suis* (1). The prevalence of *S. suis* serotype is geographically diverse, serotyping of *S. suis* is therefore necessary for the active surveillance system to monitor the outbreak of pathogenic *S. suis* strains and to prevent and control the infection (2). However, diagnosis of *S. suis* remains challenging, due to high genetic diversity. This study focused on DNA analysis based on MassARRAY technology to design and develop a specific high-throughput single assay for characterization of *S. suis* species and serotypes.

Materials and Methods

Glutamate dehydrogenase (*gdh*) and DNA repair protein (*recN*) were used as genetic markers for differentiating *S. suis* from *S. suis*-like bacteria and other bacterial species. Capsular polysaccharide (*cps*) genes were used as DNA targets for serotype-specific detection of *S. suis*. DNA sequence of target genes, extracted from a custom *S. suis* genome database (unpublished data) were used for multiple-sequence alignment. Highly conserved and specific regions of individual target genes were subsequently selected for the design of multiplexing PCR primers and single-based extension (SBE) primers, using Assay Design 2.2 software (Agena Bioscience, Inc., San Diego, CA, USA). The test condition was optimized to provide precise molecular mass of SBE products for the specific detection of DNA molecules by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS). The developed method was evaluated using 161 *S. suis* isolates collected from healthy pigs in 2022 and 5 *S. suis*-like strains.

Results

The MassARRAY-based method developed for species identification and serotyping of *S. suis* were evaluated with *S. suis* and *S. suis*-like strains, revealing 100% specificity and sensitivity. The study showed that the MassARRAY results were in accordance with the results obtained from PCR-based serotyping and DNA sequencing methods. All 29 serotypes of *S. suis* could be completely determined in a single assay platform. Based on single nucleotide polymorphism at position 483 of *S. suis cpsK* gene (3), the distinct molecular mass of SBE products could be generated and detected by MALDI-TOF MS, allowing the differentiation of two pairs of serotypes: 1 and 14, and ½ and 2 (Fig. 1).

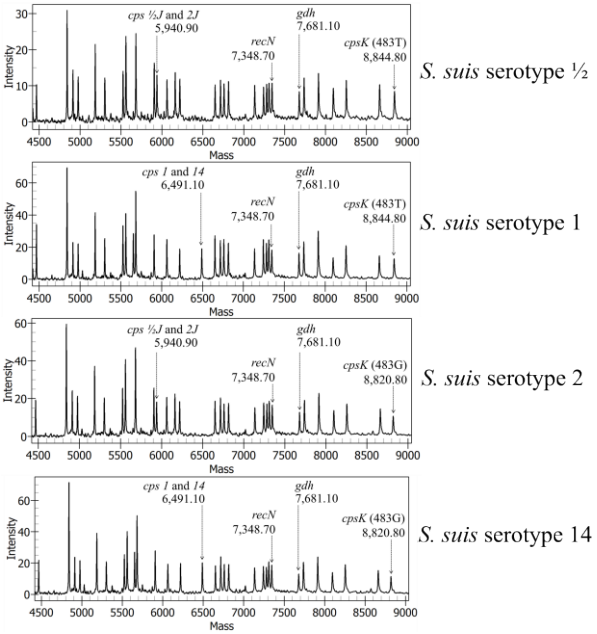


Figure 1. Representative chromatogram of MassARRAY-based method for species identification and serotyping of *S. suis* serotype ½, 1, 2, and 14.

Discussion

The MassARRAY-based method as a single-assay platform can simultaneously identify *S. suis* at the species and serotype level with high specificity and sensitivity. With the advantages of multiplexing format and high sample throughput of MassARRAY (4), the developed MassARRAY-based method could be a method of choices for *S. suis* serotype surveillance.

Acknowledgements

The present study was financially afforded by NSTDA RDI Research Grant, grant number P20-50-967. The authors would like to thank Dr. Pornchalit Assavacheep, and Mrs. Sukuma Samngamnim, Chulalongkorn University, Dr. Nattakan Lakkitjaroen and Dr. Anusak Kerdsin, Kasetsart University for providing *S. suis* and *S. suis*-like strains for the pilot study of method validation.

References

1. A Kerdsin et al. (2014) J Med Microbial 63:824-830.
2. K Wang et al. (2017) Curr Clin Micro Rpt 4:29-35.
3. Athey et al. (2016) BMC Microbiology 16:162
4. H Zhao et al. (2021) BMC Infectious Diseases 21:870

A Study of the Contamination of *Streptococcus suis* in Pig Blood from the Open Market and Convenient Store in Phuttamonthon District

K. Kaewjua[1], P. Meetam[1], W. Doemlim[1], S. Tantawet[2], N. Phumthanakorn[3], D. Laohasinnarong[2]

[1] 6th Year Student; [2] Department of Clinical Sciences and Public Health; [3] Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, 73170, Thailand

Introduction

Streptococcus suis (S. suis) is a gram-positive bacterium that is widely distributed in the pig population worldwide. It is a zoonotic pathogen that affects pigs and can be transmitted to humans. Infection with *S. suis* in humans can cause severe disease such as hearing loss, meningitis, and death [1]. In pigs, *S. suis* infections can lead to meningitis, septicemia, pneumonia, and arthritis [2]. The incident of *S. suis* infection in humans can occur worldwide, including Thailand [1,3]. The consumption of raw contaminated pig products can be a potential source of infection [3]. Therefore, this study aimed to investigate the contamination of *S. suis* in pig blood from the open market and convenient stores in Phuttamonthon district, Nakhon Pathom, Thailand.

Materials and Methods

This study was conducted in 2022 by collecting 14 samples of fresh pig blood and 13 samples of jelly pig blood from the open market and 16 samples of sterilized jelly pig blood from convenient stores in Phuttamonthon district, Nakhon Pathom, Thailand. Bacterial culture in NNCC media for 48 hrs. and biochemical methods were used to detect the presence of *S. suis*. Identification of *S. suis* was confirmed by matrix-assisted laser desorption/ionization time-offlight (MALDI-TOF) mass spectrometry at the Faculty of Veterinary Science, Chulalongkorn University.

Results

There was no bacterial growth in all sterilized jelly pork blood. A sample of jelly pork blood showed βhemolysis while 6 samples showed α-hemolysis. Eleven out of the 14 samples of fresh pig blood were found α-hemolysis. All colonies were confirmed that they were not *S. suis* by MALDI-TOF methods.

Discussion

In Thailand, some human cases of *S. suis* infection are related to consume uncooked or semi-cooked pig products, especially blood. In many festivals, villagers in rural areas slaughter animals for feasts. Pig slaughtering methods are unhygienic and easily contaminated with pathogens. The most popular food is Laab, which in the northern and northeastern cultures of Thailand often likes to pour raw pork blood before eating. Because slaughter by slitting the pig's neck makes the pig's blood risk contaminated with *S. suis*, resulting in infection and illness among the eaters.

The potential risk of *S. suis* infection from pig products, particularly pig blood, is of concern to both the pig industry and public health authorities. There is limited information available on the prevalence of *S. suis* contamination in pig blood sold in open markets and convenience stores in Thailand. Recently, a study found *S. suis* contamination in pork and pig organs by LAMP assay [4].

This study revealed that the pig blood sold in the open market and convenient stores in Phuttamonthon district is free from *Streptococcus suis* contamination. However, a larger study with a larger sample size is required to confirm these findings and ensure the safety of consuming pig blood. This study emphasizes the need for continuous monitoring of pig products in the market to ensure the safety of consumers and support promotion of Thai food as soft power.

Acknowledgements

This study was financed by research project grant 2022 from the Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand.

References

1. Lun, Z.-R. et al. (2007). Lancet Infect. Dis., 7, 201–209
2. Goyette-Desjardins, G. et al. (2014). Emerg. Microbes Infect., 3, e45
3. Takamatsu, D. et al. (2008). Emerg. Infect. Dis., 14, 181–183
4. Boonyong, N. et al. (2019). Vet. World, 12, 165–169

A novel SOS response related transcription factor regulates prophage transfer in *Streptococcus suis*

Wang Liping, Li Aijuan

Department of basic veterinary medicine, Faculty of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210000, China

Introduction

Many environmental insults can cause DNA lesions that pose a direct threat to bacterial survival. The bacterial SOS response is a widespread transcriptional regulatory mechanism to DNA damage [1]. In *E. coli*, the expression of SOS genes is controlled by a repressor protein, LexA [2]. But *Streptococcal* species lack a classical SOS response of *lexA*, and the research on the mechanism of SOS response is not yet clear [3]. Currently, many studies show that SOS responses affect antibiotic resistance by modulating mobile genetic elements. In this study, we report that a novel family of LexA-like repressors in *Streptococcus suis*. We propose that the novel transcription factor HdiR identified represents a SOS response, and it can inhibit excise and integrate phage from the genome and provide a new way to facilitate the transmission of antibiotic resistance.

Materials and Methods

Streptococcus suis SC70731 was isolated and identified from a pig farm. Genomic DNA was harvested by DNA Purification Kit（Omega）. The whole genome was sequenced by Illumina Hiseq 2500 sequencer. HdiR deletion bacterial strain △HdiR was constructed in this study. To determine UV sensitivity, overnight cultures were diluted 1:500 in THB broth and grown at 37°C until an optical density at 600 nm (OD600) of 0.3, and then cells were spotted onto THA agar plates and exposed to the indicated intensity of UV light 20s. Surviving colonies were observed after overnight incubation at 37℃. The excision and circle forms of prophage detected by PCR.

Results

In this study, we report the identification of a potential self-regulated SOS response gene *hdiR* in *S. suis*. HdiR is located on the prophage of the *S. suis* chromosome genome. HdiR monomers contain an N-terminal helix-turn-helix (HTH) DNA-binding domain and a C-terminal the S24 serine peptidase domain. In the absence of *hdiR*, *S. suis* cells were sensitive to UV light (Fig. 1). And in the absence of *hdiR*, the excision and circle forms of prophage will increase (Fig. 2).

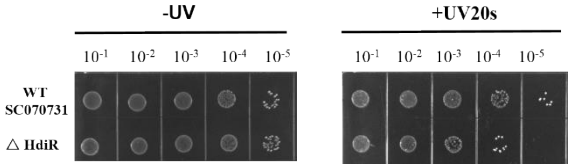


Figure 1. Survival by UV light exposure

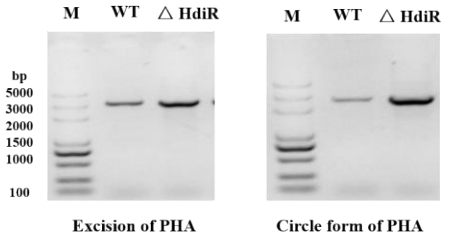


Figure 2. The excision and the circle forms of prophage.

Discussion

In this study, we show the existence of an novel SOS response-like DNA repair mechanism in *S. suis*. Recently, articles have emphasized the relevance of the SOS response to the emergence of antibiotic resistance. Some external environmental stimuli, such as ultraviolet and antibiotics, can cause SOS response and may induce the transfer of phages. The study showed that HdiR is related to SOS response like LexA in other bacteria. And HdiR is associated with excision and circle forms of prophages like CI repressor in phages [4].

Acknowledgements
The present study was financially afforded by Faculty of Veterinary Medicine Research Fund, 2022, Nanjing Agricultural University, Nanjing, China.

References

1. Baharoglu Z et al. (2014) FEMS Microbiol Rev 38, 1126–1145.
2. Zhang A P P et al. (2010) Nature 466, 883–886.
3. Sánchez-Osuna M et al. (2021) Nucleic Acids Res 49, 11050-11066.
4. Bose B et al. (2008) Molecular microbiology 70, 570-82.

Analysis of the capsular polysaccharide synthesis locus in a collection of *Streptococcus suis* field isolates isolated in the Czech Republic

N. Kralova[1,2]

[1] *Veterinary Research Institute, Brno 62100, Czech Republic;* [2] *Department of Experimental Biology, Faculty of Science, Masaryk University, Brno 62500, Czech Republic*

Introduction

Streptococcus suis is considered an important bacterial swine pathogen responsible for various diseases such as meningitis, endocarditis, arthritis, and septicemia leading to sudden death and therefore contributes to significant economic losses in the swine industry. In addition, *S. suis* is a zoonotic pathogen that can be transmitted to humans and cause disease, mainly arthritis, meningitis and streptococcal toxic shock-like syndrome. One of the main virulence factors is the presence of capsular polysaccharide (CPS). There are 29 serotypes based on serological reactions against the CPS, but serologically untypeable strains are often found, which suggests that *S. suis* has more serotypes than has been reported so far [1]. Recently, the novel *cps* loci (NCLs) were identified in non-serotypeable isolates [2]. Strains of *Streptococcus suis* are genotypically and phenotypically very different, and strains of the same serotype can differ significantly at the genomic level. We performed an analysis of capsular polysaccharide synthesis loci in 123 field samples isolated from diseased farm animals in the Czech Republic in 2018-2020.

