
4th International
Workshop on
Streptococcus suis
12th Annual
Symposium
of the CRIPA
CRIPA
Université de Montréal
2019 CANADA
June 3-5 Montréal
June 3 | 4th IWSs
June 4 | 4th IWSs & 12th CRIPA joint meeting
June 5 | 12th CRIPA
Concordia University Conference Center
9th floor of the
John Molson School of Business
Concordia University
1450 Guy St.
Montreal, Quebec, CANADA H3H 0A1

Words of the Presidents of 4th International Workshop on *Streptococcus suis*

The 4th International Workshop on *Streptococcus suis*

In 2013, with the aim to unify the growing "*S. suis* family" we organized the "1st International Workshop on *Streptococcus suis*", as one of the sub-objectives of a Canada-China Joint Health Research Initiative operating grant financed by the Canadian Institutes of Health Research and the National Natural Science Foundation of China. The workshop was jointly organized with the State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention (CDC, Beijing, China), which also served as host to this first international event on this important emerging zoonotic agent. We successfully reunited for the first time more than 80 researchers and students working on *S. suis* in the same room. Following the success of this workshop, we organized the "2nd International Workshop on *S. suis*" in Argentina within the context of the highly prestigious XIX Lancefield International Symposium on Streptococci and Streptococcal Diseases (Buenos Aires, Argentina, November 2014). Prof. Peter Valentin-Weigand (Hannover University) organized the "3rd International Workshop on *S. suis*" in Germany in September 2016. This event was jointly organized with the German Pneumococcal and Streptococcal Symposium.

CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

Since this zoonosis is an increasing health risk, the Canadian Institutes of Health Research supports the 2019 event in order to sustain and strengthen the international collaboration on *S. suis* research. Therefore, the 4th International Workshop is held in Montreal, Quebec, Canada. We expect over 150 researchers and trainees from all over the world to present and discuss recent advances on this challenging swine and zoonotic pathogen.

An important aim of the workshop is building an opportunity for knowledge exchange involving stakeholders (veterinarians and swine producers) on the risk posed by *S. suis* infection, diagnostics, and preventive measures. Thus, the "Ordre des médecins vétérinaires du Québec (OMVQ)" has recognized this event as part of the Continuing Professional Development (CPD) program.

APPROUVÉE
Ordre des MÉDECINS VÉTÉRINAIRES du Québec
FORMATION CONTINUE

OECD
BETTER POLICIES FOR BETTER LIVES

A novel aspect of the 4th Workshop is the inclusion of a dedicated session aimed to discuss policy on autogenous vaccine manufacturing and use for veterinary medicine thanks to the financial support of the Organisation for Economic Co-operation and Development (OECD), an international organisation that works to build better policies for better lives.

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

Prof. Mariela Segura **Prof. Marcelo Gottschalk**

Chairs of the 4th International Workshop on *Streptococcus suis*

▶ The 4th International Workshop on *Streptococcus suis* is jointly organized with the CRIPA 12th Annual Symposium.

The CRIPA

The **Swine and Poultry Infectious Diseases Research Centre** or **CRIPA** is a strategic cluster of the province of Quebec, financed by the "Fonds de recherche du Québec - Nature et technologies". The CRIPA mission is to fight against the swine and poultry infectious diseases.

Fonds de recherche Nature et technologies Québec

The CRIPA intends to create a dynamic environment for interaction among 50 teams headed by scientists devoted to basic or applied research at six universities and four government institutions. It also seeks to stimulate communication between these researchers and potential users of their findings, primarily veterinarians and pork and/or poultry producers in Québec. Therefore, the center published monthly scientific popularization writings on its blog named Dash of science. Find more about on our Website or our Blog.

During the CRIPA 12th Annual Symposium, the recent breakthroughs of our researchers and their teams will be presented. Mainly, their fundamental and apply advances on swine and poultry infectious diseases will be addressed and focused on four areas, which are:

- the infectious agents and their interactions with the host immune system;
- their diagnosis, molecular epidemiology and antibiotic resistance; and
- the prophylactic anti-infection measures, in particular, vaccines and other alternatives to antibiotic use.
- transposition our discoveries on agriculture economy and human health in relation to hog production and poultry, in order to select effective decisions and actions with regard to animal infectious diseases.

