6th 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

6th International Workshop on Streptococcus suis (6th IWSs): Emergence of pathogenicity and antimicrobial resistance with intensive farming - challenges and solutions

SEPTEMBER 5-6, 2025

Pembroke College, Cambridge University, UK

UNIVERSITY OF CAMBRIDGE

Pembroke College

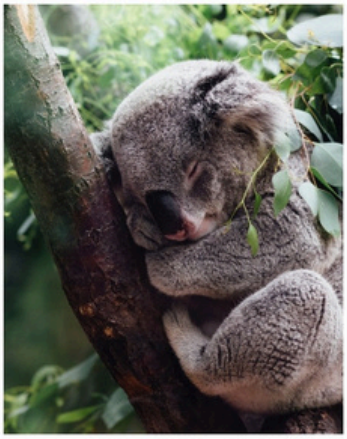
CONTENTS

This workshop has been possible thanks to the support of

GOLD SPONSORS

SILVER SPONSORS

TOGETHER, BEYOND ANIMAL HEALTH



ceva

At MSD, known as Merck & Co., Inc., Rahway, N.J., USA in the United States and Canada, we are unified around our purpose: We use the power of leading-edge science to save and improve lives around the world. For more than a century, we've been at the forefront of research, bringing forward medicines, vaccines and innovative health solutions for the world's most challenging diseases. MSD Animal Health, a division of Merck & Co., Inc., Rahway, N.J., USA, is the global animal health business of MSD. Through its commitment to The Science of Healthier Animals®, MSD Animal Health offers veterinarians, farmers, producers, pet owners and governments one of the widest ranges of veterinary pharmaceuticals, vaccines and health management solutions and services as well as an extensive suite of connected technology that includes identification, traceability and monitoring products. MSD Animal Health is dedicated to preserving and improving the health, well-being and performance of animals and the people who care for them. It invests extensively in dynamic and comprehensive R&D resources and a modern, global supply chain. MSD Animal Health is present in more than 50 countries, while its products are available in some 150 markets. For more information, visit www.msd-animal-health.com

MSD
Animal Health
The Science of Healthier Animals™

Caring for animals together

At Virbac, we are constantly exploring new ways to prevent, diagnose and treat the majority of animal pathologies. We develop care, hygiene and nutrition products to offer complete solutions to veterinarians, farmers and pet owners around the world. Our purpose: advancing the health of animals with those who care for them every day, so we can all live better together.

Shaping the future of animal health

Virbac

Chairs of the
6th International Workshop on *Streptococcus suis*

A.W. (Dan) Tucker, MA VetMB PhD DECPHM
Professor of Veterinary Public Health
Department of Veterinary Medicine
University of Cambridge (Pembroke College)

Mariela Segura, MSc PhD
Professor, Canada Research Chair in Immunoglycobiology of infectious diseases
Director of the Swine and Poultry Infectious Diseases Research Center (CRIPA)
Faculty of Veterinary Medicine, University of Montreal

Marcelo Gottschalk, DMV PhD Dr. hc
Professor
Director of the research and diagnostic laboratory of *Streptococcus suis*
Faculty of Veterinary Medicine, University of Montreal

Lucy Weinert
Associate Professor, University of Cambridge
Doctor, Pembroke College of the University of Cambridge

KEYNOTE SPEAKERS

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Navigating host barriers: Sensory systems and phase-variable epigenetics in *Streptococcus suis*

JERRY WELLS
Wageningen University
Netherlands

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Adaptive survival and infection of *Streptococcus suis*

LU LI
Huazhong Agricultural University
China

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Deciphering the unknown: Analysis of *Streptococcus suis* cell wall glycans and glycolipids

NICOLAS GISCH
Research Center Borstel
Leibniz Lung Center
Germany

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

In the shadow of *Streptococcus suis:* A genomic look at emerging streptococcal lineages

NAHUEL FITTIPALDI
University of Montreal
Canada

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Zoonotic potential of *Streptococcus suis*

CONSTANCE SCHULTSZ
University of Amsterdam
Netherlands

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Disease emergence and livestock intensification

JAMES WOOD
University of Cambridge
United Kingdom

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Phages infecting *Streptococcus suis*: are they there and should we care?

JOHN KENNY
Teagasc Food Research Centre
Ireland

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Insights into *Streptococcus suis* prevention and control: progress, pitfalls and perspectives

MARCELO GOTTSCHALK
University of Montreal
Canada

5-6 September 2025

Pembroke College

6th International Workshop on *Streptococcus suis*

UNIVERSITY OF CAMBRIDGE

Pembroke College

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

Dissemination of antimicrobial resistance genes by chromosomal mobile genetic elements in *Streptococcus suis*

SOPHIE PAYOT
INRAE
France

5-6 September 2025

Pembroke College

CONFERENCE ABSTRACTS

CONFERENCE ABSTRATCS

DAY 1
SEPTEMBER 5TH, 2025

THEME 1

STREPTOCOCCUS SUIS HUMAN AND SWINE INFECTIONS: VIRULENCE FACTORS AND HOST-PATHOGEN INTERACTIONS

Keynote lecture

Navigating host barriers: Sensory systems and phase- variable epigenetics in *Streptococcus suis*

Prof. Jerry Wells (Wageningen University Research, THE NETHERLANDS)

Jerry M. Wells is a professor and the Chair of Host-Microbe Interactomics at Wageningen University & Research. He is a prominent researcher in the field of host-microbial interactions, mucosal immunology, bacterial infection and immunity, and intestinal health. He graduated from the University of Cambridge, where he earned his PhD. In addition, he holds a Master of Business Administration (MBA) with distinction from the University of Nottingham, UK. He has over 20 years of research experience in intestinal permeability, the microbiota-mucosal interface, and the regulation of intestinal barrier function. He is an inventor named on 17 patent applications and has authored more than 140 scientific articles, including publications in high-impact journals such as Nature, Nature Biotechnology, Nature Reviews Microbiology, and PNAS.

Prof. Wells' research is internationally recognized and widely cited, with a particular emphasis on the gut microbiota, probiotics, mucosal delivery systems, and the mechanisms of host-microbe mutualism. His academic influence includes serving as a tutor and lecturer at both the University of Cambridge and Wageningen University, mentoring doctoral students, and organizing international conferences.

Keynote lecture

Adaptive survival and infection of *Streptococcus suis*

Prof. Lu Li (Huazhong Agricultural University, CHINA)

The research themes of Professor Lu Li, at the College of Veterinary Medicine, Huazhong Agricultural University, China, include the pathogenesis of animal bacteria, host anti-infectious immunity and control technologies for animal infectious diseases. She mainly focuses on the interactions between pig respiratory pathogens and their hosts. For anti-infectious immunity, she studies the roles of natural immune molecules in pigs in resisting pathogens and immune regulation. Based on these studies, she is interested in the identification of drug targets, screening of anti-infection compounds, and vaccine developments. Finally, she carries out studies on breeding of disease resistant pigs using anti-infection genes.

Environmental conditions steer lung immune responses and susceptibility to *Streptococcus suis* infection

Sandra Vreman[1]*; Susanna Commandeur[1]; Manouk Vrieling[1]; Dirkjan Schokker[1]; Norbert Stockhofe-Zurwieden[1]

[1] Wageningen University Research, the Netherlands.

Background. Environmental conditions and microbiological colonisation can be critical for disease susceptibility to *Streptococcus suis* (*S. suis*) infections. Caesarean-Derived Colostrum-Deprived (CDCD) pigs are free of *S. suis* and other swine specific microbes. This study compared conventional (Conv) farm raised pigs with CDCD pigs before and after *S. suis* infection with e.g. focus on lung immune status. **Materials and Methods**. Eight week old, CDCD derived piglets and Conv pigs were either infected intranasally after sedation with *S. suis* serotype 2 (10^9 CFU of strain ST1; 1.5 mL in each nostril) or were kept as non-infected control (n=6/group). After challenge pigs were followed for 7 days or until they reached a humane endpoint (HEP). Lung tissue transcriptome from CDCD and Conv pigs and related histopathology before and after infection were compared. **Results**. Lung transcriptome analysis prior infection revealed 684 differentially expressed genes (DEGs) between CDCD and Conv pigs mainly related to immune pathways. Lung histopathology of these CDCD pigs lungs showed reduced cellularity compared to Conv piglets, particularly in alveolar macrophages and perivascular/bronchiolar infiltrates. After *S. suis* infection, all Conv pigs survived throughout the study, while all infected CDCD pigs reached a HEP three days post-infection. Infected Conv pigs showed no histopathologic lung changes compared to controls and no DEGs between both groups. Conversely, lungs of *S. suis* infected CDCD pigs developed a pleuritis and interstitial pneumonia and transcriptome analysis showed 435 DEGs compared to uninfected CDCD pigs. **Conclusion**. The exclusion of passive maternal immunity and porcine microbiome in CDCD pigs influences the immunological (lung) responsiveness increasing susceptibility to *S. suis* disease and pneumonia compared to Conv pigs. The transcriptome analysis indicated relevant immune genes related to disease protection in pigs.

Streptococcus suis OseR, a redox sensor, regulates ergothioneine uptake via a Cys thiol switch, enhancing oxidative stress resistance and virulence

Zongfu Wu[1]*; Xinchi Zhu[1]

[1] Nanjing Agricultural University, China.

Ergothioneine (ET), a low-molecular-weight (LMW) thiol, serves as a potent antioxidant. While only a limited number of Actinomycetes and fungi can synthesize ET, most microorganisms acquire it from external sources. Recently, a microbial ET transporter system (EtUV) was identified in *Helicobacter pylori* and *Streptococcus pneumoniae*, but the regulatory mechanisms controlling EtUV in bacteria remain unknown. In this study, we identified and characterized OseR, a novel MarR family repressor in *Streptococcus suis*, a significant pathogen causing systemic diseases such as septicemia and meningitis in pigs and humans. We demonstrated that OseR senses oxidative stress through a thiol switch at Cys35, which regulates the ET transport system EtUV. Under oxidative stress, OseR dissociates from the promoter region of the ET transport operon due to the formation of an intermolecular disulfide bond, leading to the activation of EtUV expression. Our findings reveal that OseR not only controls ET transport but also modulates other LMW thiol transport pathways, including glutathione and cysteine, as well as genes involved in oxidative stress responses. Deletion or mutation of OseR significantly impairs oxidative stress tolerance, survival in mouse macrophages, and virulence in mice. Similarly, deletion or mutation of EtU, which encodes a transmembrane permease essential for ET uptake, markedly reduces oxidative stress tolerance and virulence in mice. Importantly, our results suggest that OseR-mediated regulation of the ET transport system, driven by a thiol-based switch, may be conserved across bacterial species, highlighting a broader role for OseR in bacterial adaptation to host environments. This study advances our understanding of the regulatory mechanisms governing ET uptake in bacteria and provides new insights into the link between ET and bacterial pathogenicity.

Keynote lecture

Deciphering the unknown: Analysis of *Streptococcus suis* cell wall glycans and glycolipids

Dr. Nicolas Gisch (Research Center Borstel, Leibniz Lung Center, GERMANY)

Dr. Nicolas Gisch is Principal Investigator at the Research Center Borstel – Leibniz Lung Center in Germany, and since 2013 deputy head of the Bioanalytical Chemistry group and head of NMR spectroscopy analytics. His research focuses on purification and structural characterization of bacterial cell wall components—mainly lipoglycans and complex carbohydrates—linked to infectious and inflammatory processes. He develops and optimizes methods for isolating and analyzing such molecules from Gram-positive bacteria (mainly streptococci), Gram-negative bacteria, and mycobacteria, using mass spectrometry and NMR. Dr. Gisch collaborates nationally and internationally, contributes to multidisciplinary projects and mentors doctoral candidates at the University of Lübeck, where he recently completed his habilitation.

Oral inoculation of pigs with *Streptococcus suis*

Matheus Costa[1]*; Mariana Meneguzzi[1]

[1] University of Saskatchewan, Canada.

Streptococcus suis has long been regarded as an upper respiratory tract (URT) early colonizer of pigs. There is a general consensus that infection arises from the URT as well, following immunosupressing events. However, humans are susceptible to infection through the gastrointestinal tract. Here we hypothesized that pigs can be infected and develop disease following oral inoculation with *S. suis*. Six pigs at 4-weeks of age were sourced from a high-health herd, free of PRRSv or IAV-S. Animals were transported for 4 hours to a bio containment level 2 facility, to simulate weaning stress. Upon arrival, four animals were inoculated, via oral gavage, with 10 mL of a pure culture of *S. suis* (10ˆ7 CFU/mL, serotype 1). Two pigs were sham inoculated, serving as controls. Animals were clinically monitored for 7 days. At the end of the study, euthanasia and a complete necropsy was performed and samples collected for bacterial culture. Control pigs remained healthy throughout the study. None of the inoculated pigs developed pyrexia. One inoculated pig (630) was reluctant to move 48 hours post-inoculation, but would move upon stimulation. A second pig (635) was lame on right hind leg 5 days post-inoculation. Upon necropsy one pig (633) had mild meningeal congestion and reactive intestinal lymph nodes. *S. suis* was isolated from a meningeal swab (1+) and ileal lymph nodes (3+) from this pig (633). Necropsy of pig 635 identified ascites, splenomegaly and lymphadenopathy. Bacterial culture identified *S. suis* from submandibular lymph node (1+), thoracic fluid (1+), and spleen (1+). All other necropsies were unremarkable. This work presents initial evidence that pigs are susceptible to infection by *Streptococcus suis* following oral inoculation. Further studies to clarify this new route of infection should be conducted, particularly to investigate management risk factors and potential association with serotypes.

MSD Animal Health

GOLD SPONSOR PRESENTATION

The IgM protease of *S. suis*, a cross-protective vaccine antigen: prevalence, subtyping and pig vaccination-challenge studies

A.A.C. Jacobs*[1]; A.W.G. Grommen[1]; S. Badbanchi[2]; T.J. van Kasteren-Westerneng[1]; L. Garcia-Morales[1]; R.P.A.M. Segers[1]

[1] MSD-AH, The Netherlands; [2] MSD-AH, Germany.

The IgM protease of *S. suis* has been shown to be a cross-protective vaccine antigen that induces serotype independent protection (Jacobs et al 2024 PHM). The IgM protease can be classified into three groups A, B and C. Groups A and B have been shown associated with clinical isolates whereas group C is more often associated with carrier isolates. Recombinant group A IgM protease rIde-14009-1, formulated in a vaccine, induced protection in pigs against all group A strains tested i.e. strains of different serotypes 1, 2, 7, 9 and 14, but not against group B strains. In the Netherlands most group B strains are associated with st9 ST16. Investigations of sequences available in the public domain (n=2000, status 2021) indicated that 90% of the clinical isolates are of group A, 5% group B and 5% group C. There appear to be differences within regions and countries e.g. group B is more prevalent in the EU and in particular in the Netherlands. As vaccine protection is only within group A strains it is relevant to know the current group A, B and C distribution in the different regions. Therefore, recent clinical isolates from The Netherlands (two studies), Germany, Denmark, Italy, Spain (two studies), Brazil, USA, Canada (two studies) and China were classified by using the genome sequence or by specific PCR. The results will be presented and discussed.

THEME 2

GENOMICS, EVOLUTION OF PATHOGENESIS AND MOLECULAR EPIDEMIOLOGY (Part I)

Keynote lecture

In the shadow of *Streptococcus suis*: A genomic look at emerging streptococcal lineages

Prof. Nahuel Fittipaldi (University of Montreal, CANADA)

Nahuel Fittipaldi is an Associate Professor at the Université de Montréal (Faculty of Veterinary Medicine, Saint-Hyacinthe, Canada), where he leads a research program focused on the genomic epidemiology, taxonomy, and host-pathogen interactions of *Streptococcus suis* and other streptococci of veterinary and zoonotic relevance. Trained as a microbiologist in Argentina and Canada, he combines comparative genomics, molecular microbiology, and surveillance approaches to address questions at the interface of animal and public health.

Genome-scale metabolic model atlas of *Streptococcus suis*

Karl Kochanowski[1]*; Pau Obregon-Gutierrez[1]; Ines LeFranc[2]; Ayelen Perez-Falcon[1]; Alexander W. Tucker[2]; Virginia Aragon[1]; Lucy Weinert[2]

[1] IRTA-CReSA, Spain; [2] Cambridge University, UK

Streptococcus suis is a gram-positive bacterium with an intriguing dual role: it is an important member of the healthy porcine airway microbiota, but it can also cause severe systemic infections in pigs as well as humans. Mounting evidence suggests that metabolism plays a key role in *S. suis* pathogenicity. However, currently the relationship between genomic variability, metabolism, and pathogenicity in *S. suis* is poorly understood. To tackle this issue, we developed the first large-scale atlas of strain-specific genome-scale metabolic models in *S. suis*, which includes over 3000 strains from multiple pathogenic and non-pathogenic lineages. In my talk, I will discuss how we can use these models to examine the metabolic capabilities of *S. suis* more broadly. For example, we find that while amino acid auxotrophies are largely conserved in *S. suis*, there are some lineages with very different auxotrophy patterns, suggesting that they occupy distinct metabolic niches *in vivo*. Moreover, I will demonstrate how we can leverage these models to identify new metabolic vulnerabilities that are specific to pathogenic lineages and relevant *in vivo*, thus paving the way for new treatment strategies which directly target *S. suis* metabolism.

Genomic and Antimicrobial Resistance Traits of Invasive *Streptococcus suis* in Spain

Cristina Uruén[1]; Clara Marín[2]; Marcelo Gottschalk[3]; Vigirnie Libante[4]; Sophie Payot[4]; Miguel Arenas[5]; **Jesús Arenas[1]***

[1] University of Zaragoza, Spain; [2] CITA, Spain; [3] University of Montreal, Canada; [4] INRAE, France; [5] University of Vigo, Spain.