Materials and Methods

123 field isolates from diseased pigs were serotyped by agglutination tests using reference antisera and Multiplex-PCR [3]. All isolates were sequenced by Illumina sequencing. The *cps* synthesis loci were extracted from draft genome sequences, annotated and then compared to the reference serotypes and novel *cps* loci sequences.

Results

The genomes of 123 field isolates were sequenced. 24 isolates were identified as *S.suis*-like and 99 isolates were identified as *S. suis* and *cps* loci analysis was performed for these isolates. We found that 64 isolates in our collection had a *cps* locus similar to that of reference strains of different serotypes, but in 31 of these strains the *cps* locus differ from the canonical reference sequences by mutations or gene insertion/deletion. 11 strains had *cps* loci identical to recently described NCLs. 24 strains had NCLs not yet described.

Discussion

We analyzed the *cps* loci of 99 *S. suis* isolates from different farms in the Czech Republic. All strains shared a highly conserved 5'region of the *cpsA*, *cpsB*, *cpsC* and *cpsD* genes and a conserved *glf* gene at the 3'region, while the central region was variable. All isolates had polysaccharide polymerase (*wzy*) and flippase (*wzx*) genes and various genes encoding glycosyltransferases, acetyltransferases, and other transferases. This implies a great diversity of *S. suis* strains in the Czech Republic.

Acknowledgements

This work was supported by the Ministry of Agriculture of the Czech Republic (Institutional Support No. MZE-RO0518, MZE-RO0523) and by the National Agency for Agricultural Research (Project No. QK1810193).

References

1. Okura et al. (2016) Pathogens 5(3), 45.
2. Zheng et al. (2015) Appl Environ Microbiol 81(12), 4111–4119.
3. Kerdsin et al. (2014) J Med Microbiol 63, 824–830.

Characterization of a novel lysin Ply691 and its lytic activity against *Streptococcus suis* in vitro and vivo

Xinyi Li, Yanhong Shang, Chenglong Li, Xiang Dang Du*

College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, 450046, P. R. China

*xddu@henau.edu.cn

Introduction

Streptococcus suis (*S.suis*) is a zoonoticpathogen, which not only causes huge economic losses to the pig industry, but also threatens human health and even life. With the long-term and improper use of antibiotics, bacterial resistance to antibiotics has become increasingly severe. Therefore, the demand for finding new strategies to treat *S. suis* infection is increasing. Lysin, as a novel antibacterial protein, has unique advantages in preventing and treating bacterial infections and is a highly promisingantibacterial agent.

Materials and Methods

The prophage lysin Ply691 from the genome of the *S. suis* SC267 strain was identified. Ply691 was subsequently characterized to determine its temperature and pH stability, host range, lytic activity, and therapeutic potential in an *in vivo* mouse bacteremia model.

Results

Ply691 consists of an N-terminal Amidase-5 catalytic domain, a C-terminal Glucosaminidase catalytic domain, and two CW-7 binding domains located in the middle. It has a widespectrum lytic activity against 11 serotypes of *S. suis*, including types 2, 3, 5, 9, 10, 12, 17, 18, 19,29, and 30. Ply691 can reduce the number of *S. suis* colonies by about 1 Log within 20 minutes. In addition, Ply691 has a wide temperature adaptation range (4ºC -37ºC) and good alkaline tolerance (pH 7-10) in vitro. By injecting the mice 2×MLD *S. suis* SC267 into their abdominal cavity, and then treated with 2 mg Ply691 after an hour. The mice survived 100% and the bacterial load in their blood and organs (heart, liver, spleen, lung, kidney, brain) were significantly reduced. After treatment, the heart, liver, lung, kidney, spleen, and brain tissues of the mice were almost similar to those of thenormal group (Fig.1), indicating that the lysin Ply691 has potential application in the treatment of *S. suis* infection.

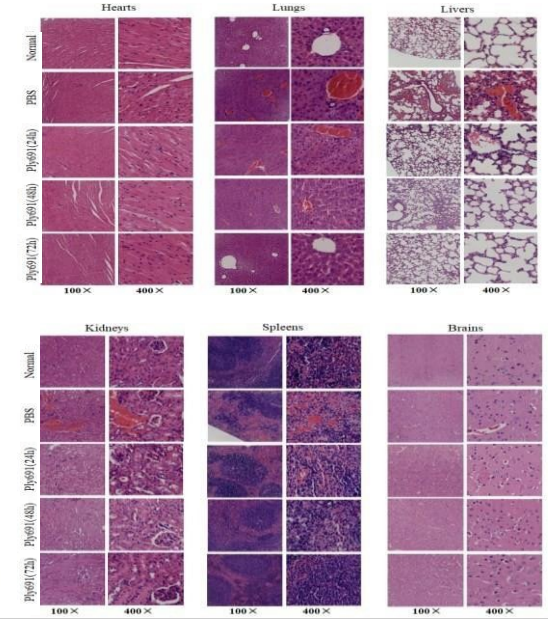


Fig.1 Histopathology of different tissues

Discussion

To sum up, a prophage encoded lysin Ply691 was identified from *S. suis* SC267. Ply691 has lytic activity against 11 serotypes of *S. suis*, and has a wide temperature adaptation range (4ºC -37ºC) and good alkaline tolerance (pH 7-10). In the mouse model of bacteremia, Ply691 showed good therapeutic effects in vivo. Therefore, the lysin Ply691 has very broad applicationprospects in clinical treatment.

References

1. Wang et al. (2015) Vet Microbiol. 2022268:109425.

Antimicrobial Susceptibility of *Streptococcus suis* Isolated from Diseased Pigs in Thailand during 2018-2020

K. Lunha[1], W. Chumpol[1], S. Samngamnim[2], S. Jiemsup[1], P. Assavacheep[2], S. Yongkiettrakul[1]

[1] *National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani 12120, Thailand*

[2] *Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*

Introduction

Streptococcus suis is a porcine zoonotic pathogen causing severe systemic infection in humans and pigs. Therapeutic and prophylactic medication for *S. suis* infection in humans and pigs mainly rely on antibiotics. However, the imprudent use of antibiotics has led to antimicrobial resistance (AMR) problems worldwide. The widespread resistance of streptococci to commonly used antibiotics has occurred while the antibiotic susceptibility of zoonotic *S. suis* distincts among different countries, serotypes, and time periods [1]. The information of antibiotic susceptibility of *S. suis* strains are relatively limited in Thailand. This study addressed the AMR surveillance in *S. suis* to provide current AMR situation for supporting Thailand's National Strategic Plan on Antimicrobial Resistance 2022-2027, aligned with WHO's Global Action Plan on Antimicrobial Resistance.

Materials and Methods

During 2018-2020, a total of 225 *S. suis* were collected from specimens of diseased pigs in 99 farms, localized in 14 provinces across Thailand. The presumptive alpha hemolytic colonies with Gram-positive staining and catalase negative results were confirmed as *S. suis* by using PCR targeting *gdh* and *recN*. The identification of *S. suis* serotype was conducted using multiplex PCR-based method [2]. The antimicrobial susceptibility was determined by standard broth microdilution method using two sets of commercially prepared antibiotic plates including Sensititre Vet Bovine/Swine BOPO6F and *Streptococcus* species STP6F for veterinary and human usages, respectively.

Results

The study revealed lung tissues as the predominant isolation site of *S. suis* (81.7%). The most abundant serotypes were serotype 2 or ½ (26.7%), followed by serotype 8 (8.4%), and 29 (7.6%). The antimicrobial susceptibility test (AST) revealed the most resistance to clindamycin, tilmicosin, tetracycline, tylosin tartrate, erythromycin, azithromycin, oxytetracycline, and chlortetracycline (Fig. 1a). The data also indicated a tendency of reduced efficacy of available veterinary medicines including ampicillin, amoxicillin/clavulanic acid, cefepime, cefotaxime, ceftiofur, ceftriaxone, chloramphenicol, florfenicol, gentamicin, penicillin, and tiamulin for the treatment of *S. suis* infection (Fig. 1b).

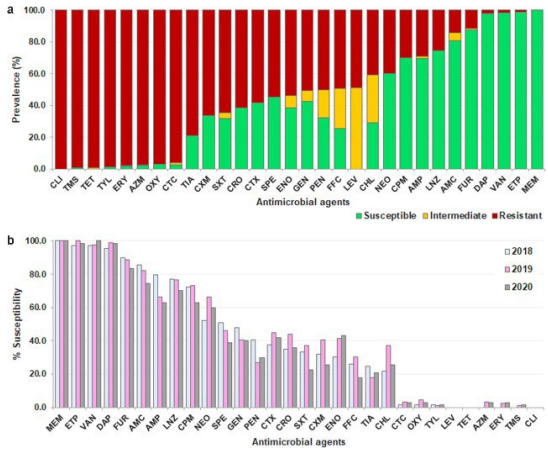


Figure 1. Antimicrobial susceptibility of *S. suis* isolated from diseased pigs during 2018-2020 (a) and comparison of the percentages of isolates that were susceptible to the tested antimicrobials in individual years (b).

Discussion

The predominance of serotype 2 or ½ and 8 was identified from diseased pig-isolated *S. suis*, in different regions of Thailand, supporting that the distribution of different serotype *S. suis* in pigs could be varied by geographical localizations [1]. The AMR surveillance confirmed the widespread of AMR and multidrug-resistant *S. suis* strains in all serotypes, from different time periods, and in different regions of the country. The data suggested the emergence of AMR problem in diseased pig-isolated *S. suis* population in Thailand. Although beta-lactams were the most effective agents [1], a tendency of reduced efficacy of available antibiotics and increased proportion of intermediate susceptibility and resistance to many antibiotics over the time were occurred. Consequently, effective antibiotics for the treatment of *S. suis* infection in both of animals and humans could be limited in the near future. The sustainable and effective AMR surveillance and infection control strategies should be made to prevent the dissemination of AMR and MDR in *S. suis* in the country

Acknowledgements

The present study was financially afforded by NSTDA RDI Research Grant, grant number P20-50-967.

References

1. Segura et al. (2020) Pathogens 9, 374.
2. Kerdsin et al. (2014) J Med Microbiol 63, 824-830.

Genes of antimicrobial resistence of *Streptococcus suis* Isolates from pigs in the Czech Republic

K. Matiaskova, K. Nedbalcova [1], M. Zouharova [1], K. Matiaskova [1], N. Kralova[1,2], J. Matiasovic [1]

[1] *Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic;* [2]*Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic*

Introduction

Streptococcus suis is currently considered as one of the most serious bacterial pathogens in pig industry and is widespread worldwide [1]. Currently, 29 serotypes of *S. suis* were determined (out of 35 originally described) [2]. Monitoring of antimicrobial resistance (AMR) is currently supported by many national and international policy agendas due to spread of the AMR genes in the environment [3].

Materials and Methods

A total of 173 *S. suis* isolates were obtained from pigs in the Czech farms. Identification of *S. suis* was performed using MALDI-TOF method. Multiplex PCR was used for determination of all serotypes. The phenotypic resistances of isolates were detected using the microdilution method according to the Clinical and Laboratory Standards Institute methodology. Genes of AMR were determined by searching the whole genome sequences of the isolates using databases ResFinder, CARD and ARG-ANNOT.

Results

Dependence of resistance to antimicrobials upon serotype assignment could not be proven in all but serotype 31, wherein all isolates (n = 17) were resistant or intermediately resistant to clindamycin, tilmycosin, tulathromycin, and tetracycline. AMR genes were predominantly present for macrolides and lincosamides (*erm(B)*), tetracyclines (*tet(O))* and aminoglycosides (*ant(6)-Ia*) (Table 1). These results are consistent with results of antimicrobial susceptibility testing (AST) where the resistance to the commonly used antimicrobials of tetracycline, tiamulin, tilmicosin (macrolide), tulathromycin (macrolide), and clindamycin (lincosamid) was high. However, the presence of the AMR gene in the isolate did not always correspond to the AST result.

Discussion

The high frequencies of resistance to tetracycline correlate with the frequent use of this antibiotic for the treatment *S. suis* infections in pig farms in the past [4]. Resistance to tiamulin and macrolides is reported very often. We tested them due to their common use in pig farms against outbreaks of other diseases caused by Gram-negative bacteria [5]. The high degree of resistance to these antimicrobials in *S. suis* isolates may be due to the horizontal transfer of resistance genes between different bacterial populations [6].