Centre de Recherche en Infectiologie Porcine et Avicole

Swine and Poultry Infectious Diseases Research Center

CRIPA

Université de Montréal

Welcome to our symposium!

Prof. Carl Gagnon
Director of CRIPA

We thank our generous sponsors for supporting the 4th IWSs!

Gold

Fonds de recherche Nature et technologies Québec

CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

Sponsored by The OECD Co-operative Research Programme:
Biological Resource Management for Sustainable Agricultural Systems

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This event is also possible with the support of *Tourisme Montréal*.

CONFERENCE CENTER

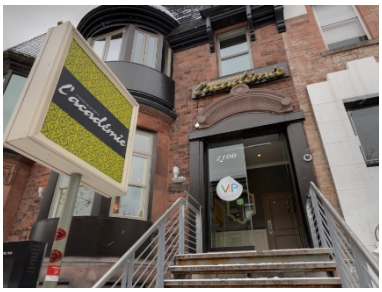
The two events will be held on the Concordia University Conference Center,
9th floor of the **John Molson School of Business; Concordia University**
1450 Guy Street; Montreal, Quebec, CANADA H3H 0A1

INFORMATIONS ABOUT June 4th EVENING EVENTS

▶ Gala dinner for researchers

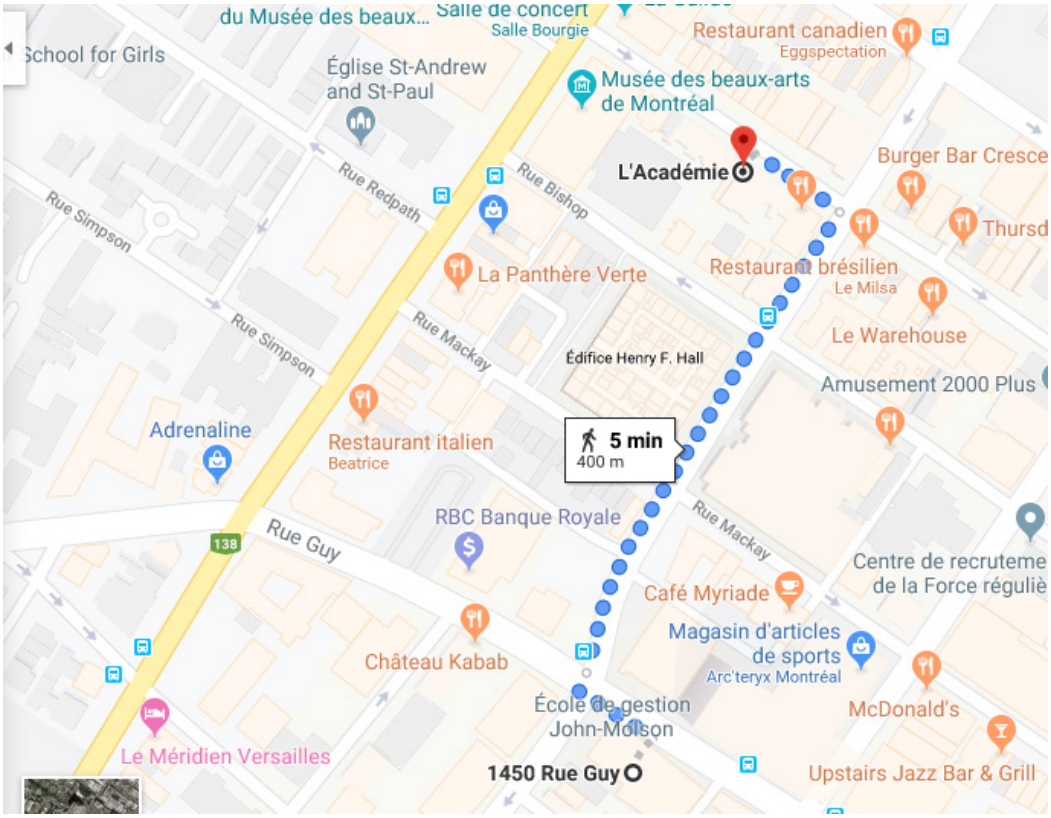
19:30 h: The complimentary gala dinner will take place at the restaurant "L'Académie" at 5 minute-walk distance from the Conference center.