Spain is the leading pig-production country in Europe and ranks third globally, where *S. suis* is a major swine pathogen. We aimed to characterize invasive *S. suis* isolates representing the major pig-producing autonomous communities in Spain, focusing on genomic features, antimicrobial resistance (AMR), and gene dissemination. 156 invasive isolates were tested, revealing 47 STs, most belonging to ST1 (26%), ST123 (18%), ST29 (9%), and ST3 (7%). Whole-genome sequencing of 19 representative isolates revealed a pangenome distributed into 7 Bayesian clusters. Around 70% of the isolates were tested for susceptibility to 18 antibiotics of 9 families. High resistance rates (>80%) were detected against tetracyclines, and lincosamides. Intermediate resistance (20-40%) was recorded for sulfonamides, trimethoprim, tiamulin, enrofloxacin, and penicillin, and low for florfenicol and some β-lactams. 23 AMR genes were detected, including 4 not previously reported in *S. suis*. Some chromosomal genes were detected and bioinformatic analysis using RDP4 showed traits of recombination events. Other genes were located on mobile genetic elements, mainly ICEs and IMEs. tet(O) and *erm(B)* genes, conferring tetracycline and macrolide resistance, were often located in these elements. Antimicrobial resistance to tetracyclines and macrolides of more than 2,000 clinical isolates of human- pathogenic streptococci collected from Hospitals in Aragón (Spanish´s highest pig production region), revealed moderate resistance to the cited antibiotics (~30%). Genetic analysis of some resistant strains carried *tet(O)* and *erm(B)* genes in ICEs/IMEs with > 98% identify to some of *S. suis*. Except for *S. mitis*, *S. suis* transferred ICEs to all *Streptococcus* species tested under laboratory conditions, however the transference rates varied, in part caused by interspecies growth inhibition. Our results reveal a highly diverse *S. suis* population in Spain with significant potential to disseminate AMR genes.

Keynote lecture

Zoonotic potential of *Streptococcus suis*

Prof. Constance Schultsz (University of Amsterdam, THE NETHERLANDS)

Professor Constance Schultsz is an MD, Medical Microbiologist and Professor of Global Health, in particular for emerging infectious diseases and antibiotic resistance, at the Amsterdam UMC of the University of Amsterdam (UvA) Her research interests include zoonotic and emerging infectious diseases, in particular Streptococcus suis, and antibiotic resistance. She is interested in molecular epidemiology and pathogenesis, next-generation sequencing applications, smart sampling strategies for antimicrobial resistance surveillance, as well as behavioural and socio-economic drivers of antimicrobial resistance. Prof. Schultsz has previously worked as a Research Fellow at the International Centre for Diarrheal Diseases Research, Bangladesh and worked as a consultant microbiologist at the VU University Medical Centre. From 2003 until 2008 she headed the Microbiology department at the Oxford University Clinical Research Unit, Vietnam, at the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. In 2008 she joined the Amsterdam UMC. She was appointed Deputy Head of the Department of Global Health in 2016 and at the same time became an executive board member of the The Amsterdam Institute for Global Health and Development.

Population biology of *Streptococcus suis* and emergence of pathogenic lineages

Peter van Baarlen[1]*; Maria Juanpere Borras[1]; Jerry Wells[1]

[1] Wageningen University, the Netherlands.

The cosmopolitan bacterial species *Streptococcus suis* is among the most abundant bacteria associated with wild and domesticated pigs, characterised by high population diversity and presence of different genetic lineages in porcine tonsillar microbiota samples. In farmed pigs, most *S. suis* lineages are common inhabitants of porcine upper airways and are carried asymptomatically, but some *S. suis* lineages cause serious diseases in pigs including meningitis. Although *S. suis* does not appear to colonise humans, zoonotic human infections by porcine disease-associated *S. suis* lead to meningitis and other serious conditions. The genetic basis correlating with pathogenic or commensal carriage lifestyles has been subject of intensive research. Accessory genomes of disease-associated *S. suis* include genes that are not part of *S. suis* core genome nor of non-disease-associated (carriage) *S. suis*. We found that *S. suis* accessory disease-associated genes appear to occur especially in host-associated bacteria and may be horizontally transferred in microbiomes. Using coalescent theory and bayesian inference, we investigated, across branches of phylogenetic trees, the origin and ages of exemplary genes that appear to have been horizontally acquired by disease-associated *S. suis* lineages. We hypothesise how transfer and acquisition of disease-associated genes in natural host-associated microbiomes may contribute to emergence of pathogenic lineages.

Emergence of serotype 9 *Streptococcus suis* in Italy and Spain: genomic insights into sequence type 123 with a reduced susceptibility to beta-lactams

Francesca Romana, Massacci[1]*; Lucilla Cucco[1]; Elisa Albini[1]; Elisa Russo[1]; Francesca Piersanti[1]; Giulia Dilio[2]; Marta Paniccià[1]; Giovanni Pezzotti[1]; Jose Francis Fernandez-Garayzabal[3]; Ana Isabel, Vela[3]; Chiara Francesca Magistrali[1,2]

[1] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati', Perugia, Italy; [2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy; [3] Department of Animal Health, Veterinary Faculty, Complutense University, Madrid, Spain.

A reduced susceptibility to beta-lactams was recently reported in *Streptococcus suis* serotype 9 (SS9), a dominant serotype in Italy and Spain (Cucco et al., 2022, Massacci et al., 2024, Uruén et al., 2024, Vilaró et al., 2025). This study aims at investigating the population structure and beta-lactams susceptibility in SS9 isolates from Italy and Spain from 2002 to 2024. One hundred sixty-six SS9 isolates from clinical cases of streptococcosis in pigs were analyzed. Antibiotic susceptibility was assessed using minimal inhibitory concentration (MIC). To assess the sequence type (ST), the presence of genes coding for antibiotic resistance and substitutions in the Penicillin Binding Protein (PBP) at the PBP1A, PBP2B and PBP2X, all the isolates were whole genome sequenced and analyzed using bioinformatics tools (Cucco et al., 2022). ST16 (N=19) included susceptible isolates to beta-lactams except one isolate from Spain. Most isolates (61.5%; 102/166) belonged to ST123, without clear geographical clustering. More than half of these isolates (58.8%; N=60) exhibited a reduced susceptibility to penicillin and MIC values exceeding 1 mcg/ml recorded exclusively after 2017. A reduced susceptibility to penicillin was also observed in ST1953 (N=2), ST94 (N=1), ST16 (N=1), ST1650 (N=1) and a new-ST (N=1). ST1540 (N=7) was identified only in Italy showing a complex resistance profile, including a reduced susceptibility to beta-lactams. In ST123 isolates, the same substitutions described in Massacci et al. (2024) at PBP2B (K479T, D512E, K513E, T515S, T625R, D587E) and PBP2X (A627S/T, T551S, I568T, D541E, V547M, M437L, S445T, Y525F, N595S, N569K, T467S, Q405E) were found. Conversely, the substitutions at PBP1A described by Lunha et al. (2023) (P409T, S477D/G, M587S/T) were not detected. In conclusion, the reduced susceptibility to beta-lactams of ST123 isolates may have contributed to its spread among the pig population in both countries.

Special lecture:

Disease emergence and livestock intensification

Prof. James Wood (University of Cambridge, UK)

James Wood is Alborada Professor of Equine and Farm Animal Science. He is an infectious disease epidemiologist in the Disease Dynamics Unit at the University of Cambridge; he is also co-chair of Cambridge Infectious Diseases Interdisciplinary Research Centre and his research focuses on One Health approaches to the investigation of disease emergence, especially from wildlife and its control. He is involved in collaborative multidisciplinary studies of the ecology and emergence of RNA viruses from fruit bats in Ghana and control of bovine tuberculosis in Ethiopia, India and UK. He has published widely on infectious disease emergence and its drivers, especially considering zoonoses in sub-Saharan Africa and on bovine TB transmission and its control in cattle. He chairs the Cambridge Africa Strategic Advisory Group.

DAY 2
SEPTEMBER 6TH, 2025

THEME 3

MOLECULAR EPIDEMIOLOGY (Part II), FARM MANAGEMENT AND DISEASE CONTROL IN THE ERA OF ANTIMICOBIAL REDUCTION

Keynote lecture

Phages infecting *Streptococcus suis*: are they there and should we care?

Dr. John Kenny (Teagasc Food Research Centre, IRELAND)

John Kenny is a Senior Research Officer at Teagasc the Irish Agri-Food Development Authority, and a Funded Investigator at the APC Microbiome Ireland and VistaMilk Research Ireland Centres. Prior to that he managed the Centre for Genomic Research at the University of Liverpool, where he led the team applying a variety of sequencing technologies to a diverse range of biological topics, including human, crop, outbreak and microbiome projects. Research in John's lab primarily focusses on the application of 'omics techniques to better understand and apply food fermentation and bacteriophages. The various projects combine genomics, metagenomics, metatranscriptomics, and metabolomics.

Incorporation of whole genome sequencing of *Streptococcus suis* into national pig disease surveillance at the Animal and Plant Health Agency, in collaboration with the University of Cambridge

Claire Scott[1]*; Rowan Morris[1]; Richard Ellis[1]; Muna Anjum[1]; Miranda Kirchner[1]; Susanna Williamson[1]; Dan Tucker[2]; Lucy Weinert[2]; Geng Zou[2]

[1] APHA (Animal and Plant Health Agency), Rougham Hill, Bury St Edmunds, Suffolk, IP33 2RX, England; [2] University of Cambridge, Department of Veterinary Medicine, Madingley Road, Cambridge, CB3 0ES.

Background: Global shortages of *Streptococcus suis* antisera necessitates the identification and implementation of an alternative method to serotype *S. suis* at the Animal and Plant Health Agency (APHA). Whole genome sequencing (WGS) presents opportunities to provide improved surveillance intelligence as well as greater insights into the likely significance of isolates to disease processes than traditional serotyping methods. This includes establishing the multilocus sequence type (MLST) of isolates and presence of antimicrobial resistance-associated genes. **Methods**: In collaboration with the University of Cambridge, funded by a UKRI Impact grant, APHA are developing a WGS pipeline for *S. suis*. This has involved holding a stakeholder workshop to understand the potential of the tool for surveillance and explore the perceived value of the WGS among submitting veterinarians. WGS of archived isolates with known serotypes has been carried out as an initial validation step. **Results**: Initial validation work has been successful: WGS showed 100% correlation with traditional subtyping and 100% repeatability; fewer isolates were untypable in terms of serotype; and further genetic characteristics of isolates were ascertained (including virulence-associated gene presence and MLST). This indicates greater potential for WGS to detect and characterise new or re-emerging threats - a key goal for endemic disease surveillance, a nation-wide duty for APHA. Further, this greater granularity of information will enable veterinarians submitting diagnostic samples to APHA to come to more evidence-based interpretations of the clinical significance of *S. suis* isolates, which will facilitate informed herd health decisions. By collaborating with academic experts in the field of *S. suis* and incorporating stakeholder opinions into the design of the pipeline, APHA plans to implement genomic surveillance of *S. suis* in a way which is both evidence-based and meets the needs of national surveillance.

The antiviral defence profile of *Streptococcus suis*

Emmet B. T. Campbell[1]*; Timofey Skvortsov[1]; Christopher J. Creevey[1]

[1] Queen's University Belfast, UK.

S. suis is a major pig pathogen that significantly impacts the global swine industry while also posing a zoonotic risk to humans, particularly those in occupational settings. As bacteriophages present a promising alternative to antibiotics in the fight against antimicrobial resistance, understanding the antiviral defence mechanisms of *S. suis* is crucial for characterising phage-host interactions. In this study, we annotated 57,180 antiviral defence genes across 2,119 *S. suis* genomic assemblies from BV-BRC using PADLOC (v2.0.0), DefenseFinder (v2.0.0), and a BLAST-based comparative approach from *B. cereus*. We performed a pangenome analysis with Roary (v3.13.0) and identified coincident genes using COINFINDER (v1.2.1). Our findings reveal that *S. suis* harbours an extensive array of antiviral genes, with restriction modification (RM) dominating the defence landscape. The majority of defences enable non-abortive infection, with 32,779 genes, compared to 12,334 genes linked to altruistic outcomes. Furthermore, pathogenic serotypes exhibited a bimodal distribution in the number of unique defence systems detected, indicating that more virulent strains may rely on diverse sets of antiviral strategies to evade phage predation. These insights enhance our understanding of *S. suis* phage resistance, informing future efforts in antimicrobial alternatives and pathogen control. Understanding these defence profiles is critical for advancing phage therapy applications, guiding antimicrobial resistance management, and predicting bacterial adaptability in response to viral threats.

Can shared antibiotic use promote host jumps? Evidence from zoonotic *Streptococcus suis* in birds

Muriel Dresen[1,2]*; Gemma G. R. Murray[3]; Peter Valentin-Weigand[4]; Marcus Fulde[2]; Lucy A. Weinert[1]

[1] University of Cambridge, Cambridge, UK; [2] Freie Universität Berlin, Berlin, Germany; [3] University College London, London, UK;
[4] University of Veterinary Medicine Hannover, Hannover, Germany.

The interconnectedness of human, animal, and environmental health is a challenge for emerging health threats, such as antimicrobial resistant bacteria. For example, widespread use of similar antimicrobials in both human and livestock may play a role in interspecies bacterial transmission by disrupting natural microbial communities and creating a common survival environment that favours resistant bacteria. Pigs and poultry receive the highest levels of antimicrobials and consequently frequently harbour multidrug resistant bacteria. One ubiquitous pig pathogen, *Streptococcus suis* that also spills over to cause disease in humans, commonly exhibits frequent multidrug resistance. Here we show from a sample of over 3000 *S. suis* isolates from pigs, wild boar, humans, cats, dogs, cattle, and birds that a multidrug resistant lineage, distinct from the lineage responsible for most zoonoses, shows signatures of adaptation to birds. We find long-term phylogenetic persistence in poultry populations, with multiple host jump events and subsequent transmission to wild and pet birds. Moreover, we identified unique mobile genomic islands and greater survival in chicken versus pig blood. While chickens may not be a primary source of zoonotic *S. suis* infections, our results suggest that shared antibiotic usage in pigs and poultry may have promoted a host jump of these antimicrobial-resistant bacteria. Increasing intensification of livestock production may enhance transmission of antimicrobial bacteria to other animals or humans, emphasising the need of a One Health approach.

Assessment of economic and antibiotic usage impacts of endemic *Streptococcus suis*-related disease in the United Kingdom

Geng Zou[1]; Lola Pailler[2]; Lucy Weinert[1]; Michael Jarvis[3]; **AW (Dan) Tucker[1]***

[1] Univeristy of Cambridge, UK; [2] IRTA, Spain; [3] The Vaccine Group, UK.

Background: While case reports of human disease are reasonably well documented in most countries, data on the impact of *S. suis*-related disease in pigs is sparse. Robust quantitative evidence of prevalence, economic and antibiotic usage impacts of *S. suis* disease is crucial to better prioritise government funded veterinary surveillance and vaccine development programmes, while also enabling evidence-based decisions on management and prevention at farm level. Previous work (Niela-Ibanez et al 2021) found a significant economic burden from *S. suis* disease approaching 1 Euro / pig averaged across national production. However, impacts on antibiotic usage (AMU, mg/kg bodyweight) are unknown. Here we describe the quantitative estimation of economic and AMU impacts of *S. suis* in pigs in the UK. Methods: Structured interviews were conducted with specialist pig veterinarians with oversight of commercial farrowing, weaning, and grow-finish production sites in the UK. Data was collected using a survey format based on Niela-Ibanez *et al* 2021 and included number and type of units under care, % units affected, average % batches affected in those units, average % individual pigs affected in suspected batches, % mortality in suspected batches. Data on therapeutic and metaphylactic treatments, and vaccine-based prevention was combined with consensus valuations to generate costs/animal on affected farms, and costs per animal across all farms. Antibiotic usage (mg antibiotic / kg body weight) attributable to *S. suis* on affected farms was compared against over all average AMU values recorded in the national electronic medicines book (eMB) database. A stochastic model was used to account for uncertainty and variability in the data. Results: In total 9 veterinarians were interviewed, representing 8 of the largest UK-wide specialist pig veterinary practices in the UK, and capturing data from a total of 1499 production units. Full data and conclusions will be presented at the workshop.

Streptococcus suis serotype 2 is the most contagious respiratory pathogen in piglets

Flemming Thorup[1]*; Nicole Bakkegård Goecke[2]; Elisabeth Okholm Nielsen[1]

[1] SEGES Innovation, Agro Food Park 13, 8200 Aarhus, Denmark; [2] Department of Veterinary and Animal Science, Copenhagen University, Stigbøjlen 4, 1870 Frederiksberg, Denmark.

Background & Objectives. Porcine Respiratory Disease Complex is frequently observed in pigs in the nursery and grower-finishing units. The pathogens involved are often present in the piglets already at weaning. This investigation analyzed the presence of respiratory pathogens in the farrowing unit. **Material & Methods**. The investigation comprised all litters in three farrowing sections in each of three herds (Herds 1-3). Ten and 21 days after farrowing (n = 359 and 339 litters, respectively), five randomly selected piglets per litter were tested by nasal swabs. The five swab samples were pooled and tested for 14 pathogens using a high-throughput qPCR platform (Biomark HD). All three herds were free of PRRS-virus.

Results. The nine farrowing sections contained 27-48 lactating sows. All litters in all three herds were positive for *S. suis* type 2 on both day 10 and day 21 after farrowing. No other pathogens were present in all litters. Haemophilus parasuis was observed in 48-95% of the litters on day 10 and in 85-100% of the litters on day 21. Influenza A virus (IAV) was present in six of nine sections on day 10 and in eight sections on day 21. Only in four of the nine sections, 90% or more of the litters were infected with IAV on day 10 or day 21. *Bordetella bronchioseptica* was only present in one herd, and less than 50% of the litters were infected on day 10, while 90% were infected by day 21. *Mycoplasma hyorhinis* was present in two herds with 0 and 50% presence day 10 and 10 and 100% presence day 21, respectively. Porcine cytomegalovirus, Porcine circovirus type 3, *Pasteurella multocida* and *Actinobacillus pleuropneumonia* were found in less than half of the litters. **Discussion & Conclusion**. *S. suis* serotype 2 was the only pathogen present in all litters on both day 10 and day 21. It is unclear, if all litters were infected from their mother or if *S. suis* from infected litters was transmitted to the remaining litters by management procedures.

Keynote lecture

Insights into *Streptococcus suis* prevention and control: progress, pitfalls, and perspectives

Prof. Marcelo Gottschalk (University of Montreal, CANADA)

Prof. Gottschalk has been a full professor at the Faculty of Veterinary Medicine of the University of Montreal since 2001. He is the director of the International Reference Laboratory for Swine Pleuropneumonia and the North American Reference Laboratory for *Streptococcus suis*. According to Expertscape's PubMed-based algorithms, he ranks among the top 0.02% of published authors worldwide on human and veterinary streptococcal infections. In recognition of his career, Ghent University awarded him an Honorary Doctorate (Doctor Honoris Causa) in 2018. Prof. Gottschalk has published more than 450 articles in peer-reviewed journals and over 250 articles in professional journals. He has been invited as keynote speaker to give more than 350 presentations in 37 countries around the world.