Antibiotic group	AMR gene	Percentage of strains carrying the gene
Macrolides	*mef(A)*	3.47
	msr(D)	2.89
Aminoglycoside	*ant(6)-Ia*	15.61
	ant(6)-Ib	5.78
	aph(3')-III	5.20
	aac(6')-aph(2'')	1.73
	aad(6)	4.05
	SAT-4	1.73
	(AGly)spw	6.36
Macrolides, Lincosamide	*erm(B)*	53.76
Lincosamide	*lnu(B)*	6.36
	lnuC	0.58
	lsa(E)	6.94
Amphenicols	*optrA*	1.73
Tetracyclines	*tet(W)*	4.62
	tet(L)	0.58
	tet(44)	2.31
	tet(40)	3.47
	tet(O)	49.13
	tet(M)	12.72

Table 1. AMR genes in *S. suis* isolates (n=173).

Acknowledgements
The study was supported by the Ministry of Agriculture of the Czech Republic (Projects No. MZE-RO0518, RO0523 and No. QK1810193).

References

1. Goyette-Desjardins et al. (2014) Emerg Microb Infect 3, e45.
2. Okura et al. (2016) Pathogens 5(3), 45.
3. Varela et al. (2013) Vet Res Com 21, 381-407.
4. Vela (2005) Vet Microbiol 105, 143–147.
5. Burch and Duran (2008) Blackwell Publish 02 -25
6. Charpentier et al. (2012) Curr Opin Microbiol 15, 570–576.

Conjugated vaccine against *Streptococcus suis* infection in pig

J. Matiašovic[1], N. Králová[1,2], H. Štěpánová, J. Gebauer[1], A. Norek[1], K. Matiašková[1], M. Zouharová[1], K. Nedbalcová[1], V, Babák[1], R. Jarošová[1,3], P. Makovický[1,4], I. Kucharovičová[5], B. Šimek[5], H. Plodkova[5], T. Pecka[5]

[1] Department of Infectious Diseases and Preventive Medicine, Veterinary Research Institute, Brno, Czech Republic; [2] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic; [3] Department of Morphology, Physiology and Animal Genetics, Faculty of AgriSciences, Mendel University in Brno, Czech Republic; [4] Department of Histology and Embryology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic; [5] State Veterinary Institute Jihlava, Jihlava, Czech Republic

Introduction

Immunization against streptococcal pathogens is challenging due to the nature of the interaction between the pathogen and host immune system. Fortunately, vaccines based on conjugates of capsular polysaccharide and carrier protein were proven to efficiently induce robust IgG immune response. However, the manufacturing cost is limiting for their use in animals. We thus tested an experimental vaccine against Streptococcus suis serotype 2 based on capsular polysaccharide conjugated to chicken ovalbumin, a cheap alternative conjugate protein, and compared its immunogenicity and protectivity with a vaccine based on CRM197 conjugate.

Materials and Methods

The isolated *S. suis* serotype 2 field strain capsular polysaccharide was conjugated [1] to the carrier protein OVA (chicken ovalbumin, Merck, Germany) and CRM197 (Fina BioSolution, USA). The immunization dose per animal was 0.25 mg of antigen in 2 mL of emulsion antigen:adjuvant (Montanide ISA 50 V2) 1:1.

Three groups of weaned piglets, each group consisting of ten animals, were used in the experiment at 28 day of life. One group was immunized with the CPS-OVA vaccine and the second group with the CPS-CRM197 vaccine, both containing the ISA50V2 adjuvant, and the third group remained not immunized. Three weeks after the first dose (D21), all vaccinated animals received the second dose of the same vaccine.

Two weeks after the second dose of vaccine (D35), pigs in all three groups were infected intraperitoneally with 2 x 10^9 CFU/2 mL of homologous *S. suis* strain. Day 42 was the end of the experiment.

Results

Immune response

In the CPS-OVA and CPS-CRM197 groups, the IgG serotype 2 specific antibody level was significantly increased ($p \leq 0.05$; two-way ANOVA) from three weeks after the first dose (D21) and two weeks after the second dose (D35), respectively, compared to the day of immunization (D0). In the control group, a significant increase in specific IgG level was found only after the infection challenge on D42 when compared to D0 (Fig. 1).

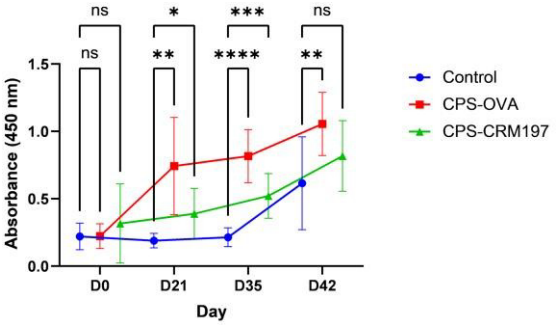


Figure 1. IgG antibody response to vaccination.

Bacteriology examination

Five animals in the control group were culture-positive at least in one organ.

In the CPS-OVA group, organ samples from two animals were culture-positive.

In the CPS-CRM197 group, only one animal was culture-positive and only for the swab from the pericardium. A statistically significant ($p < 0.05$; Fisher's exact test) difference in culture-positive samples was found between the CPS-CRM197 and both the control and the CPS-OVA groups.

Discussion

Apart from one animal, the individuals in the CPS-OVA group were well protected against the infection, similar to animals vaccinated with the CPS-CRM197 conjugate. This result is comparable to previous experiments with other conjugated vaccines against *S. suis* [1]. The CPS-OVA is thus promising as an alternative to CRM197 carrier protein for the further development of vaccines against *S. suis* infection in pigs.

Acknowledgements

This study was supported by the Ministry of Agriculture of the Czech Republic by grants NAZV QK1810193 and RO0518.

References

1. Goyette-Desjardins et al. Infect Immun 2016, 84(7), 2059-2075

Resistance of *Streptococcus suis* Isolates from the Czech Republic during 2018–2022

K. Nedbalcova [1], I. Kucharovicova [2], M. Zouharova [1], K. Matiaskova [1], N. Kralova[1,3], M. Brychta [2], B. Simek [2], T. Pecka [2], H. Polodkova [2],J. Matiasovic [1]

[1] Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic; [2] State Veterinary Institute, Rantirovska 93, 586 05 Jihlava, Czech Republic; [3] Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic

Introduction

Diseases caused by *Streptococcus suis* are currently a major economic problem in pig farms worldwide. Although *S. suis* is primarily considered to be the causative agent of pig infections, it is increasingly being identified as the zoonotic agent responsible for serious human infections [1]. Antimicrobials are used to treat *S. suis* infections. However, the inappropriate or careless use of antimicrobials to treat infections in human and veterinary medicine has led to antimicrobial resistance (AMR), which has become a global problem in recent years. AMRs significantly increase the risk of therapeutic failure. Due to the increase in AMR in recent decades, the susceptibility of bacterial pathogens to antimicrobials should be carefully monitored to ensure the long-term efficacy of authorized antibacterial drugs [2].

Materials and Methods

A total of 506 *S. suis* isolates were obtained from the systemic organs of dead pigs or from nasal swabs of diseased pigs in the Czech farms during 2018–2022. *S. suis* was either the primary pathogen or part of the multifactorial infectious disease with respiration symptoms in the pigs. Each of the tested isolates was derived from a different animal. Identification of S. suis was performed using MALDI-TOF method. The antimicrobial susceptibility testing (AST) was performed by determining the minimum inhibitory concentrations (MICs) using the microdilution methodaccording to internationally recognized methodology accredited by the Clinical and Laboratory Standards Institute.

Results

The results of the AST of 506 field isolates of *S. suis* are shown in Fig. 1.. None of the tested isolates were resistant to to amoxicillin, in combination with clavulanic acid and sulfamethoxazole potentiated with trimethoprim antimicrobial substances. Only one or two intermediately resistant isolates were found to ceftiofur and enrofloxacin in individual years. On the contrary, high or very high levels of resistance were found to tetracycline, tiamulin, tulathromycin, and clindamycin.

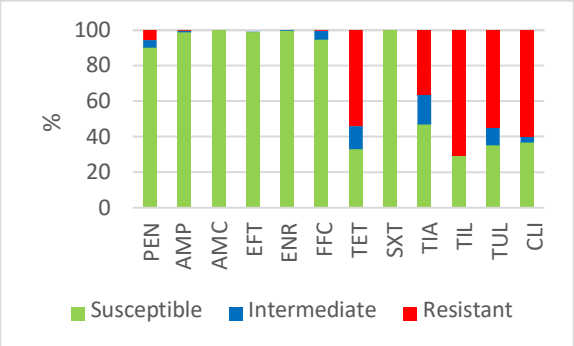


Figure 1. The results of AST of *S. suis* isolates (n=506). PEN=Penicillin; AMP=Ampicillin; AMC= Amoxicillin/Clavulanate 2/1; EFT=Ceftiofur; ENR= Enrofloxacin; FFC=Florfenicol; TET=Tetracycline; STX=Trimethoprim/Sulfamethoxazole 1/19; TIA= Tiamulin; TIL=Tilmicosin; TUL=Tulathromycin; CLI=Clindamycin.

Discussion

Knowledge of the resistance of pathogens present in the population is important for making decisions about which antimicrobial to choose in field conditions. With respect to the frequent import and export of pigs between countries of the European Union, presented results can be informative for neighboring countries. The high frequencies of resistance to tetracycline correlate with the frequent use of this antibiotic for the treatment *S. suis* infections in pig farms in the past [3]. In the past, the first choice to control the occurrence and spread of *S. suis* was feeding mixtures supplemented with beta-lactams, such as penicillin or amoxicillin. Nowadays, due to the presence of bacterial populations resistant, or even multi-resistant, to antibiotics, this usage must be well justified [4].

Acknowledgements

The study was supported by the Ministry of Agriculture of the Czech Republic (Projects No. MZE-RO0518 and No. QK1810193).

References

1. Goyette-Desjardins et al. (2014) Emerg Microb Infect 3, e45.
2. Varela et al. (2013) Vet Res Com 21, 381-407.
3. Vela et al. (2005) Vet Microbiol 105, 143-147.
4. Correa-Fiz et al. (2020) Sci Reports 10, 371-391.

STK affects the location and contraction of the Z-ring in *Streptococcus suis* cell division

Minghui Ni, Liangsheng Zhang, Qi Huang, Rui Zhou*
State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, 430070, China, e-mail: rzhou@mail.hzau.edu.cn

Introduction

Cell division is one of the important physiological activities of bacteria, which is an important target of antibiotics [1]. Recently, eukaryotic-like Ser/Thr kinases (STKs) in bacteria have been reported as important regulators involved in the regulation of cell division of several important bacterial pathogens. Previous studies in our laboratory found that STK in *Streptococcus suis* mediates the phosphorylation of several important cell division proteins and deletion of *stK* leads to severe defects on cell division [2,3]. However, the exact process of how STK affects cell division is still unclear. In this study, our results indicate that FtsZ and STK are both localized in the centre of the septum and STK plays an important role in the Z-ring localization and contraction.

Materials and Methods

S. suis SC-19 is a pathogenic strain isolated in an outbreak in China in 2005 and Δ*stK* is a *stk* gene deletion strain. *S. suis* SC-19 and Δ*stK* were cultured in TSB liquid medium or TSA plate. Two fluorescent D-amino acid HADA and TAT were used as fluorescent dye to label the nascent peptidoglycan (PG). The membrane dye AF-647 was used to label the outline of cells. For protein subcellular localization study, FtsZ or STK were fused with a GFP at the C or N terminus using anhydrotetracycline (ATc) inducible plasmid. The cells were imaged using a structural light illumination super-resolution microscope (SIM).

Results

Previous studies in our laboratory found that STK disruption leads to cell elongation and the formation of multiple unclosed septa [3]. In this study, the subcellular localization of FtsZ and STK were examined by fusing a GFP followed by SIM analysis. It was shown in Fig. 1A and Fig. 1B that FtsZ and STK arrived at the mid-cell at the early stage (stage I) of cell division. The septum slowly closes as the peptidoglycan of the septum is gradually synthesized (stage Ⅱ) and then septum peptidoglycan begins to hydrolysis and the cell divides to form two daughter cells(stage III and IV). STK and FtsZ displayed co-localization in these four stages. We also observed localization of FtsZ in Δ *stK* showing that depletion of STK leads to mislocalization of FtsZ at the septum and appears early localization at the mid-cell of the nascent daughter cell, forming a structure with multiple Z-rings, and their contraction also appears to be affected (Figure 1C)

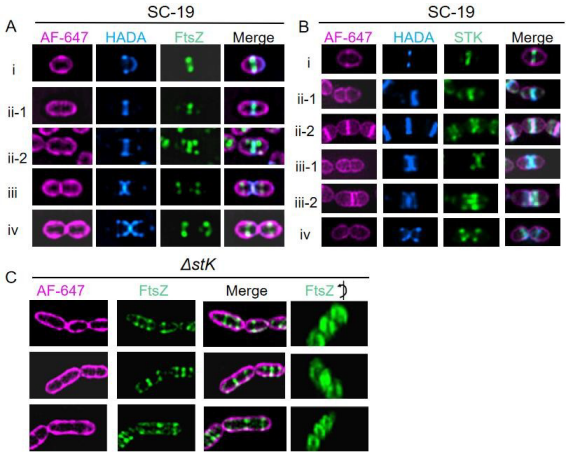


Figure 1. Subcellular localization of FtsZ-GFP (A) and STK-GFP (B) in SC19 and FtsZ-GFP in STK deletion strain (C).