2100 Rue Crescent, Montreal, H3G 2B8



Please note that the restaurant is located in one of the most interesting streets in Montreal, with many pubs, so you can sit, relax and take a drink after the conferences, while waiting for dinner time.

Registration is mandatory for this activity.



▶ Activity for students, post-docs and research assistants

18 h: BEER and SCIENCE

Our invited speaker will be Dr. **Yves Gingras**, professor at the Université du Québec à Montréal, Chairholder of the Canada Research Chair in History and Sociology of Science (2004-2018) and Scientific Director of the "Observatoire des Sciences et des Technologies".

He will give an informal talk (in English) about the excesses and dysfunctions of the world of scientific publishing.

Free transport from the Workshop/Symposium to the BEER and SCIENCE is provided for anyone who registered. Also, a free beer or soft drink and appetizers will be offered by the organizing committee to registered participants.

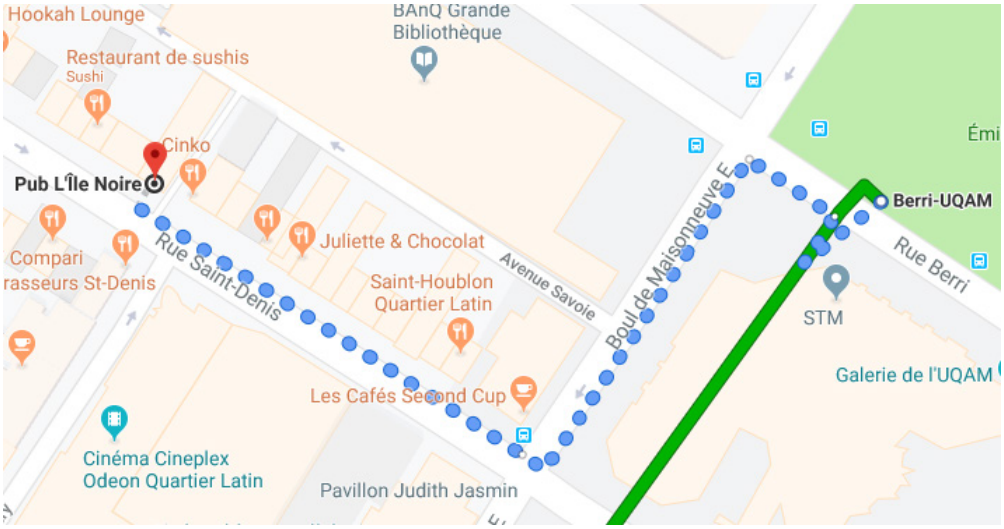
Two volunteer students are mandated to guide the participants to the subway (metro) station, and they will provide on June 4th after the conferences the subway one-way ticket to all registered participants.

Our edition will be held in the center of downtown Montreal at the **Pub L'Île Noire**.
1649 Rue Saint-Denis, Montréal, QC H2X 3K4

Instructions:

Take subway green line.

- From station Guy-Concordia, follow the **direction Honoré Beauregard** to take the train.
- Stay in the train until 5 stops, leave the train at station Berri-UQAM,
- Go to street Berri in direction of Boulevard Maisonneuve East (Boul. Maisonneuve E.),
- Go left on Boul. Maisonneuve E.,
- Got right on Rue Saint-Denis (St-Denis); find the Pub.