Ceva Animal Health

GOLD SPONSOR PRESENTATION

High diversity of *Streptococcus suis* isolates from diseased pigs in Europe and its effective management by autogenous *S. suis* bacterins

Dr. Paloma Suarez (CEVA Animal Health)

Dr. Paloma Suárez is the Swine Franchise Manager for Europe at CEVA Animal Health, with over 25 years of experience in swine health, vaccines, and livestock innovation. She leads strategic development for swine products, promoting modern vaccination and health management solutions. Known for her collaborative approach, Dr. Suárez plays a key role in advancing animal health across Europe through product innovation, stakeholder engagement, and industry leadership.

Keynote lecture

Dissemination of antimicrobial resistance genes by chromosomal mobile genetic elements in *Streptococcus suis*

Dr. Sophie Payot (French National Institute for Agriculture, Food, and Environment, FRANCE)

Dr. Sophie Payot is a Senior Researcher at the French National Institute for Agriculture, Food, and Environment (INRAE) in Nancy, and Deputy Director of the Genome Dynamics and Microbial Adaptation unit in Nancy. With a PhD in molecular biology, she specializes in bacterial genome plasticity, focusing on horizontal gene transfer mechanisms such as integrative and conjugative elements in streptococci. Her work addresses the molecular basis of bacterial adaptation and antibiotic resistance dissemination, contributing to agriculture and public health. Dr. Payot leads multiple research projects and serves on scientific committees related to bacterial resistance epidemiology.

Invited conference:

Adaptation to farming practices: the evolution of pathogenicity and drug-resistance in *Streptococcus suis*

Dr. Gemma Murray (University of Cambridge, UK)

Dr. Gemma Murray is an evolutionary biologist formerly based at the University of Cambridge, where she held positions as a Research Associate in the Department of Veterinary Medicine and a Junior Research Fellow at Newnham College. Her research focuses on how bacterial pathogens evolve to cause disease, infect new hosts, and resist antibiotics. With a background in genetics, quantitative genomics, and theoretical biology, Dr. Murray applies population genetics and phylogenetic methods to understand microbial evolution. Her interdisciplinary work has advanced knowledge on pathogen adaptation, antibiotic resistance, and bacterial ecology.

Understanding the development of penicillin resistance in *Streptococcus suis* through Adaptive Laboratory Evolution

Chenxi Liu[1]*; Lucy Weinert[1]

[1] Department of Veterinary Medicine, University of Cambridge.

Streptococcus is a zoonotic bacterium commonly found in the upper respiratory tract of pigs. While typically a commensal bacterium, certain pathogenic strains, can cause serious infections in humans, such as pneumonia, meningitis, and sepsis. Beta-lactam antibiotics, especially penicillin, are widely used to treat *S. suis* infections. However, the increase of penicillin resistance in *S. suis* jeopardises the efficiency of this approach, therefore understanding of penicillin resistance evolution is crucial. To model how penicillin resistance may develop in *S. suis* populations, we subjected a diverse panel of *S. suis* strains to gradually increasing concentrations of penicillin over 30 passages. We then performed whole-genome sequencing to identify mutations arising during the evolution process and assessed whether similar genetic changes occurred across different lineages. All strains successfully achieved a fourfold increase in their minimum inhibitory concentration (MIC) for penicillin. Across the evolved lineages, we identified five non-synonymous single nucleotide polymorphisms (SNPs) in penicillin-binding proteins (PBPs) 1A and 1B, and 21 non-synonymous SNPs in PBP2B and PBP2X, suggesting a strong association between penicillin resistance and mutations in PBP2B and PBP2X. However, no consistent SNPs were found across all lineages or among lineages sharing the same genetic background. Notably, five out of the 18 evolved lineages did not exhibit any SNPs in PBP genes, indicating that resistance can emerge through multiple, distinct evolutionary routes. Additionally, evolved lineages with reduced susceptibility to penicillin exhibited slower growth compared to their ancestral strains, pointing to a fitness cost associated with resistance.

Invited conference:

Antibiotic use in pigs in the UK: Progress, solutions and barriers to the global AMR issue

Dr. Mandy Nevel (UK Agriculture and Horticulture Development Board)

Dr. Mandy Nevel is the Head of Animal Health and Welfare at the UK Agriculture and Horticulture Development Board (AHDB). With over 35 years of experience in livestock veterinary medicine, she leads efforts to improve animal health, welfare, and responsible antibiotic use across the UK's livestock sectors. Dr. Nevel's background includes academic research, vaccine development, veterinary pathology, and disease surveillance. She is recognized for her leadership in promoting sustainable agriculture and advancing animal welfare policy and practices.

Invited conference:

Insight into *Streptococcus suis* in Thai Pigs: genetic diversity and antimicrobial resistance surveillance

Dr. Suganya Yongkiettrakul (National Center for Genetic Engineering and Biotechnology, THAILAND)

Dr. Suganya Yongkiettrakul is a Principal Researcher at Thailand's National Center for Genetic Engineering and Biotechnology (BIOTEC), specializing in molecular microbiology and biochemistry of infectious diseases, particularly zoonotic pathogens like *Streptococcus suis*. Her work focuses on developing molecular diagnostics, studying antimicrobial resistance mechanisms, and applying high-throughput omics technologies for pathogen detection and food safety. Dr. Yongkiettrakul leads and collaborates on international research projects, contributing to advances in public health, food security, and biotechnology in Thailand and beyond.

Kamonwan Lunha[1]; Wiyada Chumpol[1]; Surasak Jiemsup[1]; Nattakan Meekhanon[2]; Pornchalit Assavacheep[3]; Piroon Jenjaroenpun[4]; Thidathip Wongsurawat[4]; **Suganya Yongkiettrakul[1]***

[1] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani, Thailand; [2] Department of Veterinary Nursing, Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand; [3] Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; [4] Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Streptococcus suis is a significant zoonotic pathogen in Thailand, responsible for severe infections in both swine and humans. Recent studies have advanced understanding of its genetic epidemiology and antimicrobial resistance across diseased pigs and healthy slaughtered pigs. Whole-genome sequencing enabled comprehensive characterization of serotypes, sequence types (STs), and clonal complexes (CCs), revealing substantial genetic diversity. The most prevalent serotypes identified were 2, 8, and 29. A total of 189 distinct STs were detected, including 169 novel STs, with ST104 being the most common, followed by ST233 and ST87. A total of 29 distinct clonal complexes (CCs) were identified. While CC104 was mainly associated with serotype 2, other CCs showed diverse serotype profiles, reflecting complex serotype-CC relationships with 62 unique combinations observed. Antimicrobial susceptibility testing against 30 agents representing 12 antimicrobial classes revealed high rates of non-susceptibility to macrolides, tetracyclines, and tiamulin. In contrast, meropenem, vancomycin, daptomycin, and ertapenem maintained high efficacy against most isolates. Multidrug resistance was widespread, compromising the effectiveness of key antimicrobials such as ampicillin, cephalosporins, chloramphenicol, florfenicol, gentamicin, penicillin, and tiamulin. Significant correlations between specific CCs and antimicrobial non-susceptibility patterns were observed, with CC233, for example, being linked to beta-lactam non-susceptibility. These findings highlight the substantial genetic diversity and AMR burden of pig-isolated *S. suis* in Thailand, emphasizing the critical need for sustained surveillance, improved biosecurity measures, targeted control strategies, and prudent antimicrobial use in swine production to mitigate zoonotic transmission risks and safeguard the therapeutic effectiveness of antibiotics in both veterinary and human health settings.

Factors correlating with human infection via the whole genome sequence analysis of *Streptococcus suis* serotype 2

Nguyen Xuan Truong[1]; Nguyen Trung Thanh[1]; Nguyen Thi Huong Lan[2]; Dinh Huy Man[2]; Nguyen Thanh Vinh[1]; Phung Le Kim Yen[1]; **Ngo Thi Hoa[1]***

[1] Oxford University Clinical Research Unit, HCMC, Vietnam; [2] Hospital for Tropical Diseases, HCMC, Vietnam.

Streptococcus suis continues to be one of the most common causes of acute bacterial infections in meningitis patients in Vietnam. Most of the cases were sporadically reported. We employed whole genome sequence (WGS) analysis to investigate if there are factors potentially associated the human infections. A database of virulence factors was constructed. The WGS of over 500 *S. suis* serotype 2 strains isolated from patients and pigs from Vietnam are analysed. The phylogeny tree constructed using core -SNPs was presented with two major clades of strains. Most of strains isolated from patients clustered in one clade of 528 isolates, so called high-pathogenic clade, and the other lower pathogenic clade included 64 strains mostly isolated from pigs or pig products. The median of the genome sizes of strains in the high pathogenic clade was significantly smaller than that in the lower pathogenic clade. Within the high-pathogenic clade, the larger genome size was correlated with higher number of virulence and antimicrobial resistance genes, while the larger genome size of the lower pathogenic clade contained fewer virulence genes but still carried many antimicrobial resistance genes. We identified the presence of more than twenty genes exclusively carried in the high-pathogenic clade including three known virulence genes. While all of the isolates in the lower pathogenic clade exclusively carried one gene, the strains caused diseases in pigs or humans carried additional virulence genes which were not found in any isolate in the high-pathogenic clade. The findings suggested the identification of the factors associated with human infections for further investigation.

POSTER ABSTRACTS

Streptococcus suis endocarditis: evidence in Thailand

Wilawan Thipmontree[1]; Rattagan Kajeekul[1]; **Anusak Kerdsin[2]***

[1] Division of Infectious Diseases, Department of Medicine, Maharat Nakhon Ratchasima Hospital, Nakhon Ratchasima, Thailand;
[2] Faculty of Public Health, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand.

Streptococcus suis is an important zoonotic pathogen worldwide, responsible for a range of clinical syndromes, with meningitis and sepsis being the predominant clinical manifestations. Several previous studies on human cases of *S. suis* in Thailand have indicated that infective endocarditis (IE) ranks third to fourth among clinical presentations. This study aimed to characterize IE caused by *S. suis* in a tertiary hospital in rural Thailand. Between 2021 and 2023, 200 patients with *S. suis* bacteremia who underwent echocardiograms were enrolled. Among these, 102 were diagnosed with IE and 98 were non-IE. In the multivariable analysis, predisposing factors for *S. suis* bacteremia in patients who are more likely to develop IE included penicillin-resistant strains (adjusted prevalence ratio [aPR]: 7.94, 95% CI: 1.13–55.80, p-value 0.037), persistent bacteremia (aPR: 1.58, 95% CI: 1.27–1.96, p-value <0.001), an illness duration of more than 14 days (aPR: 2.19, 95% CI: 1.66–2.89, p-value <0.001), and age under 60 years (aPR: 1.42, 95% CI: 1.09–1.83, p-value 0.008). The Kaplan-Meier survival curve showed that IE patients had a lower survival probability from admission to death compared to those without IE. The risk of death included embolic stroke (aPR 3.28, 95% CI: 1.82–5.91, p-value <0.001) and acute kidney injury (aPR 2.87, 95% CI: 1.06–7.79, p-value 0.038). While IE followed by valve surgery seemed to be a protective factor for patients with IE (OR 0.02, 95% CI: 0.00-0.08; p-value <0.001). Among the 67 IE-associated *S. suis* strains, the novel lineage serotype 2-ST1688 was significantly associated with IE (OR=2.96, 95%CI=1.13-7.72; p-value=0.027). In conclusion, routine echocardiography for patients with *S. suis* bacteremia is recommended, and heart valve surgery can decrease mortality. However, managing antimicrobial-resistant *S. suis* infections presents a significant challenge.

Development of a murine model for *Streptococcus suis*-induced arthritis

Hulin Yang[1]; Yali Li[1]; Tingting Feng[1]; Rui Zhou[1,2,3]; Qi Huang[1,2]*

[1] National Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, China; [2] International Research Center for Animal Disease (Ministry of Science & Technology of China), Wuhan 430070, China. The Cooperative Innovation Center of Sustainable Pig Production, Wuhan 430070, China; [3] The Cooperative Innovation Center of Sustainable Pig Production, Wuhan 430070, China.

Arthritis is a common clinical manifestation of *Streptococcus suis* (*S. suis*) infection in both humans and swine, yet its pathogenesis remains poorly understood and effective therapeutic strategies are lacking, highlighting the need for appropriate animal models. In this study, we developed a murine arthritis model using clinical *S. suis* isolates obtained from the knee joints of pigs showing characteristic arthritis symptom. It was demonstrated that intra-articular rather than intravenous or intra-peritoneal inoculation of *S. suis* induced significant joint inflammation in mice. Acute arthritis developed within four hours post-infection (hpi) as evidenced by swelling and lameness. Clinical symptoms started to resolve by three days post-infection (dpi), with complete functional recovery and weight restoration achieved by nine dpi. Histopathological examination showed extensive inflammatory infiltration in fibrous and synovial layers at one dpi, which markedly subsided by five dpi. Furthermore, to enable dynamic infection monitoring, two novel bioluminescent *S. suis* strains expressing NanoLuc or AkaLuc luciferase, which exhibited strong substrate-dependent luminescence, were constructed. Using an *in vivo* imaging system (IVIS), infection dynamics of these bioluminescent strains were able to be monitored in real time, and a strong positive correlation between the bioluminescence and bacterial load was observed. This newly developed arthritis model as well as the IVIS-based real time infection tracking system provides a powerful platform for both elucidating the pathogenesis of *S. suis*-induced arthritis and assessing potential therapeutic interventions.

Unveiling the division of labor of class A PBPs in *Streptococcus suis*

Liujun Wu[1]; Ping Chen[1]; Xinhui Jia[1]; Shenye Yu[1]; Wanjiang Zhang[1]; Siguo Liu[1]; Gang Li[1]; **Yueling Zhang[1]***

[1] State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China.

Class A penicillin-binding proteins (aPBPs) are essential for bacterial cell wall synthesis and have long been regarded as the primary enzymes in this process. However, recent studies have shifted the focus to class B PBP (bPBP)/SEDS pairs, prompting a reevaluation of aPBPs' roles. Due to functional redundancy among aPBPs, defining their individual contributions to peptidoglycan (PG) synthesis in ovococci has remained challenging. To address this, our study introduces a novel strategy by investigating aPBP functions in *Streptococcus suis*. Unlike the well-studied model strain S. pneumoniae, *S. suis* possesses a non-essential bPBP, PBP2b, enabling its specific depletion alongside each aPBP. This approach—which halts peripheral PG (pPG) synthesis upon PBP2b depletion—generates clearer phenotypic distinctions, facilitating the elucidation of individual aPBP roles in PG synthesis. Using a multi-faceted methodology, including *in vivo* protein fusion, the bacterial adenylate cyclase two-hybrid system, PG labeling, and high-resolution microscopy, we uncovered a cooperative role for PBP1a and PBP2b in pPG synthesis and septal PG (sPG) remodeling, which is critical for integrating septal and lateral cell walls. In contrast, PBP1b primarily contributes to PG repair, while PBP2a participates in sPG synthesis to reinforce the core PG layer produced by PBP2x/FtsW. Based on these findings, we propose an extended model detailing the division of labor among aPBPs in ovococcal PG synthesis. This study advances our understanding of the molecular mechanisms governing bacterial cell wall synthesis and provides new insights into bacterial cell division.

Effects of *Streptococcus suis* infection on immune organs

Siyu Pan[1]; Tong-Qing An[1]; Haijuan He[2]; **Shujie Wang[1]***

[1] Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences,China; [2] Institute of Animal Husbandry, Heilongjiang Academy of Agriculture Sciences, China.

Streptococcus suis (*S. suis*) is a significant porcine pathogen associated with substantial economic losses in the global swine industry. It represents one of the primary causes of bacterial mortality in postweaned piglets and is also recognized as an emerging zoonotic pathogen capable of causing infections in humans. Our research discovered for the first time that *S. suis* can induce thymus atrophy in mice and piglets. Specifically, *S. suis* triggers apoptosis of CD3+ T cells in the thymus, leading to a marked reduction in the number of CD4+CD8- and CD4-CD8+ T cells in peripheral blood, as well as significant alterations in the cytokine profile, particularly the dysregulation of pro-inflammatory factors such as IL-2, IL-4, IL-6, IL-10, IFN-β, and TNF-α. These changes affect the host's immune response and result in immunosuppression, suggesting a novel virulence mechanism of S. suis. Furthermore, we investigated the damage and mechanisms caused by *S. suis* to peripheral immune organs, including spleen and lymph nodes. Our results showed that *S. suis* induces splenomegaly, lymphadenectasis, and triggers apoptosis in B cells as well as pyroptosis in macrophages. Our study provides a comprehensive summary of the damage and mechanisms associated with *S. suis* infection on immune organs.

Analysis of the structural diversity of lipoteichoic acids in *Streptococcus suis*

Anna Nehrmann[1]*; Dominik Schwudke[1,2]; Nahuel Fittipaldi[3]; Mariela Segura[3]; Marcelo Gottschalk[3]; Nicolas Gisch[1]

[1] Division of Bioanalytical Chemistry, Priority Area Infections, Research Center Borstel, Borstel, Germany; [2] German Center for Infection Research, Thematic Translational Unit Tuberculosis, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany; German Center for Lung Research (DZL), Airway Research Center North (ARCN), Research Center Borstel, Leibniz Lun; [3] 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.

S. suis strains are classified into 29 serotypes based on their capsular polysaccharide[1] and belong to numerous sequence types (STs).[2] Lipoteichoic acids (LTA) have been described as major molecules of the *S. suis* cell wall and their contribution to virulence has been suggested.[2,3] The absence of LTA led to significant morphological defects, including defective separation of bacterial cells and an impaired ability to maintain a proper coccus shape.[4] Previous structural studies so far focused exclusively on the LTA of serotype 2, as this is the predominant serotype worldwide, and a deviating structure was found in strains P1/7 (ST1) and SC84 (ST7) compared to strain 89-1591 (ST25).[3] The investigated strains produced two distinct types of LTA, a type I LTA with a poly-glycerol phosphate (poly-GroP) chain, and a second, more complex LTA containing glycosylated repeating units in addition. However, the glycosylation pattern of this second LTA type was different in the ST25 strain compared to the other two strains.[3] The aim of this study is now to explore LTA structures present in other serotypes on the molecular level. We structurally characterize the LTA from six selected *S. suis* strains belonging to serotype 1 (ST1), 1/2 (ST1), 1/2 (ST 28), 7 (ST29), 9 (ST16), and 14 (ST6). To this end, we apply our expertise for the isolation and structural characterisation of Gram-positive bacterial cell wall components using mass spectrometry and nuclear magnetic resonance spectroscopy.[3,5] The obtained results will provide broader information on the diversity of LTA structures in S. suis.