Discussion

In this study, we investigated the localization of FtsZ and STK in wild-type strain and STK deletion strain. Our results show that STK and FtsZ are both localized in the centre of the septum. We also found that the location and contraction of FtsZ became abnormal in STK-deficient cells. Taken together, our data suggest that STK may regulate the location and contraction of FtsZ in cell division. Also, our previous phosphoproteomics results suggested that FtsZ is a potential substrate of STK [3]. However, whether STK can regulate Z-ring activity directly through phosphorylation requires further study, which may provide more understanding of the function of the STK and cell division in *S. suis*.

Acknowledgements

This study was supported by the National Key Research and Development Plans of China (No. 2021YFD1800401).

References

1.Battaje et al. (2023) Biosci Rep.43(2) BSR20221664.
2.Li W et al. (2021) Vet Microbiol. 258:109102.
3.Hu Q et al. (2021) Microorganisms. 9(12):2442.

The role of lipoprotein Lmb ("Laminin-Binding protein") in the pathogenesis of infection caused by *Streptococcus suis* serotype 2

S. Payen[1], J. Aranda Rodriguez [2], M. Segura[1] and M. Gottschalk[1].

[1]Faculty of Veterinary Medicine, University of Montreal, Quebec, Canada.
[2] Department de Genètica i Microbiologia, Universitat Autònoma de Barcelona (UAB), Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Espagne

Introduction

Streptococcus suis serotype 2 is an important bacterial pathogen of swine, responsible for substantial economic losses to the swine industry worldwide [1]. The knowledge on the pathogenesis of the infection caused by *S. suis* is still poorly known. It has been previously described that *S. suis* possesses at least one lipoprotein with double laminin and zinc (Zn)-binding properties, which was described in the literature as either laminin-binding protein (Lmb, as in the current study), lipoprotein 103, CDS 0330 or AdcAII [2,3]. In the present study, the role of the Lmb in the pathogenesis of the infection caused by *S. suis* serotype 2 was dissected.

Materials and Methods

Using isogenic mutants derived from the virulent *S. suis* serotype 2 P1/7 strain that are impaired in either Lmb lipoprotein only (Δ*lmb* mutant) or Lmb and capsule expression (Δ*lmb*/Δ*cps*) [2], we evaluated the role of the Lmb in bacterial adhesion to laminin and adhesion/invasion to NPTr cell (Newborn porcine tracheal epithelial cells). Virulence comparisons were also carried out using a mouse model of systemic infection. Finally, considering the limited role observed for the Lmb as laminin-binding protein, the second characteristic described for this lipoprotein (Zn uptake) was evaluated, by performed growth curves in different media.

Results

Results showed that Lmb does not play an important role in the laminin-binding activity of *S. suis*, even when clearly exposed at the bacterial surface (absence of capsule). In addition, this protein does not play a role in bacterial adhesion to and invasion of porcine respiratory epithelial cells. Nevertheless, the *Δlmb* mutant induced significant lower virulence and bacteremia in mice in comparison to the wild-type strain. Finally, we showed that the Δ*lmb* mutant grew well in rich media but not in plasma (in vivo-like conditions). When ZnSO4 was added to the media, growth of the Δ*lmb* mutant was restored. Finally, addition of the chelating agent TPEN in plasma supplemented with ZnSO4 impaired the growth of the *Δlmb* mutant strain (Fig 1).

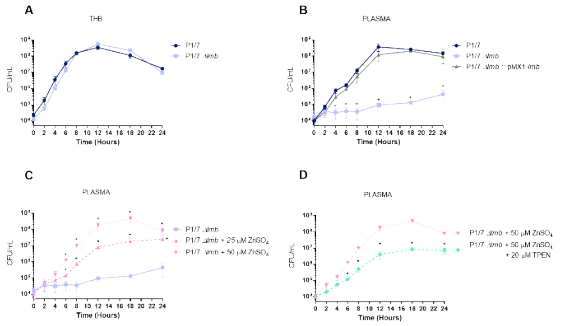


Figure 1. Growth of *Δlmb* mutant strain under different conditions**.** Growth of the wild-type P1/7 strain (dark blue), *Δlmb* (light blue) and *Δlmb*::pMX1-lmb (grey, plasma only) in rich medium (A), plasma (B), plasma supplemented with 25 µM ZnSO4 (pink dash) or 50 µM ZnSO4 (pink dot) (C) and plasma supplemented with 50 µM ZnSO4 (pink dot) added with 20 µM TPEN (green).

Discussion

Results from the current study showed that Lmb does not play an important role in the laminin-binding activity of *S. suis* serotype 2. In addition, the presence of this lipoprotein does not influence bacterial adhesion to and invasion of porcine respiratory epithelial. Finally, the Lmb plays a critical role in Zn acquisition. Indeed, the *Δlmb* mutant could not grow under Zn-restricted conditions (plasma) and the addition of Zn restored this defect. In addition, the complemented-mutant was able to grow at similar levels than the wild-type strain (results not shown). The lower virulence of the *Δlmb* mutant can be explained by a lower survival (lower bacteremia) *in vivo* as shown in the current study. This would be directly related to the in-vivo Zn-restricted conditions.

Acknowledgements

This study was funded by the Natural Sciences and Engineering Research Council of Canada to MG (grant #-2022–03730), MS (Grant # 2021–03020) and the International Affairs Department of the University of Montreal (S.P. travel scholarship).

References

1.Gottschalk et al. (2010) Future Microbiol 3,371-91.
2.Aranda et al. (2012) Can J Vet Res 76(1): 72–76.
3.Zhang et al. (2014) Microbial Res (5-6):395-401.

Streptococcus suis research update: serotype distribution and genotypic-phenotypic resistance to antimicrobials in swine isolates recovered in Spain between 2020 and 2022

M. Petrocchi Rilo[1], V. Acebes Fernández[1], C.B. Gutiérrez Martín[1], A. Aguarón Turrientes[2], A. González Fernández[1], R. Miguélez Pérez[1], S. Martínez Martínez[1]

[1] Microbiology and Immunology Laboratory, Animal Health Department, Faculty of Veterinary Science, University of León, León 24007, Spain; [2] Laboratorios SYVA, Leon Technology Park, León 24009, Spain

Introduction

Streptococcus suis is one of the main post-weaning pathogens in the porcine industry. It commonly causes meningitis, septicemia, and arthritis in diseased pigs (1). The most prevalent serotypes in Europe are 2 and 9, which seem to be the most virulent (2). This work addresses the study of 302 *S. suis* isolates, which have been collected in Spanish pig farms between 2020 and 2022. It has been performed with the intention of raising awareness about antimicrobial resistance (AMR) in *S. suis*. If no urgent action is taken, AMRrelated infections are expected to cause ten million deaths a year by 2050 (3).

Materials and Methods

Isolates were obtained from pigs with meningitis, arthritis, or pneumonia. A monoplex PCR was carried out to detect *S. suis* in farm samples. Four sets of multiplex PCRs were performed to identify different serotypes. Six AMR genes were studied: lincosamide resistance genes (RGs) (*lnuB* and *lsaE*), macrolide RGs (*ermB* and *mefA/E)* and tetracycline RGs (*tetM* and *tetO*). They were detected via multiplex PCR. Antimicrobial susceptibility testing was performed inoculating *S. suis* suspensions in FBS supplemented Mueller Hinton Broth, which was then distributed into Sensititre™ BOPO6F microplates. After incubating the plates for 24 hours at 37ºC, the results were annotated using the Sensititre™ Manual Viewbox.

Results

Seventeen different capsular types were detected. Serotypes 9 (21.2%), 2 (19.9%), 1 (16.9%) and 3 (6%) were the most prevalent ones. 78.1% of the isolates tested positive for *tetO,* 57% for *ermB*, 6% for *lsaE,* 6% for *lnuB,* 4.6% for *tetM*, and 1% for *mefA/E*.

xAntimicrobial susceptibility testing showed overall high resistances. For six of the tested antibiotics, at least 80% of the isolates showed resistance to the highest tested antimicrobial concentration (Fig. 1).

Furthermore, association of the presence of *ermB* and *tetO* with phenotypic resistance to macrolides and tetracyclines, respectively, was statistically significant (α=0.05; $p < 0.001$ using Fisher Exact Test). On the other side, only four antibiotics proved to be effective at the lowest concentration, inhibiting growth on at least 50% of the isolates. Ampicillin was the most effective one, with more than 85% of effectiveness inhibiting bacterial growth.

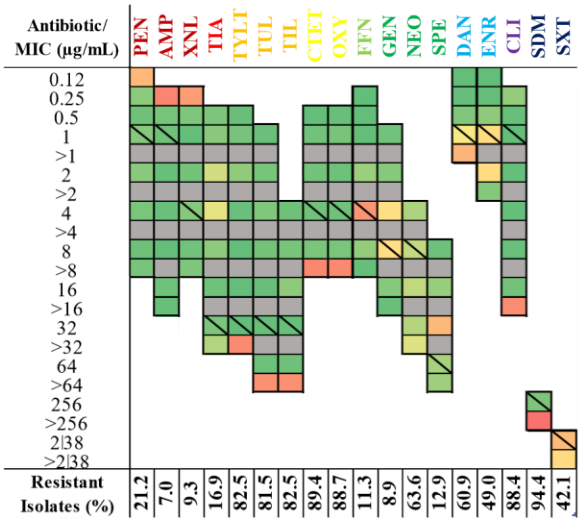


Figure 1. MIC distribution heatmap of the 302 *S. suis* isolates. Colored boxes represent the number of isolates (green: 0-99; yellow/orange: 100-249; red: 250-302) exhibiting the different MIC values for each antimicrobial. Diagonal lines indicate the breakpoints for each antimicrobial. Resistant isolates (%) for each antimicrobial are shown at the bottom of the figure.

Discussion

Most of the *S. suis* isolates used in this study exhibited worrisome AMR patterns. Seven out of the eighteen antimicrobials showed AMR levels greater than 80%. Only seven antibiotics exhibited AMR below 40%. However, two of the latter presented the highest MIC distribution at the breakpoint concentration, which could potentially lead to an eventual inefficacy of those antimicrobials to inhibit the growth of *S. suis.* Moreover, 58.3% of the isolates were resistant to nine or more antibiotics, which considerably reduces the treatment options for those isolates.

Acknowledgements

We acknowledge Laboratorios SYVA for supplying the 302 *S. suis* isolates used in this study.

References

1. Vötsch et al. (2018) Front Microbiol 9:480.
2. Segura (2020) Pathogens 9, 707.
3. O'Neill (2016) Rev Antimicr Resist 1-84.

Adhesion and invasion of human and porcine choroid plexus epithelial cells by ST104 serotype 2 *Streptococcus suis*

P. Prasopthum[1], J. Thanongsaksrikul[1], O. Khantisitthiporn[1], B. Archanachan[1], H. Ishikawa[2], H. Schroten[3], C. Schwerk[3], P. Srimanote[1]

[1]Department of Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathum Thani 12121, Thailand; [2]Department of Neurosurgery, Faculty of Medicine, University of Tsukuba, Tsukuba 305-875, Japan; [3]Department of Pediatrics, University Hospital Mannheim, Mannheim 68167, Germany.

Introduction

Zoonotic *Streptococcus suis* serotype 2 (*SS2*) is almost the sole serotype to cause meningitis and septicemia in humans. Multi-locus Sequence Typing (MLST) classifies into *SS2* into many sequence types (STs). ST1 *SS2* strains were most frequently isolated from humans and pigs with invasive diseases worldwide [1]. Surprisingly, ST104 *SS2* strains were reported to cause human infections only in Thailand. Disease manifestations of ST1 and ST104 *SS2* human infection were difference. Most ST1 *SS2* infections are resulting in the combination of septicemia and meningitis while 91% of ST104 human infections are apparently septicemia without meningitis [2]. The prevalence of ST104 *SS2* infections in pig is currently unknown. Furthermore, virulence factors and mechanism involving in ST104 *SS2* human and pig infections has not yet been studied. Therefore, the purpose of this study is to determine and characterize the ability of ST104 *SS2* to adhere and invade human and porcine choroid plexus epithelial cells compared to the well-established ST1 *SS2* strains.

Materials and Methods

One human and two disease pig ST104 *SS2* isolates were used in this study. The ST1 *SS2* P1/7 and HE06 strains isolated from disease pig and human, were also included as a comparison. For adhesion and invasion assays, human and porcine choroid plexus epithelial cells (HIBCPP and PCP-R, respectively) were seeded into a 24-well plate [3,4]. Upon 80-95% confluence, cells were infected with *SS2* at a multiplicity of infection of 10 (MOI 10; approx. 2×10^6 CFU/ml) for 4 hours. For enumeration of cell associated bacteria, the wells were then washed to remove unbound bacteria, cells were lysed to release cell associated bacteria, and the mixtures were serially diluted and further dropped on THY agar plates. For invasion assay following 4 hours infection and washed, the wells were subjected to penicillin/gentamycin exclusion to kill extracellular bacteria. The number of viable bacteria was then counted and presented as CFU/ml similar to adhesion assay. The genomic DNA of ST1 and ST104 *SS2* strains were extracted. The presence of 22 known virulence genes were investigated by multiplex PCR. The comparative whole genome sequence of ST1 and ST104 *SS2* Thai isolates were also analyzed.

Results

The ability of ST1 and ST104 *SS2* human and disease pig strains to adhere and invade HIBCPP and PCP-R cells were compared with *SS2* P1/7 reference strain. It was found that ST1 and ST104 human isolates, as well as ST104 disease pig isolates exhibited a significantly higher cell association and invasion of both HIBCPP and PCP-R ($p < 0.05$). Moreover, all tested strains had the higher abilities to associate with HIBCPP than PCP-R cells. However, the tested strains demonstrated the higher invasion capability to PCP-R cells. Surprisingly, there was no different in invasion ability of ST104 human isolate to HIBCPP cells compared to P1/7 and ST1 human *SS2* isolates. Multiplex PCR analysis indicated that there were no difference in virulence gene profile of ST1 and ST104 *SS2* strains. Furthermore, comparative pathogenomic analysis of ST1 and ST104 *SS2* Thai strains were analyzed using MGC database (http://www.mgc.ac.cn), however none of the difference in known virulence gene composition revealed.

Discussion

ST1 and ST104 *SS2* isolated from disease pig and human origins had the higher abilities to associate and invade HIBCPP and PCP-R compare to reference strain P1/7 regardless of origin of bacterial strain isolation. Despite to the disease manifestations of ST104 *SS2* in human their invasion ability of HIBCPP were equivalent to human ST1 *SS2* isolate. Currently, preliminary pathogenomic analysis revealed no distinct virulence factors for ST104 *SS2*. Therefore, further detailed analysis of whole genome sequence is required for the better understanding of ST104 *SS2* pathogenesis.

Acknowledgements

The present study was supported by doctoral scholarship for research academic, Thammasat University, Pathum Thani, Thailand.

References

1. Aradanas et al. (2021) Front Vet Sci 8, 1-17.
2. Kerdsin et al. (2011) Emerg Infect Dis 17, 835-842.
3. Lauer et al. (1998) Front. Cell Infect Microbiol 11, 1-18.
4. Schroten et al. (2012) PLoS ONE 7, 1-8.

Translocation across a human enteroid monolayer by zoonotic *Streptococcus suis* is driven by the presence of Gb3-positive cells

T.J. Roodsant[1,2], K. van der Ark[1,2] and C. Schultsz[1,2]

[1] *Amsterdam UMC, location University of Amsterdam, Department of Global Health, Amsterdam Institute for Global Health and Development, Meibergdreef 9, Amsterdam, The Netherlands*

[2] *Amsterdam UMC, location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Meibergdreef 9, Amsterdam, the Netherlands*

Introduction

Zoonotic *S. suis* infections are mostly caused by *S. suis* serotype 2 (SS2) isolates from clonal complex 1 (CC1) [1]. In the Netherlands, the zoonotic SS2CC20 lineage has emerged from the non-zoonotic but virulent pig lineage SS9CC16 [2]. The consumption of undercooked pig products is an important risk factor for human *S. suis* infection, implying *S. suis* potential as foodborne pathogen and identifying the gastrointestinal tract as potential entry site into the human host [3]. The recent advances in organotypic cultures allows for studying bacterial pathogenesis in a multicellular model that mimics the cellular complexity of the human small intestine, such as enteroids. To further understand the role of the gastrointestinal tract in the pathogenesis of zoonotic and potentially foodborne *S. suis* infection, we studied the adhesion, invasion and translocation of zoonotic and non-zoonotic *S. suis* in a human enteroid model.

Materials and Methods

Human 3D enteroid cultures were generated from proximal (duodenum, jejunum) or distal (ileum) small intestine fetal tissue. 3D enteroids were dissociated into single cells and grown into differentiated enteroid monolayers on transwells. Monolayers were infected with *S. suis* (MOI 50) at the luminal side and translocation into the basolateral compartment was followed in time for 6 hours. Monolayer permeability during infection was monitored by adding 4kDa FITCdextran to the luminal side. Enteroid monolayers were grown in 48 wells plates to quantify *S. suis* adhesion and invasion. (Mock) Infected monolayers were analyzed by confocal imaging and flow cytometry after immunofluorescence staining.

Results

The zoonotic genotypes (CC1 and CC20) had an increased translocation frequency across proximal enteroids monolayers compared to distal enteroids monolayers. The zoonotic CC1 genotype had an increased translocation frequency compared to the non-zoonotic CC16 genotype, without affecting the barriers permeability as measured by FITC-dextran flux. Confocal imaging showed that translocation occurred without damaging the tight junctions or adherens junctions that seal of the paracellular route.

A representative strain selection from SS2CC1, SS2CC20 and SS9CC16 (five per CC) confirmed the increased translocation of SS2CC1 compared to SS9CC16 across proximal enteroid monolayers without disrupting the monolayer's barrier function. The three CCs did not differ in adhesion and invasion capabilities. The translocation of zoonotic SS2CC1 was dependent on the presence of Gb3-positive cells within the monolayer. The *S. suis* virulence factors Streptococcal adhesin Protein (SadP) and suilysin (Sly) are known to interact with Gb3, but the translocation frequency was not decreased in D*sadP*, D*sly* and D*sadP*D*sly* knockout mutants compared to WT.

Discussion

Zoonotic *S. suis* from SS2CC1 has an increased translocation frequency across the human small intestine compared to non-zoonotic SS9CC16. Similar to observations made in the Caco-2 model [4]. In contrast to the Caco-2 model, the adhesion and invasion capabilities did not differ between the tested CCs and translocation did not affect the adherens or tight junctions. Translocation of zoonotic SS2CC1 was driven by on the presence of Gb3-positive cells cells within the monolayer.

Acknowledgements

Our work was funded by the European Union Horizon2020 grant 727966 (PIGSs).

References

1. Goyette-Desjardins et al. (2014) Emerg Microbes Infect2014/06/18. 3:e45–e45.
2. Willemse et al. (2016) Sci Rep 6:28984.
3. Ho et al. (2012) PLoS One 6:e17604.
4. Ferrando et al. (2015) J Infect Dis2014/12/20. 212:95–105.

Colorimetric Detection for *Streptococcus suis* by Specific Aptamer and Gold Nanoparticle

S. Sangboonruang[1], A. Matchawong[1], N. Semakul[2], K. Tragoolpua[1], C. Srisawat[3], C. S. Tharinjaroen[1]

[1] *Infectious Diseases Research Unit (IDRU), Division of Clinical Microbiology, Faculty of Associated Medical Sciences;* [2] *Department of Chemistry, Faculty of Sciences, Chiang Mai University, Chiang Mai 50200, Thailand*
[3] *Department of Biochemistry, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand*

Introduction

Zoonotic disease caused by "*Streptococcus suis*" has an impact on human and pig around the world, especially in the northern of Thailand. Standard methods for identification of *S. suis* are relied on culture and biochemical tests, which are time-consuming and usually found underdiagnosed cases (1). Thus, simple, cost-effective, and rapid detection are essentially required for early diagnosis. Recently, aptamer, a single-stranded DNA or RNA that specifically bind to the target based on its 3D conformation is one of attractive molecules provided advantages for diagnostic assay development (2). Hence, the assay for *S. suis* detection using *S. suis*-specific RNA aptamer (R8-su12) as recognition probe and gold nanoparticles (AuNPs) serve as a potential colorimetric indicator which can be monitored the color change of AuNPs with naked eyes (3) was developed.

Materials and Methods

The R8-su12 was developed and showed specific binding against *S. suis* as described previously (2). The AuNPs were synthesized by citrate reduction method. The UV-Vis spectra of AuNPs solution under different experimental conditions were measured in the range of 400-700 nm. The principle of colorimetric detection assay was described (Fig. 1).

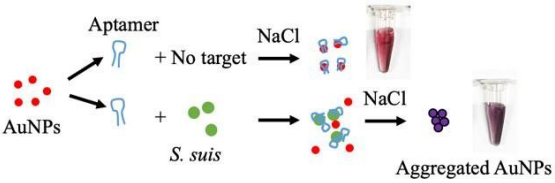


Figure 1. The principle of AuNPs based colorimetric detection assay.

Results

After eight SELEX cycles, the RNA aptamer pool showed a significant specific binding to *S. suis* compared to the random RNA library. Moreover, the 8th round cell-SELEX exhibited the highest binding affinity to the target with statistical significance (p=0.034) when compared to non-target cells (*S. pneumoniae* and *S. pyogenes*). Finally, R8-su12 exhibited the best candidate molecule.

The AuNPs were synthesized and the major peak of AuNPs solution was observed at 520 nm. Aggregation of AuNPs was induced at the optimized concentration of NaCl, resulting in color changing from red to purple. In the presence of R8-su12, AuNPs were protected against NaCl-induced aggregation, indicating by no change in color and the absorbance peak. The change in color from red to purple was observed in the presence of *S. suis* by visualization within 30 min and spectral shift at 630 nm was demonstrated (Fig. 2). This indicated that the developed assay based on R8-su12 aptamer and AuNPs had a potential that could be further applied for detection of *S. suis* with a limit of detection (LOD) in the range 10^4-10^7 CFU.

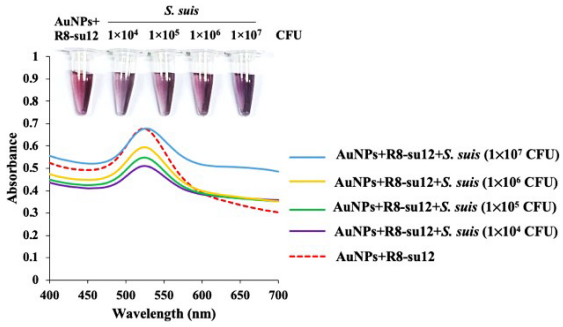


Figure 2. Colorimetric detection assay in the presence of various cell number of *S. suis*.

Discussion

This study introduced a simple and rapid colorimetric method to detect *S. suis*. However, optimization and assay evaluation with clinical specimens are further examined.

Acknowledgement

The grant was supported by CMU Junior Research Fellowship Program, 2022, Chiang Mai University, Chiang Mai, Thailand.

References

1. Thu et al. (2021) Pathogens 6, 996.
2. Matchawong et al. (2022) Molecules 27, 3894.
3. Mondal et al. (2018) Front Microbiol 9, 179

The glycosyltransferase ScwpI affects the composition and function of the rhamnose-rich cell wall polysaccharides of *Streptococcus suis*

Y. Shi[1 2], T. J. Roodsant[2 3], B. C. L. van der Putten[2 4], C. C. Domínguez-Medina[2], K. C. H. van der Ark[2 3], A. Stegeman[1], C. Schultsz[2 3], N. M. van Sorge[2 4] and L. Benedictus[1]

[1] Department of Population Health Sciences, Utrecht University, Utrecht, The Netherlands; [2] Department of Medical Microbiology and Infection Prevention, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands; [3] Department of Global Health-Amsterdam Institute for Global Health and Development, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands;[4] Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands

Introduction

The cell walls of Streptococcus spp. are decorated with cell wall-associated polysaccharides, including capsular polysaccharides (CPS) and rhamnose-rich cell wall polysaccharides (RhaCWP). RhaCWP are abundant structures, important for bacterial homeostasis, growth, and cell division. Furthermore, they are also important for Streptococcal host cell adhesion, resistance to antibacterial peptides, evading phagocytosis, and antibiotic resistance [1,2]. However, in the zoonotic and major pig pathogen *Streptococcus suis* (*S. suis*), RhaCWP have not been studied.