[1] Okura et al., Pathogens 2016; [2] Fittipaldi et al., Infect. Immun. 2008; [3] Gisch et al., J. Biol. Chem. 2018; [4] Payen et al., Vet. Res. 2024; [5] Heß et al., Nat. Communs. 2017. This work was supported by a grant of the Deutsche Forschungsgemeinschaft to N.G. (GI 979/2-1).

In silico identification of *Streptococcus suis* serotype

Ján Matiašovic[1]*; Natálie Králová[1]; Monika Zouharová[1]

[1] Veterinary Research Institute, Czech Republic.

Introduction: Serotypes of *Streptococcus suis* are associated with various pathogenicity. The serotype is determinated by the cps locus harboring genes encoding enzymes involved in the synthesis of capsular polysaccharide. Identification of genes specific for particular serotype by PCR is already used for PCR-based serotyping. Here we present a script detecting serotype-specific genes in assembled genome and thus identifying putative serotype of sequenced isolate. **Materials and methods**: The script utilise local installation of NCBI-blast+ software and a database of serotype-specific genes. The database of serotype-specific sequences is modified from the *S. suis* Serotyping_pipeline (https://github.com/streplab/SsuisSerotyping_pipeline) by adding the genes specific for the new capsular polysaccharide serotypes (NCLs) up to NCL32. After the blast search of sequences similar to serotype-specific genes present in studied genome, the result of the blast search is filtered by score and the database gene most similar to sequence present in genome determine the probable serotype. The script also identify the presence of potential modifying genes altering the final polysaccharide structure and also the presence of recN, the gene predominantly present in *Streptococcus suis* sensu stricto isolates. **Results:** The script successfully identify serotype-specific sequence in genome of all reference serotype strains and thus infer serotype. The script also distinguish the serotypes ½ vs. 2 and 1 vs 14. Moreover, the NCL serotypes up to NCL32 can be detected. **Discussion and Conclusion:** Whole-genome sequencing is becoming an affordable solution for identifying bacterial isolates. The presented script allows to estimate the potential serotype of *Streptococcus suis* isolate. Due to its dependence only on the freely available software ncbi-blast+, its installation is very simple.

This work was supported by grants RO0523 and TN02000017.

Effect of MCFA on tonsillar *S. suis* colonisation in piglets pre- and post-weaning

Xiaonan Guan[1]; Xandra Benthem de Grave[1]; Mario Romero Ortiz[2]; Alicia Suarez Velo[2]; **Francesc Molist[1]***

[1] Schothorst Feed Research B.V., The Netherlands; [2] Golden Agri International Pte.Ltd., Singapore.

This study evaluated the effects of lauric acid in sow diets (pre-weaning) and monoglycerides of lauric acid (GML) in piglets diets (post-weaning) on the tonsillar *S. suis* colonization in piglets pre- and post-weaning. Twenty-four sows (parity 2–6) received either a control diet (NC) or the NC diet + 2 kg/T lauric acid (LA) from day 100 of gestation until weaning. In post-weaning (PW) phase, 72 piglets remained in their maternal treatment groups and were fed either a control diet or control diet +3 kg/T GML. Tonsil swabs were collected from all the 24 sows at day-100 of gestation and weaning, and from all the piglets at 24 till 48 hours after birth, at weaning, and day 21 PW. The DNA were extracted from tonsil swabs and analysed for total *S. suis* and virulent *S. suis* in DNA copies per tonsil swab. During the pre-weaning period, no significant effect was observed on the total *S. suis* colonization in sow. The prevalence of virulent *S. suis* was not affected by diets. On average, 29% of sows were positive for virulent *S. suis* at day 108 of gestation, and 17.5% at weaning, and piglets early after birth were 52.5% positive. During the PW period, no interaction between diet and time was observed for total S. suis, which decreased from log10 6.7 at weaning to log10 6.1 at day 21 PW ($P<0.01$). A tendency towards interaction between diet and time were observed on virulent *S. suis* ($P=0.08$), piglets received the control diets increased from 1.9 to 2.3 log10 DNA copies per tonsil swab, while piglets received GML diets were consistent at 2.0 log10 DNA copies per tonsil swab. The prevalence of virulent *S. suis* remained high among treatments and were averaged of 98.5%. In summary, diet had little effect on total *S. suis* levels. The prevalence of virulent *S. suis* increased in piglets from birth to post-weaning. The virulent *S. suis* levels in the tonsils appeared to increase with age after weaning, while GML might help to keep the virulent *S. suis* level stable.

Characterization of the contribution of recently described virulence associated genes to the virulence of *Streptococcus suis* serotype 2

Servane Payen[1]; Nahuel Fittipaldi[1]; Mariela Segura[1]; **Marcelo Gottschalk[1]***

[1] University of Montreal, Canada.

Streptococcus suis serotype 2 is one of the most important bacterial swine pathogens. In the last years, increasing numbers of comparative *S. suis* genome studies have been performed, resulting in the identification of a plethora of so-called virulence associated genes (VAGs). Recently, one study showed that pathogenic North American strains are more likely than opportunistic and commensal strains to possess in their genome three VAGs, namely SSU_RS09155; SSU_RS09525; SSU_RS03100, all coding for hypothetical proteins of unknown functions. To evaluate a potential contribution to *S. suis* virulence of these three VAGs, we generated isogenic mutants for each of these genes in a virulent serotype 2 *S. suis* genetic background and evaluated their virulence using an experimental infection model. When tested in vivo using a mouse infection model, the isogenic SSU_RS09525 and SSU_RS03100 mutants behaved similarly to the parental wild-type strain, while a significant decrease in both virulence and bacteremia was observed for the SSU_RS09155 mutant. Further characterization of the role of SSU_RS09155 in vitro showed that the factor does not influence adhesion and invasion of *S. suis* to swine epithelial. However, the mutant strain possesses a reduced capacity to resist both phagocytosis and blood bactericidal effect when compared to the parental strain even though it was well encapsulated. Our findings strongly suggest that the protein encoded by SSU_RS09155 can be considered an important virulence factor, probably involved in resistance to phagocytosis and killing by phagocytes. To confirm its role in virulence, experimental infection of pigs (natural host) should be performed.

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

Dominic Dolbec[1]; Mélanie Lehoux[1]; Astrid Zahn[2]; Marcelo Gottschalk[1]; Javier Di Noia[2]; **Mariela Segura[1]***

[1] University of Montreal, Canada; [2] Montreal Clinical Research Institute, Canada.

The adaptive humoral response is the result of a communication network between antigen presenting cells (APC), T cells and B cells. Antibodies play a useful role in the elimination of *Streptococcus suis*, an encapsulated bacterium that can cause severe invasive disease in pigs. Reports indicate that *S. suis* can interfere with optimal APC and T cell functions. However, the interactions between *S. suis* and B cells are largely unknown. The aim of this study was to characterize the development of the adaptive humoral immune response by evaluating GC B cell dynamics and the production and role of antibodies induced following *S. suis* infections in a mouse model. We found that mice infected with *S. suis* developed GC that peaked 13-21 days post-infection. GC further increased and persisted upon periodic reinfection that mimics real life conditions in swine farms. Anti-S. suis IgM and several IgG subclasses were produced, whereas antibodies against the *S. suis* capsular polysaccharide (CPS) were largely IgM. Somatic hypermutation or isotype switching were dispensable for controlling the infection or anti-CPS IgM production. Depletion of total IgG from the WT mice sera had no effect on bacterial killing in vitro. However, T cell-deficient (Tcrb-/-) mice were unable to control bacteremia, producing optimal anti-CPS IgM or eliciting antibodies with opsonophagocytic activity. SAP deficiency, which prevents GC formation but not extrafollicular B cell responses, ablated anti *S. suis*-IgG production but maintained IgM production and eliminated the infection. In contrast, B cell deficient mice were unable to control bacteremia. Collectively, our results indicate that a GC-independent but T cell-dependent germline IgM being the major effective antibody specificity. Our results further highlight the importance IgM and potentially anti-CPS antibodies in clearing *S. suis* infections and provide insight for future development of *S. suis* vaccines.

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

Dominic Dolbec[1]; Mélanie Lehoux[1]; Masatoshi Okura[2]; Daisuke Takamatsu[2]; Marcelo Gottschalk[1]; **Mariela Segura[1]***

[1] University of Montreal, Canada; [2] National Institute of Animal Health, Japan.

Streptococcus suis is an encapsulated bacterium that can cause severe invasive diseases in pigs. The bacterial capsular polysaccharide (CPS) is a critical virulence factor that provides resistance against host phagocytic cells. The antigenicity of the CPS defines 29 distinct serotypes of *S. suis*, with some serotypes being more commonly associated with clinical disease than others. For instance, the serotype 2 is the most prevalent worldwide. 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. Therefore, serotype-switched mutants of S. suis serotype 2 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 in a mouse model showed that 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. Furthermore, 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 (such as proteins) than the CPS of serotypes 2, 7, 8 and 9. This feature consequently affected antibody capacity to induce opsono-killing of *S. suis*. 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 the CPS structure might eventually affect the efficacy of vaccines targeting subcapsular antigens at the bacterial surface.

Influence of *Streptococcus suis* SLY and DltA on the crosstalk between innate immune cells in different host compartments

Anna Richter[1,a,*]; Tanja Wurzer[2,a,*]; Laura Schaltz[3]; Simon Lassnig[3]; Christoph Georg Baums[2]; Ralph Goethe[1]; Nicole de Buhr[3]; Sophie Öhlmann[2,b]; Désirée Schaaf[1,b]

[1] Institute for Microbiology, University of Veterinary Medicine Hannover, Hannover, Germany; [2] Institute of Bacteriology and Mycology, Center for Infectious Diseases, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany; [3] Institute for Biochemistry, University of Veterinary Medicine Hannover, Hannover, Germany; [a] These authors contributed equally to this work and share first authorship; [b] These authors contributed equally to this work and share last authorship.

S. suis is equipped with diverse virulence-associated factors that can modulate the inflammatory response. Among them are the pore-forming toxin suilysin (SLY) and the enzyme D-alanine-D-alanyl carrier ligase (DltA) that implements D-alanylation of lipoteichoic acids in the bacterial cell wall. We aim to show the influence of SLY and DltA on the innate immune response following the route of systemic *S. suis* infection through three host compartments by using in vitro models. The respiratory tract is mimicked by a co-culture of porcine respiratory epithelial cells (PRECs) and porcine alveolar macrophages (PAMs). Reconstituted whole blood or isolated leukocytes represent the blood compartment. A model consisting of porcine choroid plexus epithelial cells simulates the blood-cerebrospinal fluid-barrier (BCSFB). Infection experiments will be performed with *S. suis* serotype 2 strain 10, its isogenic mutants deficient for SLY and/or DltA and respective complemented mutants. So far, a co-culture of PRECs and PAMs was successfully established. Chromosomal complementation of *S. suis* ΔdltA was conducted. Other mutants, the BCSFB and the blood model are available from previous studies. Currently we are establishing infection experiments in the respective models, focusing on the innate immune response (phagocytosis, oxidative burst, cytokine release, degranulation) of macrophages and neutrophils. In later stages we plan NET analysis and focus on the crosstalk between the selected immune cells. We will also compare the expression of SLY and DltA in the three compartments in the presence and absence of host cells and antimicrobial peptides. This cooperation project offers the prospect of a better understanding of the influence of SLY and DltA on host cell communication during the innate immune response towards *S. suis* infection.

Thioredoxin C of *Streptococcus suis* serotype 2 contributes to virulence by inducing antioxidative stress and inhibiting autophagy via the MSR1/ PI3K-Akt-mTOR pat

Lihua Fang[1*]; Yulong Tang[2]

[1] Shenzhen Longhua District Central Hospital,China; [2] Chinese Academy of Sciences,China.

The thioredoxin (Trx) system plays a vital role in protecting against oxidativestress and ensures correct disulfide bonding to maintain protein function. Our previous research demonstrated that TrxA of SS2, a clinical strain from the lung of a diseased pig, contributes to virulence but is not involved in antioxidative stress. We identified another gene in the Trx family, TrxC, which encodes aprotein of 104 amino acids with a CGDC active motif and 22.4 % amino acid sequence homology with TrxA. Unlike the TrxA, TrxC mutant strains were more susceptible to oxidative stresses induced by hydrogen peroxide and paraquat. *in vitro* experiments, the survival rate of the TrxC deletion mutant in RAW264.7 macrophages was only one-eighth of that of TrxA mutant strains. Transcriptome analysis revealed that autophagy-related genes were significantly upregulated in the TrxCmutant compared to those in the wild-type or TrxA mutant strains. Co-localization of LC3 puncta with TrxC was confirmed using laser confocal microscopy, and autophagy-related indicators were quantified using western blotting. Autophagy deficiency induced by ATG5 knockout significantly increased SS2 survival rate, especially in TrxC mutant strains. For the upstream signal regulation pathways, we found ΔTrxC strains regulate autophagy by activation of PI3K/Akt/mTOR signaling in RAW264.7 macrophages.In the Akt1-overexpressing cell line, ΔTrxC infection significantly decreased the autophagic response and promoted ΔTrxC mutantstrain survival, while inhibition of Akt with MK2206 resulted in reduced ΔTrxC mutant strain survival and enhance the autophagic response. Furthermore, loss of TrxC increased the activity of MSR1, thereby inducing cellular autophagy and phagocytosis. Our data demonstrate that TrxC of SS2 contributes to virulence by inducing antioxidative stress and inhibits autophagy via the PI3K Akt-mTOR pathway in macrophages, with MSR1 acting as a key factor in controlling in infection.

Constitutive glucose import in zoonotic *Streptococci* enables proliferation in cerebrospinal fluid

Chen Yuan[1]; Karthik Hullahalli[2]; Yuchang Wang[1]; Hao Huang[1]; Ying Liang[1]; Hongjie Fan[1]; Matthew K. Waldor[2]; **Zhe Ma[1]***

[1] Nanjing Agricultural University, China; [2] Harvard Medical School, USA.

Proliferation of the emerging zoonotic pathogen *Streptococcus equi subsp. zooepidemicus* (SEZ) in the meninges is linked to mortality in pigs and morbidity in humans. The mechanisms underlying hypervirulent SEZ's remarkable capacity to proliferate in the cerebrospinal fluid (CSF) are largely undefined. Here, using barcoded SEZ, we found that following systemic infection only ~1-10 SEZ clones invade the meninges where they subsequently replicate ~107-fold. The mannose phosphotransferase system (PTSman), which imports glucose, was identified as essential for SEZ to proliferate in the CSF. The SEZ PTSman promoter confers species-specific constitutive transcription of PTSman, enabling glucose acquisition at low glucose concentrations and limiting activation of the stringent response, leading to robust pathogen replication in the CSF. Our findings reveal how the rewiring of PTSman in the control of SEZ metabolism enables this pathogen to adapt to and replicate in CSF during CNS infection.

A new lymph node infection model for *Streptococcus suis* in pigs

Annika Katharina Breitfelder [1]*; Tatjana Sattler[2]; Sarah Pfetzing[3]; Anna Majcher[3]; Johannes Kauffold[2]; Uwe Müller[4]; Reiner Ulrich[3]; Christoph Georg Baums[1] *et al*.

[1] Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Germany; [2] Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, University of Leipzig, Germany; [3] Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany; [4] Institute of Immunology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, BBZ, University of Leipzig, Germany.

Introduction: *Streptococcus suis* (*S. suis*) can be isolated frequently from lymph nodes (LN) of natural infected pigs (Bornemann et al., 2024), and follicular hyperplasia is a common finding in pigs in the field. The objective of this study was to establish a LN infection model in piglets to investigate the interaction between *S. suis* and immune cells in the LN in the future. **Material and Methods**: Eight 5-week-old pigs were infected with 7x10^3 CFU *S. suis* serotype 2 of clonal complex 1 in the left Ln. cervicalis superficialis dorsalis. Piglets were monitored clinically and sacrificed between 24 h and 3 d post infection. Samples from different LN, inner organs and joints were screened bacteriologically and histologically including immunohistology of the infected LN. **Results**: Seven out of 8 piglets developed clinical signs of disease such as fever or lameness. Bacteriological investigations confirmed dissemination of the challenge strain to inner organs and various LN as well as proliferation in the infected LN. Immunohistology revealed *S. suis* antigen mainly predominantly within the sinusoids, focally within the paracortex, but not within the follicles. **Discussion**: The results indicate that *S. suis* very easily disseminates after injection in a lymph node and suggests that sinusoidal macrophages are an important host cell interacting with *S. suis* in the LN in this infection model. Further studies are warranted to clarify if older piglets show follicular hyperplasia after LN infection which was surprisingly not observed in this model. **Reference**: Bornemann, N. N., L. Mayer, S. Lacouture, M. Gottschalk, C. G. Baums, and K. Strutzberg-Minder. 2024. Invasive Bacterial Infections of the Musculoskeletal and Central Nervous System during Pig Rearing: Detection Frequencies of Different Pathogens and Specific *Streptococcus suis* Genotypes.

Veterinary Sciences 11(1). doi:10.3390/vetsci11010017. **Correspondence**: Annika Breitfelder; annika.breitfelder@uni-leipzig.de

---

The prophage-encoded regulator SSU3305 coordinates prophage dynamics and capsular biosynthesis in *Streptococcus suis*

Aijuan Li[1]; **Liping Wang[1]***

[1] Nanjing Agricultural University, China.

Bacterial pathogens have evolved complex regulatory networks to balance prophage maintenance and host adaptation. In *Streptococcus suis* SC070731, the prophage-encoded protein SSU3305 acts as a master regulator coordinating phage dynamics and host capsular polysaccharide (cps) biosynthesis. Genetic deletion of SSU3305 (DLSSU3305) abolishes prophage excision and circularization, while overexpression (OESSU3305) enhances these processes. SSU3305 physically interacts with prophage integrase IntI, stabilized by a hydrogen bonding network that induces IntI conformational changes to inhibit attachment site recognition, confirmed by key residue mutagenesis reducing excision efficiency. Critically, SSU3305 also directly interacts with Cps enzymes: DLSSU3305 mutants show increased capsular thickness, while OESSU3305 strains have reduced thickness. DLSSU3305 mutants fail in horizontal phage transfer, linking capsular structure to phage propagation. SSU3305 serves as a molecular switch: (1) It autonomously controls phage mobility by binding IntI to inhibit reintegration, favoring excision and replication independent of host stress. (2) It regulates host adaptation by modulating capsular thickness via Cps interactions. The thickened capsule in DLSSU3305 aids immune evasion but impairs phage spread, establishing a feedback loop between phage mobility and host surface remodeling. This dual function reveals an evolutionary trade-off: SSU3305 optimizes bacterial fitness by maintaining an "ideal" capsular state that balances host protection (via sufficient capsule) with efficient phage propagation (via manageable capsule thickness). This represents a novel paradigm where a phage-encoded factor autonomously controls its mobility while simultaneously fine-tuning critical host surface characteristics.

Roles of MutX in mutation rate maintenance and infection processes in *Streptococcus suis*

Xi Lv[1]*; Wenbin Zhao[1]; Junjie Sun[1]; Liping Wang[1]

[1] Nanjing Agricultural University, China.

Due to widespread antimicrobial use and misuse, *Streptococcus suis* has developed multi-antibiotic resistance, partly driven by genetic mutations. Oxidative DNA damage threatens genomic integrity, with guanine oxidation forming highly mutagenic 8-oxo-dGTP, which mispairs with adenine during replication. While Escherichia coli MutT and *Streptococcus pneumoniae* MutX hydrolyze 8-oxo-dGTP to mitigate this, the function of the homologous MutX (SSU0990) in *S. suis* was unclear. This study demonstrates that *S. suis* MutX is crucial for combating oxidative DNA damage and pathogenesis. Deletion of mutX (ΔSSU0990) significantly increased the mutation rate, impaired growth, and heightened sensitivity to hydrogen peroxide-induced oxidative stress. Prokaryotically expressed and purified MutX protein demonstrated significant 8-oxo-dGTP hydrolase activity via HPLC, confirming its role in sanitizing the nucleotide pool by hydrolyzing the mutagenic lesion 8-oxo-dGTP. Beyond genomic stability, ΔSSU0990 mutants exhibited enhanced phagocytosis by RAW264.7 macrophages and reduced intracellular survival. In a murine infection model, the ΔSSU0990 strain showed significantly lower bacterial loads in brain tissue compared to wild-type, indicating attenuated virulence. Complementation (CSSU0990) restored functions. This study establishes MutX in *S. suis* as a multifunctional protein essential for maintaining a low mutation rate, resisting oxidative stress, evading host phagocytosis, and sustaining full pathogenicity during systemic infection. These findings enhance understanding of *S. suis* infection mechanisms and identify mutX as a potential therapeutic target for novel anti-infective strategies.

Both the arginine deiminase system and the fucose operon, regulated by XtrSs, contribute to the survival of *Streptococcus suis* in macrophages

Guangjin Liu[1]*; Song Liang[1]; Yuming Zhang[1]; Huochun Yao[1]

[1] Nanjing Agricultural University, China.

The arginine deiminase system (ADS) and the Fucose Operon (FCS) have been identified in various bacteria, where they function to supplement energy production and enhance biological adaptability. In our study, we found that the XRE family transcriptional regulator XtrSs negatively affected the virulence of *Streptococcus suis* (*S.suis*) and significantly repressed the transcription of ADS and FCS when the bacteria were incubated with swine blood. Electrophoretic mobility shift (EMSA) and lacZ fusion assays further demonstrated that XtrSs directly binds to the promoter of ArgR, a recognized positive regulator of bacterial ADS, and represses ArgR transcription. Simultaneously, XtrSs directly binds to the promoter of Catabolite control protein (CcpA), which is widely reported to regulate central carbohydrate metabolic pathways in Gram-positive bacteria, and represses FCS transcription. Moreover, we provided compelling evidence that *S. suis* can utilize arginine via ADS to adapt to acid stress and respond to fucose through FCS. However, *S. suis* failed to proliferate in media with fucose as the sole carbon source. Furthermore, the complete ADS-knockout of *S. suis* increased arginine and antimicrobial nitric oxide (NO) levels within infected macrophages, while the FCS mutant enhanced the autophagy processes of macrophages. Both effects can lead to a decrease in the intracellular survival of S. suis. Additionally, the ADS mutant significantly attenuated virulence in a mouse infection model. Conversely, XtrSs knockout *S. suis* consistently presented the opposite results. In conclusion, our findings have confirmed the detailed regulatory mechanism of XtrSs on ADS and FCS, and have revealed the role of ADS and FCS in the survival of *S. suis* within macrophages.

Molecular characterizations of sequence type 104 serotype 2 *Streptococcus suis*

Piyathida Prasopthum[1]*; Jeeraphong Thanongsaksrikul[1]; Onruedee Khantisitthiporn[1]; Buppa Archanachan[1]; Hiroshi Ishikawa[2]; Horst Schroten[2]; Christian Schwerk[3]; Potjanee Srimanote[1]

[1] Thammasat University, Thailand; [2] University of Tsukuba, Japan; [3] University Hospital Mannheim, Germany.

Zoonotic *Streptococcus suis* serotype 2 (SS2) is almost the sole serotype to cause meningitis and septicemia in humans. ST1 SS2 strains were the most frequent ST to cause invasive diseases in humans and pigs worldwide. Surprisingly, ST104 SS2 strains were reported to cause sepsis in humans only in Thailand. Most ST104 infections were septicemia without meningitis (91%). Virulence factors and mechanisms involved in ST104 infections have not yet been fully studied. Therefore, this study aims to characterise the genetic factor(s) of ST104 and their abilities to interact with choroid plexus epithelial cells (CPECs) . The ability of ST104 (MOI 10) to adhere and invade human and pig CPECs was compared with that of the prototype ST1; unexpectedly, no differences were found for both human and pig CPECs. The capsule production was investigated by hydrophobicity assay and transmission electron microscopy. The majority of ST104 strains had similar capsule thickness to that of ST1 SS2 strains. The 71 ST104 SS2 strains were subjected to whole-genome sequencing analysis. The genome size of ST104 strains was slightly longer than that of ST1, with a slightly lower GC content. ST104 strains harboured significantly fewer copies of rRNA and tRNA loci, three and 40, respectively. The virulence-associated genes were analysed by the Virulence Factor Database (VFDB), and no distinct virulence factors were found for ST104 compared with the ST1 SS2 prototype strains. Therefore, further detailed comparative whole genome sequence analysis will be required for the better understanding of ST104 pathogenesis.

PotD regulates biofilm formation in *Streptococcus suis*

Camila Bosch[1], Jorge Gimeno[1], Luis Saralegui[1], Clara Marín[2], **Jesús Arenas[1]***

[1] University of Zaragoza, Spain; [2] Agrofood Research and Technology Centre of Aragon, Spain.

Our previous research has identified essential genes for *S. suis* infection, including *potD*, which encodes a substrate-binding protein of a canonical ATP-Binding Transporter involved polyamine acquisition. In some bacteria, polyamines have been shown to regulate biofilm formation. Thus, the aim of this study was to investigate the role of PotD in the biofilm formation by *S. suis*, a process relevant for bacterial colonization and antibiotic tolerance. Western blotting using specific anti-PotD antiserum and proteinase K accessibility assays demonstrated that PotD is produced and surface-exposed in *S. suis* reference strain P1/7 (serotype 2, ST1). Biofilm formation was determined in P1/7 and its *potD* mutant derivative at 4 h, 24 h, and 48 h on abiotic surfaces. The *potD* mutant showed a fourfold increase in biofilm formation compared to the parent strain at all time points tested. This phenotype was nicely restored when *potD* was integrated into the chromosome of the *potD* mutant. Deletion of *potD* in strains Ss_45 (serotype 2, ST 3), Ss_72 (serotype 2, ST 1) and Ss_106 (serotype 9, ST 123) also resulted in increased biofilm formation, indicating that the effect is not specific to strain P1/7. Structural analysis of the biofilm using confocal microscopy evidenced enhanced biomass, thickness coefficient and area thickness, demonstrating that PotD production strongly influences biofilm architecture. To elucidate the mechanisms by which PotD affects biofilm formation, RNA sequencing was performed on biofilm cells of P1/7 and its *potD* mutant derivative. 1031 genes were differentially expressed between the both strains, with 567 downregulated and 463 upregulated. These genes belonging to different functional categories, including genes encoding putative adhesins and metabolic pathways related to biofilm formation. Together, our data support a regulatory role for PotD in biofilm formation through multiple mechanisms.

Survival of ST1 and ST104 serotype 2 *Streptococcus suis* in murine, porcine and human macrophages

Kittiyawan Sriyanon[1]*; Potjanee Srimanote[1]

[1] Thammasat University, Thailand.

Serotype 2 *Streptococcus suis* (SS2) is a significant zoonotic pathogen that can cause sepsis and meningitis in humans and pigs. ST1 is the most prevalent in both human and pig infections. ST104 SS2, has been reported to cause human septicemia only in Thailand. This study investigates the role of macrophages in the pathogenesis of ST104 SS2 strains. The association, internalization, and intracellular survival of ST104 SS2 were investigated using murine, porcine, and human macrophages. Additionally, the presence of 22 known virulence-associated genes was determined by multiplex PCR. The ability of ST104 SS2 (MOI 100) to associate with and be internalized by macrophages did not significantly differ from that of the prototype ST1 P1/7 strain. However, the human zoonotic ST1 strain HE06 showed significantly higher association with porcine and human macrophages compared to both P1/7 and ST104 strains, suggesting that ST104 SS2 may also evade phagocytosis. Intracellular survival assay indicated that ST104 SS2 was susceptible to killing within murine macrophages, similar to ST1. In contrast, both ST104 and ST1 strains demonstrated resistance to intracellular killing in porcine and human macrophages. No correlation was found between the virulence gene profiles and the macrophage interactions (association, internalization, and intracellular survival) of ST104 SS2. Therefore, the *in vitro* macrophage assays did not explain why ST104 SS2 causes septicemia without progressing to meningitis.

A dual histidine–glutamine transporter modulates virulence and biofilm formation in *Streptococcus suis*

Carla García[1], Luis Saralegui[1], Beatriz Morales[1], Paula Jurado[1], Mª Teresa Bes[2], Clara Marín[2], **Jesús Arenas[1]***

[1] Unit of Microbiology and Immunology, Faculty of Veterinary, University of Zaragoza; [2] Department of Biochemistry and Molecular and Cellular Biology, Faculty of Science, University of Zaragoza.

A TnSeq screen in a pig infection model identified over 300 conditionally essential genes for *Streptococcus suis* infection, including a gene coding for a putative substrate-binding protein, named ShgH, linked to an unknown ABC transporter. This study aimed to characterize ShgH function and role in pathogenesis. Proteinase K and Western blot assays confirmed that ShgH is produced and surface-exposed in strain P1/7, consistent with its role as a substrate-binding protein. A P1/7ΔshgH mutant showed impaired growth in chemically defined medium under limited histidine or glutamine concentrations. Radiolabelling with 3H-histidine or 3H-glutamine showed reduced uptake in the mutant. Isothermal titration calorimetry confirmed high-binding to histidine (Kd=0.17 µM) and lower affinity to glutamine (Kd=3.5 µM). Notably, P1/7ΔshgH formed twice as much biofilm as the wild-type, and confocal microscopy revealed notable alterations in biofilm architecture. The role of ShgH in virulence was evaluated using a murine model of chronic infection, in which CD1 mice were inoculated intranasally with either P1/7 or P1/7ΔshgH. After three days, animals were sacrificed and bacterial loads in nostrils and inner organs were quantified. In animals infected with P1/7, about 10^5 CFU/g were recovered from the lungs, spleen, brain and heart. In contrast, no bacteria were recovered from mice infected with P1/7ΔshgH, indicating that ShgH is required for systemic dissemination from the nasopharynx. Phagocytosis assays using murine macrophages revealed increased bacterial adhesion but decreased phagocytosis and intracellular survival for the *shgH* mutant, relative to the wildtype. In summary, this study identifies ShgH as the substrate-binding protein of a novel ABC transporter involved in histidine and glutamine uptake in *S. suis*, and demonstrates its important role in the pathogenesis of this bacterium.

The work was funded by ABC-VACCINESs (Ref. PID2020-114617RB-100) from Spanish Agency for Research

---

Streptococcus suis uses multiple glutamine uptake systems with differential regulation and functional specialization

Carla García[1], Luis Saralegui[1], Mª Teresa Bes[2], Clara Marín[2], **Jesús Arenas[1]***

[1] Unit of Microbiology and Immunology, Faculty of Veterinary, University of Zaragoza; [2] Department of Biochemistry and Molecular and Cellular Biology, Faculty of Science, University of Zaragoza.

S. suis is auxotrophic for glutamine/glutamate. This study aimed to characterise glutamine uptake, which had not been previously investigated in this pathogen. Three conserved genes, named glnH1, glnH2 and glnH3, encoding putative substrate-binding proteins of ABC transporters, were identified by in silico analysis. Western blotting and proteinase K accessibility assays confirmed that all three proteins are produced in strain P1/7 and are surface-exposed. Single-gene knockout mutants exhibited reduced growth under glutamine-limiting conditions compared to the wild-type, with P1/7ΔglnH3 resulting the most severely affected. Moreover, radiolabelling with 3H-L-glutamine revealed that P1/7ΔglnH2 and P1/7ΔglnH3 displayed 50% reduction in uptake, whereas P1/7ΔglnH1 almost lost uptake capacity, indicating that GlnH1 is the predominant transporter *in vitro*. The role of these transporters in virulence was assessed in a murine infection model. Three days post-infection, P1/7 reached ~10^5 CFUs/g in internal organs, while P1/ΔglnH1 and P1/7ΔglnH3 were recovered at low levels from lungs, and P1/7ΔglnH2 was not detected, demonstrating that all three transporters are important for infection, but have different roles. Western blotting revealed differential expression of the three transporters under varying glutamine concentrations, suggesting distinct regulatory mechanisms. Promoter analyses revealed hypothetical GlnR-binding motifs in the three genes, although with variations. A P1/7ΔglnR mutant overproduced all three proteins, with expression influenced by glutamine levels. In summary, this study identifies three glutamine-binding proteins involved in glutamine uptake and pathogenesis in *S. suis*, and suggest that their expression is differentially regulated by GlnR.

This work was funded by ABC-VACCINESs (Ref. PID2020-114617RB-100) from Spanish Agency for Research (AEI).

Sequence Type Distribution among Clinically Relevant Serotypes of *Streptococcus suis* from Quebec (Canada), 2020-2024

Pauline Treichel[1]*; Sonia Lacouture[1]; Nahuel Fittipaldi[1]; Marcelo Gottschalk[1]

[1] Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada.

Streptococcus suis is a Gram-positive bacterium recognized for its pathogenicity in pigs and its zoonotic potential. Among the 29 described serotypes, some are part of the porcine commensal flora, while others are associated with severe infections, including meningitis, septicemia, and arthritis, conditions that lead to substantial economic losses in the swine industry. In Canada, serotypes 1, 1/2, 2, 9 and 14 are frequently recovered from such cases. This study aimed to characterize recent changes in the distribution of sequence types (STs) within these serotypes among isolates recovered in Quebec, Canada, between 2020 and 2024. A total of 199 isolates were analyzed using the standard multilocus sequence typing (MLST), scheme for *S. suis* targeting the *aroA*, *gki*, *dpr*, *recA*, *mutS*, *thrA*, and *cpn60* genes. The dominant STs were ST1, ST28, ST25, ST94, and ST620. All ST1 isolates, associated with strain high virulence, were restricted to serotypes 1 and 14. In contrast to previous reports, we observed the emergence of ST126 and ST156 among serotypes 1 and 14, respectively. Additionally, nine serotype 14 isolates belonged to a novel, previously undescribed ST assigned to MLST clonal complex 1372. For serotype 2, the main STs were ST28 and ST25; notably we did not detect the highly virulent ST1 genotype, commonly found among serotype 2 isolates from Europe and Asia. Our findings provide new insights into the changing population structure of *S. suis* in North America and may inform future surveillance and vaccine development strategies.

A CRISPR/Cas12a-based DNAzyme visualization platform for rapid discrimination of *Streptococcus suis* serotype 2 versus 1/2 and serotype 1 versus 14

Jing Sun[1]; Jieyu Bai[1]; Yuxuan huang[1]; Paul R. Langford[2]; Yueling Zhang[1]; **Gang Li[1]***

[1] Harbin Veterinary Institute, Chinese Academy of Agricultural Sciences, Harbin, China; [2] Imperial College london, London, United Kingdom.

Streptococcus suis is a major swine pathogen with serotypes 2 and 14 posing zoonotic risks. However, distinguishing serotypes 1/2 from 2 or 1 from 14 remains challenging due to high similarity in their capsule polysaccharide (CPS) loci. Here, we developed a rapid, equipment-free discriminating platform targeting a single nucleotide polymorphism (SNP) at position 483 of the *cpsK* gene (G in serotypes 2/14 vs. T/C in 1/2/1). The method integrates recombinase polymerase amplification (RPA) with CRISPR/Cas12a and a G-quadruplex-hemin DNAzyme visualization system. RPA enables isothermal amplification, while CRISPR/Cas12a ensures single-nucleotide specificity by cleaving target DNA. Subsequent DNAzyme catalysis converts colorimetric substrates, enabling naked-eye differentiation via distinct color changes (blue for serotypes 1/2/1 vs. constrated 100% specificity across 29 *S. suis* serotypes and related strains. Compared to PCR-based or sequencing methods, our platform eliminates reliance on thermocyclers or fluorescence detectors, reducing costs and operational complexity. The entire workflow, completed within 70 minutes, offers a practical solution for point-of-care testing in resource-limited settings. By enabling rapid, accurate discrimination, this tool will become a complementary tool for resolving ambiguous serotypes and enhances outbreak management in swine populations and mitigates zoonotic transmission.

Genome-based analyses reveal hidden taxa and diagnostic pitfalls within the expanding *Streptococcus suis* complex

Fengyang Hsu[1*], Kevin Li[1], Sonia Lacouture[1], Lucy A. Weinert[2], Tsutomu Sekizaki[3,4], Daisuke Takamatsu[5], Antony T. Vincent[6], Ján Matiašovic[7], Han Zheng[8], Hubert Gantelet[9], Marcelo Gottschalk[1], and Nahuel Fittipaldi[1]

[1] Groupe de recherche sur les maladies infectieuses en production animale, and Centre de recherche en infectiologie porcine et avicole, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada.; [2] Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom; [3] Department of Microbiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [4] Research Center for Food Safety, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan; [5] Division of Infectious Animal Disease Research, National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan; [6] Département des Sciences Animales, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Québec, Québec, Canada; [7] Veterinary Research Institute, Brno, Czech Republic; [8] National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China; 9 Ceva Biovac, Beaucouzé, France.