Materials and Methods

The RhaCWP biosynthesis gene cluster was identified and annotated by searching orthologs in well-studied Streptococcus spp. A database containing 1,719 publicly available *S. suis* genomes was screened for RhaCWP genes using ABRicate.

Gene mutation was performed by allelic replacement using a Janus Cassette. Genetic complementation was done using the plasmid vector pDC123. Binding of different lectins to *S. suis* strains in the exponential growth phase was measured by flow cytometry.

Results

We identified a 5-gene rhamnose and a 14-gene RhaCWP biosynthesis gene cluster in the reference strain P1/7. Population analysis of 1,719 *S. suis* whole genome sequences revealed that the rhamnose biosynthesis cluster was highly conserved, but there was considerable diversity in the RhaCWP gene cluster. In 11.7% of isolates, the gene content of the RhaCWP cluster was variable, whilst in the remaining isolates, the genetic organization was conserved with major allelic diversity restricted to two putative glycosyltransferases.

One of the glycosyltransferases, ScwpI, had nonsynonymous mutations in the putative functional domain in the pathogenic ST-16 and ST-20 lineages. Plant lectin binding assays of *scwpI* knock-out mutants and homologous and heterologous complemented strains showed that SwcpI transfers a different sugar to RhaCWP in the ST16/ST20 lineages compared to CC1 lineages (Fig. 1A). Interestingly, *scwpI* knock-out mutants showed reduced growth (Fig. 1B), while maximal bacterial cell density and chain length were unaffected compared to wild-type strains.

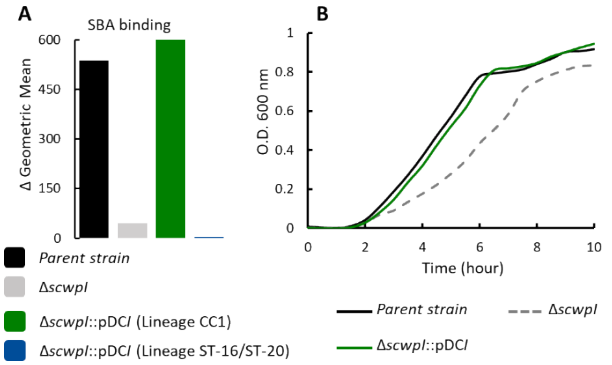


Figure 1. (A) Binding of fluorescent SBA to *scwpI* knockout and different complementation strains. SBA binds to terminal N-acetylgalactosamine (GalNAc) and, to a lesser extent, galactose residues. (B) Representative growth curves.

Discussion

Our results indicate that the genetic variation in *scwpI* results in differences in the sugar composition of RhaCWP and *S. suis* requires RhaCWP for intrinsic physiology. Planned NMR analyses will elucidate the function of ScwpI in the RhaCWP biosynthesis pathway and additional phenotypical assays will be performed to study the role of ScwpI in cell division and virulence.

Acknowledgements

The present work was part of the research Programme of the Netherlands Centre for One Health (www.ncoh.nl) and of the CANVAS research project, a public private partnership powered by Health~Holland, Top Sector Life Sciences & Health, a research and innovation funding program of the Dutch government. Y. Shi is funded by China Scholarship Council.

References

[1] Mistou *et al.* (2016) FEMS Microbiology Rev 40, 464-479

[2] van Sorge *et al* (2014) Cell Host Microbe 15, 729-740

Internalization and survival of ST104 serotype 2 *Streptococcus suis* in murine and porcine macrophage cell lines

Kittiyawan Sriyanon[1], Jeerapong Thanongsaksrikul[1], Ornreudee Khantisittiporn[1], Buppa Arechanajan[1] and Potjanee Srimanote[1]

[1]*Graduate Program in Biomedical Sciences, Faculty of allied health science, Thammasat University, Pathum Thani, 12120, Thailand*

Introduction

Serotype 2 *Streptococcus suis* (*SS2*) is an important zoonotic pathogen that can cause sepsis and meningitis in humans. *SS2* isolates are genetically subtyped into sequence types (STs) by multilocus sequence typing (MLST). ST1 *SS2* strains were the most frequent ST isolated from patients and disease pigs. In Thailand, ST1 and ST104 *SS2* were the primary causative agents of human infections. ST104 *SS2* human infection is unique to Thailand [1]. Therefore, this study aims to investigate the internalization and survival of ST104 *SS2* in murine and porcine macrophage cell lines and compare to that of well-established ST1 *SS2* strains.

Materials and Methods

ST104 *SS2* strain HS05 strain was used in this study. The ST1 *SS2* strains P1/7 and HE06 derived from disease pig and human origins were also included. J774 (murine macrophage) and 3D4/21 (porcine macrophage) cell lines were seeded into a 24-well tissue culture dish and infected with 1 x 10^7 CFU of *SS2* (MOI = 100). Following one hour of incubation at 37°C with 5% CO_2, macrophages were lysed, and the mixture was serially diluted and plated on a THY agar plate for enumeration of *SS2* association. Following infection, penicillin G (5 µg/mL) and gentamicin (100 µg/mL) was further added to kill extracellular bacteria for one hour and enumerated for the number of *SS2* internalized by macrophages [2]. Macrophage intracellular survival was performed as previously described for internalization, except the incubation in an antibiotic-containing medium was prolonged to three hours. The number of viable *SS2* presented as CFU/ml [3].

Results

There was no significant difference in the number of ST1 (strains P1/7 and HE06) and ST104 *SS2* strain HS05 to associate with murine and porcine macrophages. P1/7 could be internalized by murine macrophages 100 times higher than porcine macrophages, while the ST1 HE06 and ST104 HS05 strains could be internalized by murine macrophages only 10 times higher than porcine macrophages. Eighty-five and 15 percent of internalized P1/7 *SS2* were intracellularly killed within murine and porcine macrophages, respectively.

The internalized ST1 HE06 and ST104 HS05 were intracellularly killed by murine macrophages at 40% and 60%, respectively. On the other hand, intracellular survival of ST1 HE06 and ST104 HS05 strains within porcine macrophages was higher at 90% and 70%, respectively.

Discussion

The cell association of ST1 and ST104 *SS2* strains to mouse macrophages was no different from porcine macrophages. This result suggested no different recognition of ST1 and ST104 SS2 PAMPs by PRR on murine and porcine macrophages. In this study, ST104 *SS2* could inhibit the internalization and intracellular killing of murine and porcine macrophages, equivalent to ST1 *SS2* patient isolate. ST1 and ST104 *SS2* strains survive within porcine macrophages better than murine macrophages suggesting that the virulence factors contributing to the survival of bacteria within macrophages function more efficiently in a natural host.

Acknowledgments

This work was supported by (i) Thammasat University Fundamental Fund Fiscal year 2566, (ii) Thailand Science Research and Innovation (TSRI), and (iii) National Science, Research and Innovation Fund (NSRF) (Project number180178).

References

1. A. Kerdsin, et al. (2011). Emerging Infectious Diseases. Vol. 17, No. 5, May 2011
2. Auger J-P, et al. (2019). PLoS ONE 14(10): e0223864.
3. Auger J-P, et al. (2019). Front. Cell. Infect. Microbiol. 9:124.
4. M. Segura et al. (2002) Infection and immunity, p. 4312–4322
5. Uribe-Querol E and Rosales C (2020) Front. Immunol. 11:1066

HylS, a fragment of truncated hyaluronidase of *Streptococcus suis,* contributes to immune evasion by interaction with host complement C3b

JJ. Xu, L. Chen, SQ. Pang, L.Li*, R. Zhou*

National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, E-mail: lilu@mail.hzau.edu.cn; rzhou@mail.hzau.edu.cn

Introduction

The complement system is used as the first line to defense against pathogen infections. C3 and its fragments play essential roles in immune surveillance and clearance of pathogens, but microbes have evolved many strategies to escape it (1). Hyaluronidase (HylA) has been reported as a virulence factor in various bacterial species. In the genome of *Streptococcus suis* serotype 2 strain SC-19, we identified a region encoding a truncated hyaluronidase different from the hyaluronidase (HylA) in other strains, the product of which had no hyaluronidase activity. A fragment named HylS in this region was confirmed to be a secretory protein which could interact with complement C3b. The role of HylS in immune evasion of *S. suis* was revealed.

Materials and Methods

The strain used were the wild-type strain SC-19, *hylS* deleted mutant Δ*hylS*, the complementary strain CΔ*hylS* and the wild-type strain containing intact *hylA* in place of *hylS* (SC-19$_{hylA}$). Pulldown assay was used to detect the interaction between HylS and C3b. Depositions of C3b and membrane attack complex (MAC) on *S. suis* were detected via the FACSCalibur flow cytometer. Serum killing assays were used to compare the viability of different strains. Virulence of different strains was detected using mouse infection model.

Results

A truncated mutant of Hyaluronidase (HylA) named HylS of *S. suis* SC-19 was found to be a secretory protein interacting with complement C3b, while HylA from *S. suis* 0895 also could interact with C3b (Fig. 1). HylS was involved in the bacterial survival in human serum (Fig. 2A). Additionally, increased depositions of C3b and MAC were found on the surface of Δ*hylS* and SC-19$_{hylA}$ compared with that on SC-19 (Fig. 2B, C). In a mouse infection model, Δ*hylS* infection was found to have reduced bacterial loads in the organs of the mice (Fig. 3). Therefore, HylS contributes to the complement evasion and pathogenicity of *S. suis*.

Discussion

Comparative analysis of genes encoding hyaluronidase of *S. suis* confirmed that all ST1 isolate (high virulence) had four conservative insertions, resulting in no hyaluronidase activity (2) and also confirmed in SC-19 in this study. However, the function of the truncated product of HylA, the

HylS, was found to contribute to immune evasion. HylS was found to be a secretory protein interacting with C3b and inhibited the C3b deposition and MAC on the surface of bacteria (Fig. 1, 2). The interaction between HylS and C3b may consume C3b in the serum, thus reducing the C3 and MAC on the surface of bacteria, thereby enabling better survival of *S. suis* in serum and *in vivo*. Although the intact HylA was also found to have C3b binding ability, it showed lower degree to affect C3b deposition and formation of MAC on bacteria and survival in serum, compared with HylS.

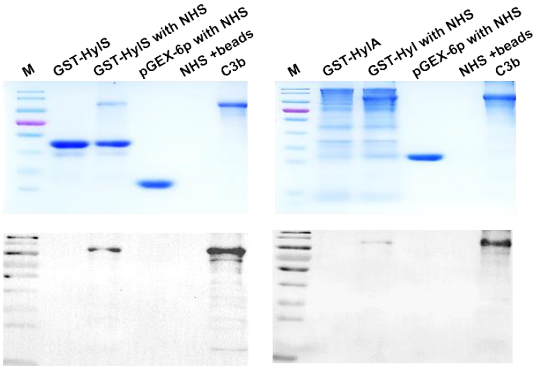


Figure.1 HylS interacts with C3b.

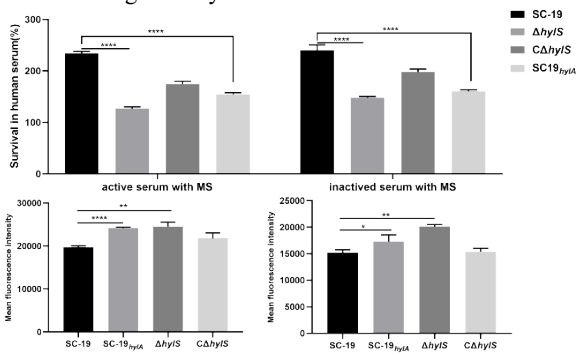


Figure.2 HylS contributes to bacterial survival in human serum and inhibits C3b deposition and MAC on *S.suis*.

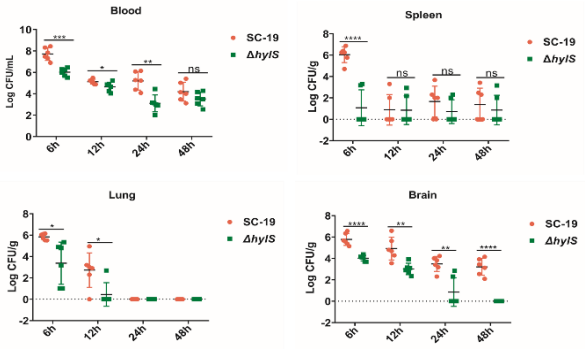


Figure.3 HylS contributes to virulence of *S. suis*.

Acknowledgements

This study was supported by the National Key Research and Development Program of China (2021YFD1800404).