Accurate delineation of species within *Streptococcus suis* and its close relatives remains challenging, owing to the suboptimal resolution of conventional diagnostic approaches and the insufficiency of comprehensive, curated reference databases. We investigated 64 isolates obtained from diseased pigs that were initially identified as *S. suis* by MALDI-TOF MS but tested negative in a *recN* gene-based PCR assay commonly used for *S. suis* species confirmation. Whole-genome sequencing revealed that only four isolates belonged to *S. suis sensu stricto*, while the majority were classified within other members of the *S. suis* complex, including *Streptococcus parasuis*, *Streptococcus ruminantium*, and several other recently proposed *Streptococcus* species. Based on core genome phylogeny, we propose a working definition of a "*S. suis* complex" as a monophyletic group of closely related species that includes *S. suis sensu stricto* and multiple emerging lineages, many of which are misidentified by standard diagnostics. These groups were consistently resolved as taxonomically coherent, while the *recN* gene showed limited resolution and evidence of recombination, reducing its diagnostic reliability. As a step toward improved tools, we identified 38 genes conserved in ≥95% of *S. suis sensu stricto* genomes that may serve as markers for future assay development. Our findings highlight substantial hidden diversity, reveal limitations in current diagnostics, and support the clinical relevance of several underrecognized taxa within this emerging pathogen cluster.

Epidemiology of pathogenic swine-derived *Streptococcus suis* on Irish farms

Emmanuel Kuffour Osei[1,3*]; John Moriarty[2]; Amy Kate O'Mahony[3]; Reuben O'Hea[4]; Áine O'Doherty[2]; Edgar Garcia Manzanilla[3]; Jennifer Mahony[1,4]; John Kenny[3]

[1] APC Microbiome Ireland; [2] Central Veterinary Research Laboratory, Backweston, Co, Kildare, Ireland; [3] Teagasc Food Research Centre, Moorepark, Ireland; [4] School of Microbiology, University College Cork.

Streptococcus suis is an important pathogen of intensive pig production industries globally including Ireland. It represents a major cause of systemic disease and mortality in post-weaned pigs aged 5 to 10 weeks, imposing significant economic losses and animal welfare concerns. In Ireland, the commercial pig sector is the third largest livestock industry, which mainly consists of integrated farrow-to-finish production systems, comprising 1,372 active herds with approximately 140,000 sows and an annual output of 4 million pigs. Despite the significant contribution of the pig industry to the Irish economy, there has been no comprehensive report of *S. suis* associated disease in pigs in Ireland. In this study, we present the prevalence and serotype distribution of *S. suis* isolates recovered from post-mortem examinations from 2005 to 2024. Furthermore, we used whole genome analysis to describe the population structure of *S. suis* circulating on Irish farms and contextualised them in the global *S. suis* landscape. In parallel, we investigated the interplay between prophages and anti-viral defence systems to contextualise the evolutionary dynamics of the *S. suis* population. The interactions between prophages and defence systems highlight an active phage-host interface that drives genome plasticity and may shape ecological success and community dynamics of *S. suis* within the swine microbiota. Exploring these dynamics is crucial for understanding pathogen evolution and carries a broader significance from a One Health perspective, given the growing interest in phage-based controls for foodborne and zoonotic pathogens. Our report represents the first population-level genomic investigation of *S. suis* in Ireland. This baseline data will inform future surveillance programmes and guide evidence evidence-based approaches to *S. suis* control in Irish pig production systems.

Sequence type distribution among clinically relevant serotypes of *Streptococcus suis* recovered from diseased pigs in Quebec (Canada)

Pauline Treichel[1]*; Sonia Lacouture[1]; Nahuel Fittipaldi[1]; Marcelo Gottschalk[1]

[1] University of Montreal, Canada.

Streptococcus suis is a Gram-positive bacterium known for its pathogenicity in pigs and its zoonotic potential. Among the 29 described serotypes, some are part of the normal porcine flora, whereas others are associated with severe infections such as meningitis, septicemia, and arthritis—conditions that result in significant economic losses in the swine industry. In Canada, serotypes 1, 1/2, 2, 9, and 14 are among the most frequently isolated from clinical cases. This study aimed to characterize recent shifts in the distribution of sequence types (STs) within these serotypes among isolates recovered in Quebec, Canada, between 2020 and 2024. A total of 199 isolates were analyzed using the standard multilocus sequence typing (MLST) scheme for *S. suis*, targeting the aroA, gki, dpr, recA, mutS, thrA, and cpn60 genes. The predominant STs identified were ST28, ST620, ST25, ST94, and ST789. All ST1 isolates—associated with highly virulent strains—were confined to serotypes 1 and 14, except for a single isolate belonging to serotype 1/2. To our knowledge, this represents the first report of a *S. suis* serotype 1/2 ST1 isolate in North America. Additionally, in contrast to previous findings, we observed the emergence of ST126 in serotype 1 and ST156 in serotype 14. Nine serotype 14 isolates were assigned to a novel ST within clonal complex 1338. A second previously undescribed ST was identified among serotype 9 isolates (n = 10). For serotype 2, the main STs were ST25 and ST28; notably, no ST1 isolates—commonly found among serotype 2 strains in Europe and Asia—were detected. These findings provide new insights into the evolving population structure of *S. suis* in North America and may guide future surveillance efforts and vaccine development strategies.

Isolates from diseased cattle suggest the presence of potential novel *Streptococcus ruminantium* serotypes

Natálie Králová[1,2]*; Ján Matiašovic[1]; Monika Zouharová[1]

[1] Veterinary Research Institute, Czech Republic; [2] Masaryk University, Czech Republic.

Introduction: *Streptococcus ruminantium*, previously classified as *Streptococcus suis* serotype 33, is frequently isolated from ruminants, particularly cattle. Due to historical misclassification, *S. ruminantium* isolates may have been inaccurately identified as *S. suis*, which complicates our understanding of their epidemiology and pathogenic potential. In this study, we identified ten *S. ruminantium* isolates from diseased or dead cattle. Notably, while some isolates corresponded to the classical serotype 33, seven others harbored distinct capsular polysaccharide (cps) loci. **Methods**: Bacterial isolates were collected from diseased or dead cattle, each isolate from a different farm. Seven isolates were from calves, and all but one isolate from a rectal swab were cultured from lung or bronchoalveolar lavage. The other two isolates were also cultured from lungs, but from one bull and one heifer. The last isolate was from mastitic milk. The isolates were initially identified by MALDI-TOF as *S. suis* isolates. Whole genome sequences revealed their close relationship to *Streptococcus suis* serotype 33, which is now classified as *Streptococcus ruminantium*. Clusters of *cps* genes were extracted and analyzed to investigate potential serotype diversity. The *cps* cluster of the five isolates was similar to the *cps* cluster of serotype 33. For the other isolates, the *cps* cluster was similar to each other but significantly different from the *cps* cluster of serotype 33, suggesting a potential new serotype of S. ruminantium. **Results and discussion**: Analysis of the *cps* gene clusters revealed potentially novel capsular type among *S. ruminantium* strains. This finding suggest a broader *cps* diversity than previously recognized, indicating the presence of additional serotypes. Detailed classification based on *cps* loci may thus contribute to a better understanding of the pathogenicity and zoonotic potential of *S. ruminantium*.

This work was supported by grants RO0523 and TN02000017.

Streptococcus suis-like bovine isolates related to *Streptococcus parasuis*

Monika Zouharová[1]*; Ján Matiašovic[1]; Natálie Králová[1]

[1] Veterinary Research Institute, Czech Republic.

Introduction: Over the past decades, substantial diversity has been revealed among bacteria previously identified as *Streptococcus suis*. Distinct *Streptococcus* species have emerged both among the original 35 *S. suis* serotypes and among strains with newly identified capsular loci. The host specificity of some of these strains is not limited to pigs, as they are frequently isolated from other species, both healthy and diseased. Here, we present an analysis of five bovine isolates collected between 2018 and 2020, originally identified as *S. suis*. **Materials and Methods**: The isolates were obtained from four mastitic milk samples and one lung sample from a ten-day-old calf suffering from bronchopneumonia. Each isolate originated from a different farm. All isolates were identified as *S. suis* using MALDI-TOF MS. Genomic DNA was extracted and subjected to Illumina whole genome sequencing. Genomes were annotated using Prokka. Subsequently, a phylogenetic tree based on core genome alignment was constructed. **Results and discussion**: Four of the isolates tested negative for the recN gene. One isolate consistently tested positive for recN by PCR, although the gene was not detected in the genome sequence data. Analysis of the cps locus revealed that each isolate harbored a unique composition of cps genes. Based on core genome alignment, all bovine isolates formed a distinct cluster closely related to S. parasuis serotypes 20, 22, and 26. These isolates may therefore represent a novel species within the *S. suis* complex. The identification and characterization of these isolates may open the way for improved discrimination of potential bovine pathogens.

This work was supported by grants RO0523 and TN02000017.

Genetic Diversity and Characterization of the Type VII Secretion System of *Streptococcus suis*

Shengrui Shi[1]; **Xiang-Dang Du**[1]*

[1] Henan Agricultural University, China.

Background and objectives: *Streptococcus suis* (*S. suis*) is an zoonotic pathogen that can cause severe diseases in pigs and humans. Recent years, the type VII secretion system (T7SS) has been widely observed in Gram-positive bacteria, which can export contact-dependent toxins to enhance the virulence and colonization capabilities of bacteria. In this study, we analyzed the genetic diversity of T7SS-encoding substrate proteins in *S. suis* and validated their functions, illustrating the role of T7SS in bacterial antagonism in *S. suis*. **Methods**: A comprehensive bioinformatics analysis was conducted on 7,777 bacterial strains collected from the NCBI RefSeq database, alongside 400 *S. suis* strains preserved in the laboratory. The T7SS locus was classified based on the diversity C-terminal sequence of *essC* gene. The effector protein domains were identified by Motif and InterProScan. The deletion and complementation mutants were constructed by natural transformation. Toxin function was validated through mouse infection model and overexpression assays. **Results**: A total of 1,247 T7SS-positive strains were identified, accounting for 16.03% (1247/7777). The T7SS locus was classified into four subtypes: *essC1*-4. The boundary of the T7SS locus was further defined, with *purR* (Pur operon repressor) at the 5' end and rluC (ribosomal large subunit pseudouridine synthase C) at the 3' end. A novel toxin, TsnA (T7SS-secreted NAD family protein A) containing the tuberculosis necrotizing toxin (TNT) domain was identified. Upon overexpression in E. coli, TsnA reduced intracellular NAD+ levels resulting in growth inhibition. Additionally, TsnA promoted the colonization of *S. suis* in the mouse infection model. **Conclusions**: This study classified T7SS locus in *S. suis* into four subtypes. We further identified a novel toxin TsnA in essC2 and confirmed its functionality, which will deepen our understanding of the T7SS effector proteins and their contributions to the bacterial antagonism.

Preliminary characterization of novel species related to *Streptococcus suis*

Daphné Cartier[1]*; Kevin Li[1]; Fengyang Hsu[1]; Sonia Lacouture[1]; Antony T. Vincent[2]; Marcelo Gottschalk[1]; Nahuel Fittipaldi[1]

[1] Groupe de recherche sur les maladies infectieuses en production animale et Centre de recherche en infectiologie porcine et avicole, Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Canada; [2] Institut de Biologie Intégrative et des Systèmes (IBIS), Département des Sciences Animales, Faculté des Sciences de l'agriculture et de l'alimentation, Université Laval, Canada.

Accurate species identification is crucial in diagnostic clinical microbiology. Yet, some isolates recovered from diseased swine and identified as *Streptococcus suis* by MALDI-TOF MS test negative in the *recN*-targeted PCR, raising concerns about misidentification, as this molecular assay is used for species confirmation. These observations, together with recent data suggesting that *S. suis* is part of a broader complex comprising several closely related species, including at least 10 not yet formally named, prompted us to begin the taxonomic characterization of selected isolates representing this diversity. Results from average nucleotide identity, average amino acid identity, digital DNA–DNA hybridization, as well as phylogenetic analyses based on core genome, 16S rRNA, and the *recN* gene, all supported the proposal of the 10 novel species, and revealed additional unrecognized diversity. While major phenotypic traits such as Gram staining, hemolysis, and motility did not differ from those observed in *S. suis*, extended biochemical testing using Rapid ID 32 Strep kits revealed minor but consistent differences between isolates, further supporting their distinction as novel taxa. Our findings expand the known diversity of the *S. suis* complex and provide a foundation for future taxonomic clarification and diagnostic refinement. Further characterization of these novel species are ongoing.

Insights in the virulence and prevalence of invasive *S. suis* serotype 9 in Dutch weaner pigs

Anita Dame-Korevaar[1], Jaime Brizuela Gabaldon[2], Helmi Fijten[1], Ruth Bossers-de Vries[1], Lisette Ruuls[1], Hilde E. Smith[1], Bart Engelen[3], Erik van Engelen[4], Marlies Olde Monnikhof[4], Eveline Willems[4,8], Chretien Gielen[5], Nico Wertenbroek[6], Ton Jacobs[6], Ries Grommen[6], Lluís Fabà[7], Norbert Stockhofe-Zurwieden[1], Astrid de Greeff[1], Manon Houben[4], Constance Schultsz[2], **Manouk Vrieling[1]***

[1] Wageningen Bioveterinary Research, The Netherlands; [2] Amsterdam UMC, The Netherlands; [3] Dopharma, The Netherlands; [4] Royal GD, The Netherlands; [5] De Varkenspraktijk, The Netherlands; [6] MSD Animal Health, The Netherlands; [7] Trouw Nutrition R&D, The Netherlands; [8] Topigs Norsvin, The Netherlands.

Background: *Streptococcus suis* serotype 9 (SS9) is the major cause of *S. suis* infections in piglets in the Netherlands. Within SS9 there are genetically distinct subpopulations associated with carriage or with invasive disease. In this study, we aimed to 1) establish a Real Time qPCR-test to determine the prevalence of invasive SS9 in Dutch weaner pigs and 2) characterize the differences in virulence between carriage and invasive SS9. **Methods**: We reconstructed a global SS9 phylogeny based on 188 publicly available genomes and compared the capsule (CPS) biosynthesis locus of invasive and carriage populations. Allelic variation in the cps9k gene was used to design a qPCR-test that would detect invasive but not carriage SS9. Tonsil samples were taken from Dutch weaner pigs on 20 farms (n=100/farm, age 5-8 weeks) in a cross-sectional field study to determine the prevalence of total and invasive SS9 by qPCR 2) Invasive and carriage SS9 isolates were assessed for differences in survival in an opsonophagocytosis and killing assay with porcine neutrophils. **Results**: SS9 was present at all farms at high prevalence (range 61-100%, median 96%). Invasive SS9 was found in 18/20 farms and the on-farm prevalence ranged between 0 and 79% (median 27%) with a high variability between batches of animals. 2) SS9 carriage strains were readily killed by porcine neutrophils, while invasive isolates were resistant to killing and required opsonisation with SS9-specific antibodies to be killed. **Conclusion**: The high variation in prevalence of invasive SS9 between farms suggests there is room for on-farm interventions to control carriage. However, the link between carriage of invasive SS9 on the tonsil and development of clinical *S. suis* disease needs further investigation. Invasive SS9 isolates differ from carriage isolates in their CPS genotype and resistance to opsonophagocytic killing, indicating that capsule may play a role in the virulence of invasive SS9.

Serotype, virulence markers and sequence type diversity of *Streptococcus suis* detected in clinical samples and isolates from Spanish pig farms (2018–2023)

Jose-Luis Arnal Bernal[1]*; Mireya Melero Pérez[1]; Desiree Martín Jurado[1]; Silvia Del Caso Yagüe[1]; Ana Belen Fernández Ros[1]

[1] Exopol, Spain

This study evaluated the epidemiology of *Streptococcus suis* in Spain between 2018 and 2023 through the analysis of 1,314 pooled clinical samples and 519 bacterial isolates from diseased pigs exhibiting signs consistent with *S. suis* infection. Real-time PCR (qPCR) assays enabled serotyping both directly from clinical samples and from bacterial isolates. Clinical samples were tested for *S. suis* and its 10 most relevant serotypes (1, ½, 2, 3, 4, 5, 7, 8, 9, and 14) by qPCR, with 55% testing positive. The most frequent serotypes were 9 (35%), 1 (18%), and 7 (16%), followed by ½ and 2 (each 9.5%). In 88% of *S. suis*-positive cases, serotyping was achieved directly from clinical material without prior isolation, demonstrating the speed, sensitivity, and specificity of this molecular tool for the diagnosis and surveillance of *S. suis* in swine. Additionally, 519 isolates were analyzed by qPCR to determine serotype and the presence of three virulence markers (epf, mrp, and sly). Serotype 9 was the most frequent, followed by 1, 7, 2, and ½. A strong correlation was observed between serotype and virulence profile, with dominant patterns identified for each major serotype. Notably, serotype distribution in isolates closely mirrored that observed in clinical samples, further supporting the value of direct qPCR-based detection as a reliable diagnostic approach. Genetic diversity was further assessed in a selected subset of 95 isolates using MLST. A moderate level of sequence type (ST) diversity was found. Serotypes 2 and 7 showed high genetic homogeneity, with 95% and 100% of isolates classified as ST1 and ST29, respectively. Serotype 1 exhibited more variability, with eight different STs, although 69% belonged to ST1. Serotype ½ was the most heterogeneous, with four STs identified, including ST1, ST3, and ST28. Five novel STs, not previously reported, were also described.

Genomic and phenotypic profiling of predominant *Streptococcus suis* lineages in Spain (ST1 and ST123) reveals differences in virulence, and antimicrobial resistance and tolerance

Cristina Uruén[1]*, Clara Marín[2], Marcelo Gottschalk[3], Luis González-Vázquez[4], Miguel Arenas[4], Jesús Arenas[1]

[1] University of Zaragoza, Spain; [2] Centro de Investigación Tecnológico y Agroalimentario (CITA), Spain; [3] University of Montreal, Canada; [4] University of Vigo, Spain.