References

1. Ricklin et al. (2016) *Immunological Reviews* 274: 33–58.

2. Haas B et al. (2015) *BMC Research Notes* 8(1): 722-722

[在此处键入]

Genome sequences of antibiotic-resistant *Streptococcus suis* strains isolated from human patients and diseased and asymptomatic pigs in Thailand

S. Yongkiettrakul [1], T. Wongsurawat [2, 3], P. Jenjaroenpun [2, 3], D. A. Acheampong [2, 4], P. Srimanote [5], K. Maneerat [6], W. Visessanguan [1], and I. Nookaew [2]

[1] National Center for Genetic Engineering and Biotechnology, Pathum Thani 12120, Thailand; [2] Department of Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, US; [3] Department of Research and Development, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand; [4] Joint Graduate Program in Bioinformatics, University of Arkansas at Little Rock and University of Arkansas for Medical Sciences, Little Rock, Arkansas 72204, US; [5] Faculty of Allied Health Sciences, Thammasat University, Pathum Thani 12120, Thailand; [6] College of Alternative Medicine, Chandrakasem Rajabhat University, Bangkok 10900, Thailand.

Introduction

Streptococcus suis, an opportunistic bacterial pathogen is recognized as an emerging zoonotic infectious disease, causing human health problems and economic losses in swine industry. Among Asian countries, China, Vietnam, and Thailand are endemic areas for *S. suis* infections [1-3]. To gain a deeper understanding of the genetic diversity and antibiotic resistance of *S. suis* strains found in Thailand and other regions, whole-genome sequencing of Thai-isolated strains and comparative genomic analysis was conducted. The findings of this study provide valuable resources for genomic epidemiology investigation of the potential life-threatening effects of *S. suis* in Thailand.

Materials and Methods

The genomes of 9 Thai-isolated antibiotic-resistant S. suis strains, including 2 from human patients, 4 from diseased pigs, and 3 from asymptomatic pigs, covering serotype 1 (2 strains) and serotype 2 (7 strains), were sequenced by Illumina NextSeq 550 platform. Genome assembly, annotation, and analysis, as well as single-nucleotide polymorphism-based phylogenetic analysis were previously described [4].

Results

The genome analysis showed significant genetic diversity among Thai-isolated S. suis strains and the transmission of virulent strains between humans and pigs. The SNP-based phylogenetic analysis indicated that S. suis isolates from Thailand are closely related to those from other countries (Fig. 1). The genome analysis revealed that (*aad*(6), *ant*(6)-Ia, *erm*B, *tet*(O), *pat*B, and *sat*4) and gene clusters (*aph*(3′)-IIIa and *aac*(6′)-Ie-*aph*(2″)-Ia) were associated with aminoglycoside, macrolide, and fluoroquinolone resistance in *S. suis*.

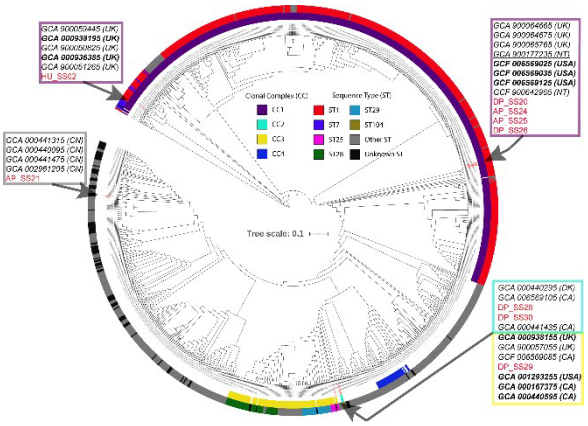


Figure 1. A SNP-based phylogenetic tree of Thai-isolated strains with 1,186 *S. suis* strains isolated worldwide, including Canada (CA), China (CN), Denmark (DK), Netherlands (NT), United Kingdom (UK), and United States of America (USA). The tree shows sequence types (STs) on the outer ring and clonal complexes (CCs) on the inner ring. The host of each isolate is indicated as human (underline), asymptomatic pig (italic), and diseased pig (italic and bold).

Discussion

SNP-based phylogenetic analysis demonstrated no geographic relationship between S. suis strains found in healthy and diseased pigs in Thailand and genetic relatedness among *S. suis* strains from pigs and humans in different locations. Genome analysis revealed *erm*B and *tet*O genes associated with resistance to macrolides and tetracyclines. The resistance of Thai-isolated *S. suis* strains to fluoroquinolones may be mediated by overexpression of the SatAB efflux pump. Further investigation is needed to identify additional mechanisms and instances of horizontal gene transfer that could lead to the development of aminoglycoside resistance in *S. suis* and whether certain mutations of 23S rRNA may result in tiamulin resistance in *S. suis* in Thailand.

References

1. Segura et al. (2020) Pathogens. 14, 374.
2. Kerdsin et al. (2022) Trop. Med. Infect. Dis. 7, 359.
3. Vu Thi Lan et al. (2006) New Eng. J. Med. 354, 44–53.
4. Yongkiettrakul et al. (2021) Infect. Genet. Evol. 87, 104674.

Acknowledgements

The present study was financially afforded by BIOTEC-RI Grant No. P1651873 to S.Y. and National Institute of General Medical Sciences of the National Institutes of Health (Award P20GM125503) to I.N.

The unique peptidoglycan synthesis model of *Streptococcus suis*

Liangsheng Zhang, Qi Huang, Rui Zhou*

State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wu han, 430070, Email: rzhou@mail.hzau.edu.cn

Introduction

The mechanism of bacterial peptidoglycan (PG) synthesis varies according to different cell shapes and species [1]. However, the current research on PG synthesis of ovococci mainly focuses on *Streptococcus pneumoniae*, while the mechanisms of PG synthesis in others ovococci are poorly understood. In *S. pneumoniae*, cells form three parallel division ring in the early stages and simultaneously complete the synthesis and division of septum [2]. Our study suggests that *Streptococcus suis*, an important ovococcal zoonotic pathogen, might have a different PG synthesis mechanism from that of *S. pneumoniae* [3]. Thus, the purpose of this research was to investigate the PG synthesis process of *S. suis* by fluorescent D-amino acids (FDAAs) labelling and 3D-SIM.

Materials and Methods

The bacterial strains used in this study is *S. suis* strain SC-19. For the FDAA labeling, cells in the logarithmic phase were diluted into fresh medium and grown for 0.5 h, then 5μl of HADA or TADA was added to 200μM and incubated for 25 min at 37°C. Finally, fluorescent dye AF647 was added to visualize membranes. For the sequential FDAA labeling, cells were incubated with HADA, washed and incubated with TADA again. Images were collected using the NIS-Elements microscope (Nikon) and cell length were measured with the software ImageJ.

Results

According the cell length as well as the location of nascent PG, the PG synthesis process were classified in different cell division stages of *S. suis* (Fig. 1 A, B). At the same time, the synthesis sites (Fig. 1 D, TADA) and dynamic synthesis of PG (Fig. 1 C) at different division stages of *S. suis* were investigated by sequential FDAA labelling. The results showed that a segment of peripheral PG was synthesized by the central PG synthesis site at the early cell division stage of *S. suis*. Then the PG synthesis site was moved to the entire septum to start the closure and thickening of the septum. After closure of the septum, the PG synthesis site gradually moved to the outer edge of the septum to start the division of the septum and the synthesis of nascent peripheral PG (Fig. 1 E). The results showed that the PG synthesis of *S. suis* was different from that of *S. pneumoniae*, indicating that *S. suis* had a unique PG synthesis model.

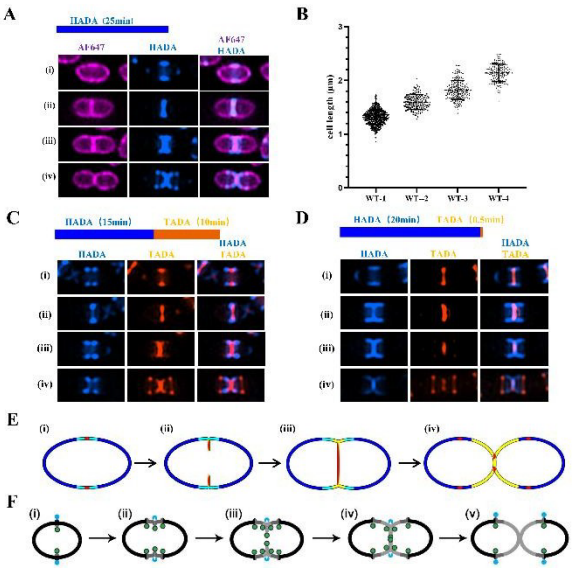


Figure 1. The PG synthesis of *S. suis* (A) HADA labeling in different stage of PG synthesis (B) Measurement of cell lengths (C, D) Sequential FDAA labeling (E) PG synthesis model of *S. suis* (F) PG synthesis model of *S. pneumoniae*.

Discussion

In this study, by studying the dynamics nascent PG synthesis, it was shown that there was only one active PG synthesis site in *S. suis*, which sequentially completes the synthesis of peripheral and septal PG. Unlike *S. pneumoniae*, the formation of division sites in newborn cells of *S. suis* occurred in the late stage of cell division, and the septum started to split until after the closure of septum. Therefore, the experimental results in this paper showed that the PG synthesis process of *S. suis* was different from that of *S. pneumoniae*, which was another PG synthesis mode of ovococci.

Acknowledgements

This study was supported by the National Key Research and Development Plans of China (No. 2021YFD1800401).

References

1. Wheeler et al. (2011) Mol Microbiol 82(5): 1096-1109.
2. Perez et al. (2019) Proc Natl Acad Sci U S A 116(8): 3211-3220.
3. Hu et al. (2021) Microorganisms 9(12): 2442.

Essential genes analysis by Tn-seq reveals potential antimicrobial drug targets against *Streptococcus suis*

Yongqing Zhang[1], Rui Zhou[1,2], Qi Huang[1,2]

[1]*College of Veterinary Medicine;* [2]*International Research Centre for Animal Disease, Huazhong Agricultural University, Wuhan 430070, China*

Introduction

Streptococcus suis is an important zoonotic bacterial pathogen causing severe economic losses to the swine industry as well as a public health threat. The growing antimicrobial resistance in *S. suis* isolates leads to the raise of clinical treatment failure [1]. Therefore, it is an urgent need to develop novel antimicrobial drugs in which identifying new drug targets is critical. In this study, we developed a new Mariner-based plasmid for Tn mutagenesis in *S. suis*. A saturated Tn library was then generated. By high throughput sequencing, a total of 193 genes were identified as essential. After excluding homologous genes and comparing these genes with already known essential genes of *Streptococcus pneumonia* and *Escherichia coli*, potential drug targets were identified.

Materials and Methods

The Tn library was constructed by transforming the competent cells of *S. suis* SC19 with pSET4s-Tn plasmid followed by growing the cells in spectinomycin (Spc)-containing medium at 28 ℃ and then growing at 37℃ to allow plasmid curation. The chromosome DNA was extracted from the Tn library and sequenced by Illumina sequencing. The sequencing results were analyzed with the Transit software using the Gumbel model [2].

Results

The plasmid pSET4s-Tn for Tn mutagenesis in *S. suis* contains a Spc resistance cassette, a temperature-sensitive ori, a Mariner transposase cassette, and a Erm resistance cassette flanked by the invert reverse site (Fig. 1A). Using this plasmid, a Tn library containing over 160,000 Tn mutants was generated. The library was subjected to Illumina sequencing which revealed that a total of 1771 genes (86.98%) contained Tn insertion (Fig. 1B).

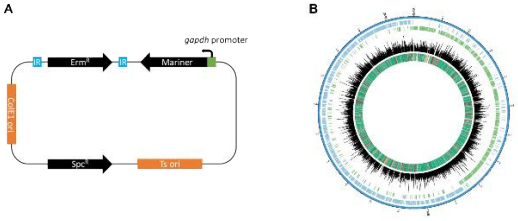


Figure 1. Construction of Tn mutagenesis library of *S. suis* SC19. (A) pSET4s-Tn plasmid. (B) Insertion sites of the Tn library.

By analysis using the Transit software, 193 genes were identified as essential genes. Among them, 114 genes do not have a homologous gene in the pig, human, or mouse. These genes were compared with the essential genes of *S. pneumonia* and *E. coli* as reported previously [3, 4] which showed that 62 genes were also essential in these bacteria (Fig. 2). Among them, genes involved in metabolism (such as *aptA*, *aptB*, *purF*, *adk*, *prs*, *purB*, *purN, znuC*, *bmrA*, *secY*) and DNA repair and replication (such as *dnaN*, *ssb*) were believed as potential antimicrobial drug targets.