Spain, the leading pig producer in Europe, faces significant challenges from *S. suis* infections. Our recent studies identified lineages ST1 and ST123 as the most prevalent among clinical isolates collected from different Spanish regions. ST1 is a globally distributed hypervirulent lineage, but ST123 emerged in Spain a decade ago. The aim of this study was to compare the virulence and resistance to antibiotic treatments of both lineages. We analysed the genome of 22 invasive isolates, 13 from ST1 and 9 from ST123. ST123 exhibited higher genetic variability (0.16%) than ST1 (0.13%). Pangenome analysis identified a shared core of ~1400 genes and ~140 lineage-specific genes for each ST. These genes belonged to different functional categories. Notably, ST123-specific genes were enriched in regulation/metabolism, whereas ST1-specific genes were enriched in virulence-associated functions. Using an intranasal mouse model of infection with 5 representative isolates from each lineage, both lineages disseminated from nostrils to internal organs by 3 days post-infection (dpi), albeit with some variability. By 7 dpi, ST123 isolates persisted more frequently in nostrils and heart tissues, while ST1 isolates were more abundantly recovered from joint and spleen tissues. *In vitro*, ST1 isolates showed ~ threefold greater adhesion, sixfold higher intracellular survival, and increased oxidative stress tolerance compared to ST123. In contrast, ST123 isolates formed approximately twofold more biofilm, which displayed a fivefold increase in ampicillin tolerance. Moreover, adaptive resistance assays revealed that β-lactam-sensitive ST123 isolates could acquire resistance to ampicillin, whereas ST1 isolates did not develop resistance under the same conditions. These findings point out that ST1 and ST123 employ distinct evolutionary strategies to adapt to host environments, evade immune responses, and respond to antibiotic pressure suggesting the need for lineage-specific control strategies.

Evaluation of polymer and emulsion adjuvants for *Streptococcus suis* vaccination

Sarah Kharief[1]*; Juliette Ben Arous[1]; Nicolas Versillé[1]; Zhao Zhanqin[2]

[1] Seppic _ Air Liquide , France; [2] Henan Science and Technology University, China.

Worldwide distributed, *Streptococcus suis* is causing considerable losses in the porcine industry. Inactivated vaccines remain the best strategy against the disease but their effectiveness is controversial and potent adjuvants are needed. In this study, MONTANIDE™ Gel 02 and MONTANIDE™ ISA 201 VG adjuvants formulated with a trivalent (serotype1 strain Z1, serotype2 strain Z2, serotype7 strain S7) inactivated *S. suis* antigen were assessed in pig trials. The *S. suis* vaccine is formulated with Gel02 (polymer) or ISA201 (water-in-oil-in-water (W/O/W) emulsion) and compared to AlOH and homemade water-in-oil (W/O) adjuvants. The safety is evaluated by vaccinating pigs intramuscularly with 4 ml of the vaccines, 5 pigs per group. Body temperature and local reactions at slaughter 14 days after vaccination were collected and scored. Efficacy was assessed by vaccinating pigs with 2 ml, twice 3 weeks apart. Antibody titers from blood samples collected at D0, D21 and D35 were monitored by ELISA. Two weeks after the boost, a challenge was performed by injecting one lethal dose of *S. suis* (serotype1 Z1 or serotype2 Z2). Clinical signs, mortality rate and specific organ lesions after necropsy are observed. Regarding safety, a weak increase of body temperature (~1°C at 24 hours) was observed in all groups except in the AlOH group. The local reactions score showed that W/O/W provided an improved safety profile compared to W/O and that the polymer adjuvant demonstrated an excellent safety profile comparable to AlOH. In terms of efficacy, Polymer provided highest efficacy among all adjuvants tested with 100% protection against both challenged *S. suis* serotypes; all pigs of the group survived. Results showed that polymer adjuvant is suited for formulating highly efficacious *S. suis* inactivated vaccines, providing high protection and balanced efficacy/safety profile, constituting a good alternative to AlOH.

Neonatal piglets can develop a protective immune response after vaccination with a *Streptococcus suis* bacterin but not with subunit-adjuvanted vaccines

Sergi López-Serrano[1], Jerry M. Wells[2], Dennis Christensen[3], Manouk Vrieling[4], Thomas Ebensen[5], Joaquim Segalés[1], Virginia Aragón[1], Norbert Stockhofe-Zurwieden[4], **Sandra Vreman[4]***

[1] Unitat mixta d'Investigació IRTA-UAB, Spain; [2] Wageningen University Research, the Netherlands; [3] Statens Serum Institute, Denmark; [4] Wageningen Bioveterinary Research, the Netherlands; [5] Helmholtz Centre for Infection Research, Germany.

Background: Vaccination shortly after birth could be essential to protect weaning pigs against invasive *Streptococcus suis* (*S. suis*) disease. In this period *S. suis* infections cause high morbidity in pigs, reduce animal welfare and contribute substantially to the use of antibiotics. In this study, we investigated if neonatal piglets could develop a protective immune response after vaccination in first week of life using adjuvanted-bacterin and subunit vaccines. **Methods:** Five groups of 5-day-old piglets were intramuscularly vaccinated with: 1; a subunit vaccine with conserved *S. suis* type 2 immunogens MRP2 and C05 adjuvanted with CAF®01 (n=8) or 2; CDA (n=8), 3; a non-adjuvanted group (only MRP2/C05, n=5), 4; a *S. suis* bacterin adjuvanted with Specol® (n=8) and 5; a non-vaccinated control group (n=4). Piglets received their booster immunization 4-weeks later. Three weeks after the booster, piglets were intranasally challenged with *S. suis* serotype 2 (ST1-10^9 CFU) and left for clinical observation up to 8 days after challenge or until they reached a humane endpoint (HEP). Blood samples and tonsillar swabs were collected throughout the study to assess humoral and cell-mediated immune responses and bacterial burden. **Results:** The subunit vaccines adjuvanted with CDA or CAF®01 elicited a weak immune response, either humoral or cell-mediated, with no protection after *S. suis* challenge. However, nearly all piglets immunized with the Specol®-adjuvanted bacterin were protected after the challenge, with an evident humoral response. **Conclusion:** This study highlights that protection against *S. suis* after neonatal vaccination could be achieved and that further research is needed to find the optimal neonatal subunit-vaccine formulation giving a broad protection against *S. suis*.

Influence of maternal antibodies on the immune response of young piglets vaccinated with a *Streptococcus suis* serotype 2 bacterin

Mariela Segura[1], Laetitia Cloutier[1], Marie-Claude Poulin[1], Annie Gaudreau[1], **Marcelo Gottschalk[1]***

[1] University of Montreal, Canada; 2 CDPQ, Canada.

Streptococcus suis is the most important bacterial pathogen affecting post-weaned piglets and is also a zoonotic agent. There is no commercial vaccine and the only alternative practitioners have is the use of autogenous vaccines (bacterins) based on the predominant strain(s) recovered in the affected farm. Depending on the farm, sows or young piglets are vaccinated; in the latter case, it is a common practice to vaccinate them at 1 and 3 weeks of age (WOA) to protect them during the nursery period (from 4 to 10 WOA). Although a potential interference with maternal antibodies (MA) has been suggested, it has not been proven yet. The objectives of the present study were: a) to compare the immune response of piglets vaccinated at 1 and 3 WOA (in the presence of high MA levels) vs those vaccinated at 3 and 5 WOA (lower MA levels); b) to use a newly developed model of colostrum-deprived conventional piglets (CDC) to evaluate the interference of MA with a bacterin-based vaccination of piglets at 1 and 3 WOA. Results showed a clear antibody response in piglets vaccinated at 3 and 5 weeks of age. On the other hand, piglets vaccinated at 1 and 3 weeks of age induced a stabilization of the decay of MA when compared to control animals. Indeed, the sum of MA and active response had as a consequence that levels of antibodies in piglets at 7 and 9 weeks of age were not statistically different between the two groups of vaccinated piglets. By using the CDC piglets, it was demonstrated that the presence of MA partially interferes with the active production of antibodies; however, as in the first study, the stabilization of the decay of MA seems to be as effective as an active immunization at 3 and 5 weeks of age.

A new generation glycoconjugate vaccine against *Streptococcus suis*

Mariela Segura*[1], Todd Lowary[2], Potjanee Srimanote[3], Mélanie Lehoux[1], Marcelo Gottschalk[1]

[1] University of Montreal, Canada; [2] University of Alberta, Canada; [3] Thammasat University, Thailand.

Streptococcus suis causes significant economic losses to the swine industry and raises concerns about animal welfare. It is also an emerging zoonotic pathogen, mainly in Asian countries. In the absence of effective commercial vaccines, disease control in pigs relies heavily on antimicrobial prophylaxis. *S. suis* is encapsulated by a capsular polysaccharide (CPS), its major virulence factor. Of the 29 serotypes described based on CPS antigenicity, serotype 2 is the most clinically prevalent in both pigs and humans. Studies have shown that CPS-specific antibodies can provide protection, making CPS an attractive vaccine antigen. However, its poor immunogenicity limits its use. Conjugating CPS to protein carriers enhances its immunogenic properties, as demonstrated by glycoconjugate vaccines in human medicine. We previously developed a CPS-tetanus toxoid conjugate that protected pigs against serotype 2 challenge. Yet, conventional production of glycoconjugates is complex and costly. Advances in chemical synthesis and formulation have led to a new generation of carbohydrate-based vaccines. In this study, we designed the first chemically synthesized glycoconjugate vaccine against *S. suis* and demonstrated its protective capacity. Eight CPS fragments (mono- to heptasaccharides) were synthesized with azido- or amino-octyl linkers for conjugation to CRM197, using maleimide–thiol chemistry. In mouse pre-trials, conjugates were selected based on high anti-CPS antibody titers, IgG subclass diversity, and opsonophagocytic activity. In pigs, antibody responses varied across conjugates, but only the heptasaccharide-based conjugate conferred strong clinical protection. Our results highlight the need to design optimal epitopes and validate their efficacy in pigs to identify effective vaccine candidates.

Field study on the role of maternal αIdeSsuis antibodies in the control of *Streptococcus suis* serotype 2 bacteraemia in piglets

Jospeha Hallbauer[1,3]*; Theresa Middendorf[1,3]; Matthias Horn[2]; Silke Lehnert[1]; Alexander Maas[3]; Christoph G. Baums[1]

[1] Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, Leipzig University, An den Tierkliniken 29, 04103 Leipzig, Germany; [2] Institute for Medical Informatics, Statistics and Epidemiology (IMISE), Faculty of Medicine, University of Leipzig, 04107 Leipzig, Germany; [3] Vivet Schweinegesundheit GmbH, Kahrweg 33, 59590 Geseke, Germany.

Independent research shows that vaccination with the Immunoglobulin M-degrading enzyme of *S. suis* (IdeSsuis) elicits protection against different serotypes. This field study investigates the role of maternal αrIdeSsuis IgG in killing *S. suis* cps 2 in piglets' blood of a herd with autogenous *S. suis* vaccination pre farrowing. Colostrum samples of 24 dams and blood samples of 72 of their piglets were collected. Survival of *S. suis* cps2 strain 10 (wt), 10ΔideSsuis and 10ΔIdeSsuis∇IdeSsuis_C195S expressing a non-functional IdeSsuis variant was analysed in bactericidal assays at week 2, 6 and 10. Specific αrIdeSsuis IgG and IgM binding to *S. suis* wt were determined in colostrum and piglets' blood. Linear mixed-effect models were run to determine factors influencing bacterial survival. Colostral αrIdeSsuis IgG levels correlated positively with those in sera of 2-week-old piglets, but decreased thereafter. At week 2 αS.suis10 IgM were close to zero and increased to week 10. Bactericidal immunity increased from week 2 to 10, regardless of the strain. The mutant ΔideSsuis showed significantly higher survival factors in 2-week-old piglets. Specific αrIdeSsuis IgG and αS.suis 10 IgM affected time-independent and time-dependent, respectively, the survival of wt and ∇ideSsuis_C195S. αrIdeSsuis IgG levels had no effect on survival of ΔideSsuis. Dams might pass specific αrIdeSsuis IgG to their piglets via colostrum. This study supports the idea that maternal αrIdeSsuis IgG play already a protective role against *S. suis* bacteraemia in the field, despite lower levels compared induced through rIdeSsuis vaccination. In week 2, expression of functional/non-functional IdeSsuis might lead to increased killing of *S. suis*, likely due to improved opsonophagocytosis mediated by αrIdeSsuis IgG rather than by neutralisation of the IgM protease activity. Accordingly, vaccination of dams pre farrowing with rIdeSsuis is a promising prophylactic approach.

Metabolic flux analysis revealed key roles of ArcB in ADI pathway and IlvC in BCAA biosynthesis during *Streptococcus suis* anaerobic growth and infection

Hao Tang[1,2]; Jiaojiao Yang[2]; Qiuhong Zhang[2]; Weiyao Han[2]; Yao Ruan[1]; Rui Zhou[2]; **Lu Li[2]***

[1] Hubei Key Laboratory of Agricultural Bioinformatics, College of Informatics, Huazhong Agricultural University, Wuhan 430070, China; [2] National Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, China.

Introduction: Since pathogens are known to reprogram metabolism to adapt to host microenvironments [1], elucidating metabolic responses under stress conditions may help to identify essential enzymes as drug targets. In this study, transcriptome-constrained genome-scale metabolic modeling (GSMM) and flux analysis were used to reveal the importance of arginine degradation (ADI) and branched-chain amino acid (BCAA) biosynthesis in anaerobic growth of *Streptococcus suis* SC19. ArcB and IlvC in these two pathways, respectively, were identified as key enzymes. **Methods**: The growth ability of SC19 was compared under aerobic and anaerobic conditions, followed by RNA-seq. Transcriptome data were used to constrain GSMMs for flux balance analysis (FBA). The gene arcB in the ADI pathway and ilvC in BCAA pathway, were deleted and complemented, respectively. Growth and metabolite levels were assessed under aerobic and anaerobic conditions. Mouse infection models were used to evaluate virulence. **Results and Discussion**: The anaerobic growth of SC19 was enhanced compared with that under aerobic condition, though the expressions of the genes involved in central metabolism were decreased. FBA showed activation of the ADI and BCAA pathways. The delta arcB mutant exhibited reduced anaerobic growth with lower ATP and NH3 levels. The delta ilvC mutant showed growth defect under valine/isoleucine limitation. In mice, both mutants showed reduced virulence, with lower mortality, bacterial loads, cytokine productions, and increased expression of the gene encoding hypoxia-inducible factor HIF1A in tissues. ArcB and IlvC are thus essential for anaerobic survival and pathogenesis of *S. suis* SC19.
References: [1] Fang F.C, *et al*., Cell Host Microbe. (2016) 20:133-143, doi: 10.1016/j.chom.2016.07.009.

Complement evasion mediated by AdcA, ApuA, HylS' and SSP5 in *Streptococcus suis*

Jiajia Xu[1]; Zhulin Qiao[1]; Siqi Pang[1]; Wenwen Song[1]; Rui Zhou[1]; **Lu Li**[1]*

[1] National Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, China.

Introduction: Bacteria have evolved multiple strategies to evade complement attack [1]. This study used bacterial two-hybrid assay and co-immunoprecipitation in the blood to identify four *Streptococcus suis* proteins that playing roles in complement evasion, the factor H (FH)-binding protein AdcA, the salivary agglutinin (SAG)-binding protein SSP5, and the C3b-binding proteins ApuA and HylS'. **Methods:** Wild-type SC19, mutants (*adcA*, *apuA*, *hylS'*, or *ssp5* gene deficient strain), and their complements strains were constructed. Protein interactions (ApuA/HylS' with C3b, AdcA with FH, SSP5 with SAG) were tested by two-hybrid assay, co-immunoprecipitation or far-western blots. C3b and MAC deposition on *S. suis* were analyzed by flow cytometry. Complement activation assays were used to evaluate the effects of ApuA and SSP5. Mouse model was used to assess bacterial virulence. **Results:** SSP5, binding to SAG's SRCR1 domain, enhanced adhesion and inhibited complement activation. AdcA interacted with FH. ApuA activated complement via both classical and alternative pathways. ApuA and HylS' interacted with C3b. All of these proteins could reduce C3b deposition and MAC formation on bacteria and promoted virulence of *S. suis*. **Conclusion and Discussion**: SSP5 enhances bacterial adhesion to epithelial cells by binding to SAG's SRCR domain, while simultaneously inhibiting SAG-mediated complement activation, promoting colonization and dissemination. After invasion, in the bloodstream, ApuA activates complements, generating C3b and C3a. But, ApuA and HylS' then bind with C3b, preventing complement deposition and MAC formation on *S. suis*. AdcA and SSP5 recruit FH and SAG to the bacterial surface, suppressing complement activation and reducing C3b and C3a production, protecting the bacteria from lysis and inflammation. Thus, these complement evasion strategies promote *S. suis* survival and pathogenicity. **References**: 1. Ricklin *et al*. Immunological Reviews. (2016) 274: 33–58.

Collateral sensitivity to gamithromycin in ciprofloxacin-resistant *Streptococcus suis* is driven by increasing intracellular antibiotic accumulation

Yu-Feng Zhou[1]*; Yue-Jun Zhang[1]; **Xiao-Ping Liao**[1]*

[1] South China Agricultural University, China.

Streptococcus suis has garnered increasing attention due to its implication in severe infections in both swine and humans, as well as its development of multidrug resistance. The phenomenon of collateral sensitivity, whereby resistance to one antibiotic leads to increased sensitivity to another, provides new opportunities for mitigating the evolution of resistance. In this study, we evolved resistance in *S. suis* to 11 clinically used antibiotics and characterized the resulting collateral sensitivity profiles, revealing a complex network of interactions. Based on our findings, we identified dozens of such drug pairs and demonstrated collateral sensitivity to gamithromycin in ciprofloxacin-resistant *S. suis* both *in vitro* and in vivo. Gamithromycin effectively limits the evolution of resistance and reduces the mutant selection window for ciprofloxacin-resistant *S. suis* strains. Mechanistic studies indicated that the heightened sensitivity of ciprofloxacin-resistant *S. suis* to gamithromycin was associated with increased intracellular gamithromycin accumulation due to membrane potential alterations and reduced functions of proton motive force (PMF)-dependent efflux pumps. Furthermore, collateral sensitivity-based treatments significantly resensitized ciprofloxacin-resistant *S. suis* strains to gamithromycin, resulting in superior efficacy, lower pharmacodynamic targets, and higher treatment success rates in a murine thigh infection model. Our results indicate that gamithromycin sensitivity in *S. suis* is a collateral consequence of resistance to ciprofloxacin, providing valuable insight for the strategic design of collateral sensitivity-based antibiotic therapies for *S. suis* infections.

Antibiotics sensitivity profiles of *Streptococcus suis* strains isolated between 2021 and 2024 in North-East of Italy from diagnostic samples

Denis Vio[1]*; Donatella Tagliente[1]; Amalia Ghiriti; Marianna Merenda[1]; Paola Fiorini[1]; Manuel Garbuio[1]; Martina Giacomelli[1]; Martina Ustulin[1], Federico Martignago[1], Marco Simioni[1]

[1] Istituto Zooprofilattico Sperimentale delle Venezie, Italy.

Introduction: This study is a retrospective evaluation of *in vitro* antimicrobial susceptibility of *S. suis* isolates from North-East of Italy in years 2021-2024 from swine diagnostic samples. **Materials and Methods:** *S. suis* isolates analyzed originates from diagnostic submissions to the laboratories of IZSVe. Isolates were subjected to MIC determination with broth microdilution method according to CLSI. MIC plates included: amoxicillin/clavulanic acid (AUG 0,25/0,12-16/8 µg/mL), enrofloxacin (ENR 0,25–4 µg/mL), cefazolin (FAZ 0,25–8 µg/mL), ceftiofur (XNL 0,25–8 µg/mL), eritromicin (ERY 0,03–8 µg/mL), florfenicol (FFN 2–8 µg/mL), tetraciclin (TET 0,25–16 µg/mL), trimethoprim/sulfadiazine (TBR 0,12/2,38-8/152 µg/mL), rifampicin (RIF 0,06–2 µg/mL), clindamicin (CLI 0,5–2 µg/mL), ampicillin (AMP 0,03–16 µg/mL), penicillin (PEN 0,03–16 µg/mL). Isolates were categorized as susceptible, intermediate and resistant according to CLSI or EUCAST veterinary breakpoints. **Results:** The evaluation of the percentage of sensitive isolates from brain (200) and lung (81), resulted respectively: 90% and 81,5% for AUG, 86,5% and 81,5% for ENR, 96% and 88,9% for FAZ, 98% and 87,7% for XNL, 27 and 28,4% for ERY, 89% and 87,7% for FFN, 1,5% and 2,5% for TET, 61% and 75,3% for TBR, 83% and 82,7% for RIF, 20,5% and 16% for CLI, 95% and 88,9% for AMP, 68% and 60,5% for PEN. **Discussion :** Schrerr *et al*. (2024), Van Hout *et al*. (2016), Werinder *et al*. (2020) reported a low percentage of susceptible to TET respectively in Netherlands, Sweden, and Swiss; this study confirms previous findings. In accordance to previous literature, incomplete cross-resistance between AMP and PEN have been evidenced. For AUG, ENR, FAZ, XNL, TBR, CLI, AMP, PEN, strains isolated from brain showed higher percentage of sensibility. Airways derived strains are more likely to be commensal and, according to a recent report, antimicrobial resistance genes may be more common in non-pathogenic isolates (Rupasinghe *et al*., 2025).

Emergence of zoonotic and multi-drug resistant *Streptococcus suis*

Jaime Brizuela[1,2*], Gemma G. R. Murray[3], Parichart Boueroy[4], Andrew J. Balmer[5,6] , Thidathip Wongsurawat[7], Piroon Jenjaroenpun[7], Peechanika Chopjitt[4], Rujirat Hatrongjit[8], Lucy Weinert[6], Anusak Kerdsin[4,$], Constance Schultsz[1,2,$]

[1] Department of Global Health, Amsterdam Institute for Global Health and Development, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands; [2] Department of Medical Microbiology and Infection Prevention, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands; [3] Department of Genetics, Evolution and Environment, University College London, London, United Kingdom; [4] Faculty of Public Health, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon 47000, Thailand; [5] Genomic Surveillance Unit, Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; [6] Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom; [7] Division of Bioinformatics and Data Management for Research, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [8] Faculty of Science and Engineering, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon 47000, Thailand; [$] Equal contributions.

Streptococcus suis is a global emerging zoonotic porcine pathogen that causes sepsis and meningitis. In Thailand, *S. suis* has become one of the leading causes of bacterial meningitis in adults due to the consumption of traditional raw pork products. While the majority of reported zoonotic infections worldwide are caused by strains from lineage CC1 carrying a serotype 2 capsule, zoonotic infections in Thailand are caused by an unusually diverse set of lineages with ~40% of reported infections caused by two Thai endemic lineages, CC104 and CC233. In this study, we aimed to identify the drivers of the emergence and recent evolution of these two lineages. We sequenced the whole genomes of 141 Thai *S. suis* zoonotic and porcine isolates and integrated them with a curated dataset of 2761 published *S. suis* genomes. Using comparative genomics, Bayesian evolutionary analysis, and multivariate approaches, we investigated the emergence of multi-drug resistance and zoonotic potential in CC104 and CC233. CC104 and CC233 emerged recently, around 1990 (95% posterior: 1987-1992) and 2002 (95% posterior: 2000-2004) respectively. Both lineages acquired a serotype 2 capsule locus from CC1 isolates prior to their emergence. Multiple independent acquisitions of antimicrobial resistance were detected in both lineages, with some isolates harbouring up to 12 resistance genes targeting eight different antibiotic classes. Most importantly, both lineages acquired increased resistance to penicillin and ceftriaxone, which form the standard therapy to treat *S. suis* infections in humans. Horizontal acquisition of multiple genomic elements can facilitate the rapid emergence of novel multidrug-resistant zoonotic *S. suis* lineages. Given that *S. suis* infections in both pigs and humans are primarily controlled and treated through the use of antibiotics, these findings highlight the urgent need for improved and enhanced surveillance, infection control, and treatments.

Comprehensive resistome analysis reveals the emergence and spread of the oxazolidinone resistance gene *optrA* in zoonotic *Streptococcus suis*

Xingyang Dai[1]*; Junjie Sun; Xiao Liu[1]; Li Chen[1]; Xiaoming Wang[1]; Jinhu Huang[1]; Zongfu Wu[1]; Liping Wang[1]

[1] Nanjing Agricultural University, China.

The emergence of optrA gene-mediated resistance to oxazolidinones has rendered linezolid clinically ineffective for the treatment of multidrug-resistant Gram-positive bacterial infections. This study aims to characterize the distribution and spread of the *optrA* gene within the zoonotic pathogen *Streptococcus suis* in China. The *optrA* gene has been detected in *S. suis* isolates since 2009, with detection rates gradually increasing to the point of national prevalence. Acquisition of *optrA* in *S. suis* has resulted in a significant increase in the minimum inhibitory concentration distribution peaks for both linezolid and florfenicol, while amino acid substitutions in OptrA had a limited effect on resistance levels. Phylogenetic analysis revealed notable clustering among *S. suis* strains from different temporal and geographical contexts, especially those isolated from patients and healthy animals. The results suggest that the *optrA* gene in *S. suis* may originate from the species itself, form compound transposons with different insertion sequence elements, particularly IS1216E, and integrate into diverse mobile genetic elements (MGEs), be prevalent in *S. suis*, and spread to other species through horizontal gene transfer events. MGEs harboring the *optrA* gene, along with other antimicrobial resistance genes, are notably prevalent in *S. suis*. This study represents the first comprehensive analysis of the origin and spread of the *optrA* gene in *S. suis*, providing insight into the observed prevalence of this gene in the pathogen.

Hydrocortisone potentiates penicillin treatment by protecting the blood–brain barrier in *Streptococcus suis* meningitis

Xiao-Ping Liao[1]*; Jun-Qi Liu[1]; Yu-Feng Zhou[1]

[1] South China Agricultural University, China.

Streptococcus suis is a major zoonotic pathogen responsible for severe diseases such as meningitis, arthritis, and septicemia in both pigs and humans. Due to the high diversity of serotypes and antigenic variation, no effective broad-spectrum vaccine is currently available, making antibiotic therapy the mainstay for treating *S. suis* infection. However, central nervous system (CNS) infections remain particularly challenging to treat, in part due to *S. suis*-induced disruption of the blood-brain barrier (BBB) and the exacerbation of host-mediated inflammatory damage. This study aimed to evaluate the therapeutic potential of combining penicillin (PEN) with hydrocortisone for the treatment of *S. suis*-induced meningitis, with an emphasis on BBB protection and modulation of inflammatory responses. Using *in vitro* assays assessing hemolysis, bacterial adhesion and invasion, and BBB translocation, alongside a murine meningitis model, we evaluated the impact of this combination on BBB integrity, bacterial clearance, and inflammatory responses. Our results showed that PEN-hydrocortisone combination therapy significantly reduced *S. suis*-induced BBB disruption *in vitro* and inhibited bacterial translocation across endothelial monolayers. In vivo, the combined therapy lowered bacterial loads in brain tissues and improved histopathological scores compared to PEN monotherapy. Enhanced BBB integrity was further confirmed by reduced Evans Blue extravasation and preserved tight junction protein expression. Additionally, treatment suppressed the transcription and expression of pro-inflammatory cytokines (e.g., IL-6, TNF-α) and cell death-related genes, indicating effective mitigation of neuroinflammation and protection of CNS structures. These findings suggest that hydrocortisone enhances the efficacy of penicillin in *S. suis* meningitis by preserving BBB function and modulating host inflammatory responses, offering a promising adjunct strategy for managing streptococcal CNS infections.

Antibiotic resistance patterns and molecular characterization of *Streptococcus suis* isolates from swine and humans in China

Hao Ren[1]*; Changzhen Wang[1]; **Xiao-Ping Liao**[1]*

[1] South China Agricultural University, China.

Streptococcus suis is a zoonotic pathogen that causes disease in humans after exposure to infected pigs or pig-derived food products. In this study, we examined the serotype distribution, antimicrobial resistance phenotypes and genotypes, integrative and conjugative elements (ICEs), and associated genomic environments of *S. suis* isolates from humans and pigs in China from 2008 to 2019. We identified isolates of 13 serotypes, predominated by serotype 2 (40/96; 41.7%), serotype 3 (10/96; 10.4%), and serotype 1 (6/96; 6.3%). Whole-genome sequencing analysis revealed that these isolates possessed 36 different sequence types (STs), and ST242 and ST117 were the most prevalent. Phylogenetic analysis revealed possible animal and human clonal transmission, while antimicrobial susceptibility testing indicated high-level resistance to macrolides, tetracyclines, and aminoglycosides. These isolates carried 24 antibiotic resistance genes (ARGs) that conferred resistance to 7 antibiotic classes. The antibiotic resistance genotypes were directly correlated with the observed phenotypes. We also identified ICEs in 10 isolates, which were present in 4 different genetic environments and possessed differing ARG combinations. We also predicted and confirmed by PCR analysis the existence of a translocatable unit (TU) in which the oxazolidinone resistance gene *optrA* was flanked by IS1216E elements. One-half (5/10) of the ICE-carrying strains could be mobilized by conjugation. A comparison of the parental recipient with an ICE-carrying transconjugant in a mouse *in vivo* thigh infection model indicated that the ICE strain could not be eliminated with tetracycline treatment. *S. suis* therefore poses a significant challenge to global public health and requires continuous monitoring, especially for the presence of ICEs and associated ARGs that can be transferred via conjugation.

Evaluation of immunological efficacy and development of *Streptococcus suis* nanovaccines utilizing ZIF-8 and I53-50 delivery platforms

Huaixia Li[1]*; Qi Huang[1]; Rui Zhou[1]

[1] Huazhong Agricultural University, China.

Streptococcus suis seriously threatens public health security. There is a pressing need to develop cross-protective multivalent vaccines offering broad and long-lasting protection. Nanoparticles employed as antigen carriers can effectively boost antigen delivery efficiency and extend their duration of action in vivo,leading to significantly enhanced immune responses. This study specifically focused on two representative nanoplatforms: the I53-50 self-assembling nanoparticle derived from endogenous proteins and the chemically synthesized metal-organic framework ZIF-8. A novel nanovaccine delivery system was developed.SEM and DLS analyses indicated that both composite particles possessed regular morphology, uniform dispersion. Significant advantages were demonstrated by this MOF-based carrier vaccine in the murine challenge model. Without exogenous adjuvants, the ZIF-8 delivery system successfully induced a potent humoral immune response. The immunoprotection efficacy achieved was comparable to that of a commercial subunit vaccine. ZIF-8 may exhibit inherent immunoadjuvant activity. Concurrently, an I53-50 icosahedral carrier vaccine was developed. *S. suis* antigens HP1036/HP0197 were genetically fused to the I53-50 B subunit, expressed, and purified. Subsequent co-incubation with the A subunit enabled self-assembly, resulting in highly purified two-component nanoparticles, HP1036@I53-50 and HP0197@I53-50, isolated by size-exclusion chromatography. Murine model evaluation demonstrated dual advantages of the I53-50 nanovaccine in the absence of exogenous adjuvants. It induced markedly elevated antigen-specific antibody titers and concurrently activated antigen-specific T-cell responses. Effective blockade of pathogen colonization was confirmed. This study provides a new promising direction for the development of effective and long-lasting vaccines against *S. suis* and other major swine pathogens.

The conserved transcription factor PrlP modulates colonization and pathogenicity of *Streptococcus suis* in response to environmental stress

Wang Yuhua[1]*; Liu Ran[1]; Lv Weihua[1]; Xia Kunlong[1]; Lu Xi[1]; Gao Xinyu[1]; Miao Shulin[1]; Zhang Anding[1]

[1] National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China.

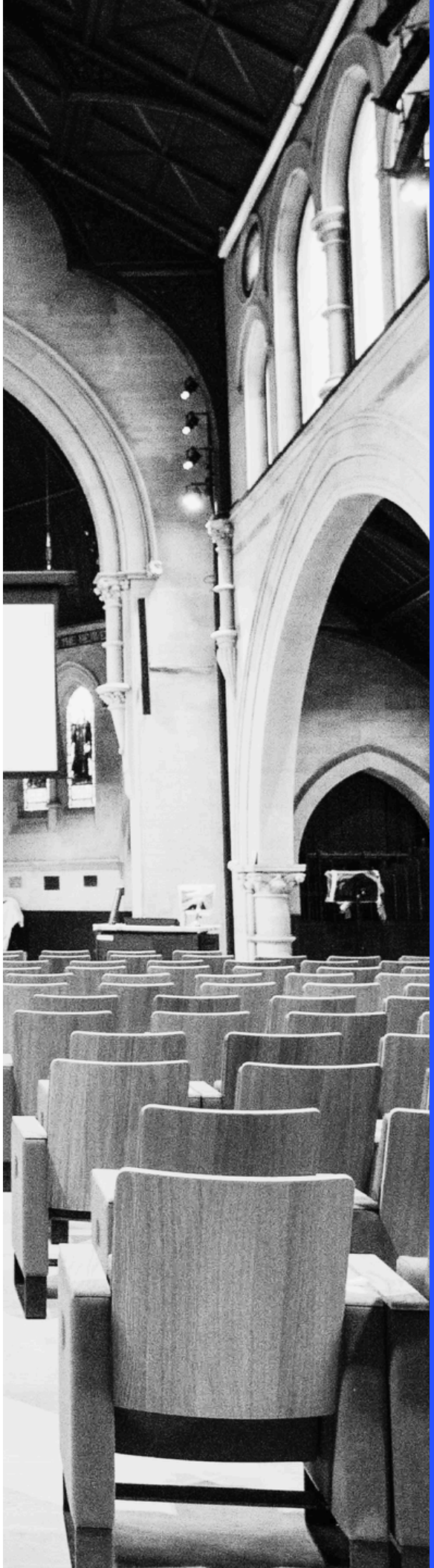
Opportunistic pathogens can cause infections when host defenses are compromised. Among them, *Streptococcus suis* (*S. suis*) colonizes the upper respiratory tract of pigs and causes severe diseases in both swine and humans. Although the pathogenic mechanisms of these bacteria have been partially elucidated, the molecular processes that govern their adaptation, colonization, and pathogenesis remain incompletely understood. In this study, we identified PrlP as a transcriptional repressor in *S. suis* that responds to mildly acidic, oxidative, hyperosmotic, and thermal stresses, and regulates bacterial growth, chain morphology, nasal colonization, and virulence. The C-terminal S24 peptidase domain of PrlP mediates stress-induced self-cleavage to control protein stability, while the N-terminal helix-turn-helix (HTH) DNA-binding domain is essential for its transcriptional regulatory function. Combined ChIP-seq and RNA-seq analyses revealed its binding motif (5'-CCTGAAWCT-3') and identified B9H01_08740 as a direct target gene, as further validated by EMSA. Notably, deletion of B9H01_08740 in the prlP-deficient background restored the associated phenotypes. These findings highlight PrlP as a key regulator in *S. suis* that maintains cellular homeostasis in response to stress conditions and modulates target genes such as B9H01_08740 to promote nasal colonization and virulence. Therefore, this study provides new insights into the regulatory mechanisms of pathogenic bacteria and may aid in the development of targeted strategies against *S. suis* infections.

Protection induced in pigs previously infected by the non-virulent strain 1330 of *Streptococcus suis* serotype 2 is not due to the secretion of the bacteriocin suicin

Samantha J Hau[1]*; Nahuel Fittipaldi[2]; Servane Payen[2]; Daniel Grenier[2]; Daniel W Nielsen[1]; Susan L Brockmeier[1]; Marcelo Gottschalk[2]

[1] National Animal Disease Center, USDA, Ames, IA; [2] Groupe de recherche sur les maladies infectieuses en production animale, Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada, Fonds de Recherche du Québec - Nature et Technologies (FQRNT), Saint-Hyacinthe, QC, Canada.

Streptococcus suis causes severe disease in swine and imposes a significant economic burden on the swine industry. Disease with *S. suis* is controlled with antibiotic treatment and inactivated vaccines, which can be derived from the strains circulating on the farm. Inactivated vaccines have shown mixed results with limited data supporting reductions in morbidity and mortality following their use. With increasing restrictions on antibiotic use and increasing concerns surrounding antimicrobial resistance, alternatives to antibiotics and improved vaccines are needed to treat and prevent *S. suis* disease. Live vaccines are a potential novel and effective method of preventing disease, as they limit pathogenic strain colonization while stimulating a protective immune response. Additionally, some strains of bacteria, including *S. suis*, produce bacteriocins, antimicrobial peptides that can be used as a targeted alternative to antibiotics. This study investigated the use of an avirulent, bacteriocin producing isolate of *S. suis* (90–1330) as an intranasal vaccine and evaluated the role of the bacteriocin by comparing protection to animals inoculated with a mutant lacking bacteriocin production (90–1330Δsuicin). Animals were protected from systemic disease when challenged with a virulent isolate 21 days after inoculation with either 90–1330 or 90–1330Δsuicin but were not protected when challenged 3 days after inoculation. Evaluation of antibody titers showed increased titers 21 days post-inoculation, and the humoral response was likely providing systemic protection. Although 90–1330 was unable to protect animals challenged 3 days post-inoculation, the strain should be considered a good candidate for vaccine development. *S. suis* 90–1330 was able to induce a protective immune response with a single intranasal inoculation and bacteriocin production may be able to contribute to protection when animals have a lower exposure dose, as in a production setting.



6th International Workshop on *Streptococcus suis*

Centre de Recherche en Infectiologie Porcine et Avicole

Swine and Poultry Infectious Diseases Research Center

CRIPA

Université de Montréal

UNIVERSITY OF CAMBRIDGE

Pembroke College