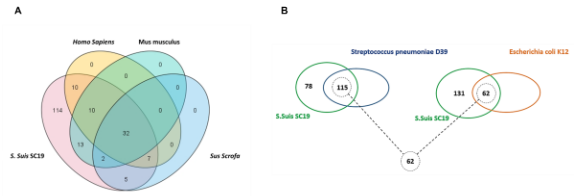


Figure 2. Essential genes analysis of *S. suis* SC19.

Discussion

Transposon-based random mutagenesis has been widely used to identify virulence genes, antimicrobial resistance genes, etc. By constructing a highly saturated Tn library followed by high throughput sequencing, essential genes can be easily identified. In this study, we developed an efficient tool for Tn mutagenesis in *S. suis* and identified the essential genes by sequencing a highly saturated Tn library. By analyzing these genes, a series of potential drug targets were revealed. Most of these targets are involved in bacterial metabolism. The tool can be further used for functional genes identification of *S. suis* and the drug targets identified in this study will facilitate further antimicrobial drug development.

Acknowledgements

The study was supported by the National Key Research and Development Program of China [2022YFD1800902].

References

1. Kittiwan et al. (2022) Microb Genom, 8(11):mgen000882.
2. DeJesus et al. (2015) PLoS Comput Biol 11(10): e1004401.
3. Liu et al. (2017) Mol Syst Biol, 10;13(5):931.
4. Goodall et al. (2018) mBio, 20;9(1):e02096-17.

Role of HtrA in Streptococcus suis serotype 2 stress tolerance and Virulence

Haodan Zhu*, Junming Zhou, Yiyi Hu, Dandan Wang, Bin Li, Yanxiu Ni, Kongwang He

Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing, PR China

Streptococcus suis (SS) is an important zoonotic pathogen worldwide, however, the pathogenesis is still not entirely known. HtrA (high-temperature requirement A) is a heat shock-induced serine protease with homologs in a wide range of bacteria and eukaryotes and is also associated with infectious diseases since the inactivation of *htrA* genes results in significantly reduced virulence properties by many bacterial pathogens. In our previous study, HtrA was identified as a down-regulated protein in the serine/threonine kinase (STK) gene mutant strain *Δstk*. In this study, we investigated the roles of HtrA homologs of SS (designed SsHtrA) during bacterial infection. The isogenic mutant strain *ΔhtrA* exhibited increased susceptibility to various stress agents including acidic pH and H_2O_2-induced oxidative stress. The mutant strain *ΔhtrA* showed greatly decreased adherence abilities to the two different cell lines (HEp-2 and PIEC) and survival rates in the whole blood and macrophage cells RAW264.7. Mouse infection experiment showed that the inactivation of HtrA greatly attenuated the high pathogenicity of virulent strain SS2-1 and the colonization in the various tissues. These virulence-related phenotypes were restored by genetic complementation. The observed results suggest that HtrA contributes to the survival of the virulent strain SS2-1 under different stress conditions and plays an important role in the survival and persistence of SS2-1 in the bloodstream and host tissues during bacterial infection.

Diversity of *Streptococcus suis* strains isolated from sick pigs from farms in the Czech Republic

M. Zouharova[1], B. Simek[2], K. Nedbalcova[1], I. Kucharovicova[2], M. Brychta[2], K. Matiaskova[1], N. Kralova[1,3], J. Gebauer[1], J. Matiasovic[1]

[1] Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic; [2] State Veterinary Institute, Rantirovska 93, 586 05 Jihlava, Czech Republic; [3] Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic

Introduction

Streptococcus suis is a significant cause of mortality in piglets and growing pigs worldwide. Some strains colonise the upper respiratory tract as commensal strains, but some strains are pathogenic, that causes meningitis, arthritis, endocarditis, polyserositis, and septicemia and are also considered an emerging zoonotic pathogen. The *S. suis* population is highly heterogeneous, as different serotypes, phenotypes, and genotypes have been found. Currently 29 serotypes of *S. suis* are recognized. Multilocus sequence typing (MLST) is a method commonly used for the genotyping of pathogens, and numerous sequence types (STs) exist within the *S. suis* species [1]. The objective of this study was to characterize the diversity of *S suis* isolates originating from sick pigs from farms in the Czech Republic.

Materials and Methods

In total, 528 isolates were obtained from 144 farms located in the Czech Republic during the years 2018–2022. *S. suis* isolates were acquired from organs and body cavity swabs of dead animals or from nasal swabs of diseased animals with suspected *S. suis* infection. Identification of *S. suis* was performed using MALDI-TOF method and verified by PCR. Multiplex PCR, was used for determination of all serotypes. PCR-RFLP method was used for differentiation of serotype 2 and ½ or 1 and 14. Allelic determination of seven housekeeping gene loci was performed using MLST, ST assignment was performed using the PubMLST database.

Results

We detected 23 different serotypes and 100 isolates were not assigned to known serotypes according to PCR method. Predominant serotype 7 (15.2%) was followed by serotypes 2, 1/2, 9, 8, 4, 3, 1, 29, 31, 16, 12, and 15. Other serotypes were identified either rarely (up to 10 cases) or not at all, 18.5% of isolates were non-typeable. Multilocus sequence typing revealed 56 sequence types, of which 29 was identified as novel. The predominant sequence types were ST29 and ST28. No direct relationship between serotypes and sequence types was detected.

Discussion

The data showed high diversity among *S. suis* isolates collected in the Czech Republic from diseased pig. We identified 23 different serotypes, 56 sequence types, and 100 combinations of serotypes and sequence types. Moreover, a significant proportion of isolates were non-typable with respect to serotypes or sequence types or both. Isolates of serotype 7 represented more than 10% of all isolates. Isolates of other serotypes (S1/2, S1, S2, S3, S4, S8, and S9) known to be present in pathogenic strains [2] were less frequent. In our study, S7 was associated significantly (after Bonferroni correction) with presence in the central nervous system, thus is considered to be highly pathogenic. Similar to serotypes, only sequence types 29 and 28 were found in more than 10% of isolates. These two sequence types, belonging to the same clonal complex [3], represented nearly one-quarter of all isolates. Moreover, ST29 was significantly associated with the central nervous system.

Acknowledgements

The study was supported by the Ministry of Agriculture of the Czech Republic (Projects No. MZE-RO0518 and No. QK1810193).

References

1. Estrada et al. (2019) J Clin Microbiol 57, e00377-19.
2. Prüfer et al. (2019) PLoS ONE 14, e0210801.3.
3. Scherrer et al. (2020) Vet Res 51, 85.

Emergence of serotype 9 *Streptococcus suis* in Italy: genomic insights into strains with a reduced susceptibility to beta-lactams

F.R. Massacci[1], L. Cucco[1], M. Paniccià[1], A. Luppi[2], E. Albini[1], A. Peruzzo[3], L. Ferroni[1], M. Ustulin[2], M. Orsini[3] & C.F. Magistrali[1]

[1] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati', Perugia, Italy; [2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Parma, Italy; [3] Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy

Introduction

Streptococcus suis is one of the most important swine pathogens and an emerging zoonotic agent. In a previous study, we described an increase of *S. suis* serotype 9 (SS9) infections in pig herds in Italy: most isolates belonged to sequence type (ST) 123 and were characterized by a reduced sensitivity to penicillin [1]. Beta-lactams represent the first-line antibiotic therapy against SS9 infections. The targets of this antibiotic class are the penicillin-binding proteins (PBPs), which are involved in the biosynthesis of the bacterial cell wall. The hypothesis behind this study is that reduced susceptibility to beta-lactams emerged in some SS9 lineages and contributed to their successful spread.

Materials and Methods

We investigated 66 isolates of SS9 isolated from clinical cases of streptococcosis collected during 2002–2021 in Italian pig farms. The antibiotic susceptibility was assessed using minimal inhibitory concentration (MIC). To assess the ST, the presence of genes coding for antibiotic resistance and virulence and substitutions in the PBP (PBP1A, PBP1B, PBP2A, PBP2B e PBP2X) the 66 isolates were whole genome sequenced (WGS) and analyzed using bioinformatics tools [1]. The genomes of the Italian isolates were compared with the SS9 genomes available in public repositories. Multiple linear regression models were employed to investigate factors associated with an increase in MIC values to penicillin, ceftiofur and ampicillin respectively, assuming the ST, the year of isolation and the substitutions of PBPs as independent variables. To analyze the effect of the substitutions on the susceptibility to beta-lactams, we grouped the substitutions characterized by a high (>0.8) tetrachoric index. The significance level was set at $\alpha = 0.05$. Statistical analyses were performed using R software.

Results

The most frequent STs were ST123 and ST16, which differed in terms of virulence factors and susceptibility to beta-lactams. All isolates were susceptible to ampicillin and ceftiofur, and 36.4% of the isolates were resistant to penicillin. A high number of isolates were resistant to clindamycin (85.2%) and all isolates were resistant to tetracycline. The MIC values (log) for penicillin increased from 2002 to 2021. This trend was confirmed by multiple regression analysis (adjusted R^2=0.661), which showed a positive association between MIC values (log) for penicillin and year of isolation (p=0.0018; slope=0.018 95%CI=0.007-0.029). Namely, the model showed an annual increase in the MIC value for penicillin of approximately 4%. Moreover, higher MIC levels (log) were associated with the ST123, ST1540, and ST1953 as compared to ST16 (ST123: p<0.001, slope=0.718 95%CI=0.534-0.903; ST1540: p=0.037, slope=0.281 95%CI=0.017-0.544; ST1953: p<0.001, slope=0.824 95%CI=0.436-1.211). ST123 was associated with MIC values for penicillin 5.2-fold higher than those observed in ST16, 1.9 times for ST1540, and 6.7 times for ST1953. All the 66 isolates showed substitutions at the PBP sequences, except for one isolate that originated from a wild boar.

The substitutions were frequently associated in the same isolates and clustered into 17 different groups, revealing a mosaic architecture of the PBPs. Group 4, including PBP2xT551S, group 5, including PBP2xA627S/T, and substitution PBP1aT606P were associated with a higher MIC value for penicillin. Higher MIC values for ceftiofur were associated with group 9, including PBP2bD587E, PBP2bK479T, PBP2bD512E, PBP2bK513E, and PBP2bT515S.

Finally, high MIC values for ampicillin were associated with group 10, including PBP2xN595S, PBP2xN569K and PBP2xT467S, and PBP1aT606T. Based on the phylogenetic tree, SS9 Italian isolates belonging to the ST123, ST1953, and ST94 clustered together in the same phylogenetic group with isolates from Spain and were distinct from isolates of other origins.

Discussion

The susceptibility to penicillin of SS9 has been progressively decreasing in the last twenty years on Italian farms, as a possible consequence of the selective pressure generated by the use of antibiotics. Moreover, higher MIC values for penicillin were associated with ST123, ST1540, and ST1953. We observed that mutations to PBPs sequences were not independent of one another, but associated in large blocks. This observation is in line with the well-known mosaic structure of PBPs [2]. Some substitutions described here as being associated with a reduced susceptibility to beta-lactams were already observed [3, 4]. The presence of SS9 characterized by a reduced susceptibility to beta-lactams is a threat to public health. We suggest that diagnostic laboratories adopt quantitative methods to assess antibiotic susceptibility in this species, as qualitative screening might not be sufficient to identify isolates with reduced susceptibility to beta-lactams. In addition, since a reduced susceptibility may be observed only in some molecules of this class, we suggest that penicillin, an aminopenicillin and ceftiofur should be all included in the MIC panel.

Acknowledgements

This study was funded by the Italian Ministry of Health (RC011/2021 IZSUM).

References

1) Cucco L. *et al.* (2022) Emerg Infect Dis 28(1):139-147;
2) Zapun A, *et al.* (2008) FEMS Microbiol Rev. 32(2): 361-85;
3) Bamphensin N, *et al.* (2021) Pathogens 10(9):1178;
4) Hadjirin NF, *et al.* (2021) BMC Biol. 19(1):191.

5th International Workshop on *Streptococcus suis*

Centre de Recherche en Infectiologie Porcine et Avicole
Swine and Poultry Infectious Diseases Research Center
CRIPA
Université de Montréal

ABSTRACT BOOK

5th International Workshop on Streptococcus suis (5th IWSs): Advanced Research in the Era of Antimicrobial Restriction

JUNE 6-7, 2023

Sapphire Room 201, IMPACT Forum, Muang Thong Thani, Thailand

This workshop has been possible thanks to the support of"Innovative Veterinary Solutions for Antimicrobial Resistance" program (InnoVet-AMR) of the International Development Research Centre

GAMRIF
Global AMR Innovation Fund

IDRC · CRDI
International Development Research Centre
Centre de recherches pour le développement international

UKaid
from the British people