DAY 1 - JUNE 3, 2019 | AM
4th INTERNATIONAL WORKSHOP ON *STREPTOCOCCUS SUIS*

8:00 - 8:30	Registration
8:30 - 8:45	Welcome talk; Acknowledgements (**Prof. Mariela Segura** and **Prof. Marcelo Gottschalk**)
8:45 - 9:00	**Dr. Rafael Blasco (SPAIN)** Overview of the OECD Cooperative Research Program

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THEME 1 PATHOGENESIS OF THE INFECTION: HOST-PATHOGEN INTERACTIONS

CHAIRS: **Prof. Tsutomu Sekizaki** (JAPAN) and **Dr. Astrid de Greeff** (THE NETHERLANDS)

9:00 - 9:30	**Keynote speaker – Prof. Jerry Wells** (THE NETHERLANDS) Interactions of *Streptococcus suis* with the lympho-epithelial tissues of palatine tonsils
9:30 - 9:50	**Peter Valentin-Weigand (GERMANY)** Viral co-infection replaces effects of suilysin on adherence and invasion of *S. suis* into respiratory epithelial cells grown under air-liquid conditions
9:50 - 10:10	**Ogi Okwumabua (USA)** Replacement of the glutamate dehydrogenase gene of *S. suis* serotype 2 compromises growth and virulence
10:10 - 10:30	**Gemma Murray (UK)** The link between genome reduction and pathogenicity in *S. suis*
10:30 - 11:15	**Coffee** break and **poster session** (45 min)
11:15 - 11:45	**Keynote speaker – Dr. Masatoshi Okura** (JAPAN) Switching of capsular type impacts on *Streptococcus suis* virulence in mice and pigs
11:45 - 12:05	**Thomas Roodsant (THE NETHERLANDS)** A human organoid model to study the translocation of *S. suis* in the human small intestine
12:05 - 12:25	**Maria Laura Ferrando (THE NETHERLANDS)** Growth of *S. suis* in normal porcine serum induces natural competence and increased expression of virulence factors and metabolic pathways predicted to promote survival in the blood and organs
12:25 - 14:00	**Lunch** and **poster session** (95 min)

DAY 1 - JUNE 3, 2019 | PM
4th INTERNATIONAL WORKSHOP ON *STREPTOCOCCUS SUIS*

THEME 2 DIAGNOSIS AND EPIDEMIOLOGY OF THE *STREPTOCOCCUS SUIS* INFECTION IN HUMANS AND PIGS (1st part). CHAIRS: **Dr. Nahuel Fittipaldi** (CANADA) and **Dr. Daisuke Takamatsu** (JAPAN)

14:00 - 14:30	**Keynote speaker – Prof. Constance Schultsz** (THE NETHERLANDS) Novel insights in epidemiology and pathogenesis of zoonotic *Streptococcus suis* infections
14:30 - 14:50	**Anusak Kerdsin (THAILAND)** Situation of *S. suis* isolated from humans in Thailand
14:50 - 15:20	**Keynote speaker – Dr. Mark O'Dea** (AUSTRALIA) The genomics of Australian *Streptococcus suis*: where do we fit in the global scene?
15:20 - 15:40	**José Luis Arnal (SPAIN)** Updated study of characterization of *S. suis* isolates from Spanish pigs with meningitis
15:40 - 16:30	**Coffee** break and **poster session** (45 min)

THEME 3 ANTIMICROBIAL RESISTANCE

CHAIRS: **Dr. Nahuel Fittipaldi** (CANADA) and **Dr. Daisuke Takamatsu** (JAPAN)

16:30 - 17:00	**Keynote speaker – Dr. Lucy Weinert** (UK) The genetic basis of antimicrobial resistance and virulence in *Streptococcus suis*
17:00 - 17:20	**Virginie Libante (FRANCE)** Key role of chromosomal mobile genetic elements transferred by conjugation in the dissemination of antimicrobial resistance in *S. suis*

DAY 2 - JUNE 4, 2019 | AM
4th INTERNATIONAL WORKSHOP ON *STREPTOCOCCUS SUIS*

8:15 - 8:30 Registration (New participants)

THEME 4 DIAGNOSIS AND EPIDEMIOLOGY OF THE *STREPTOCOCCUS SUIS* INFECTION IN HUMANS AND PIGS (2nd part). CHAIRS: **Dr. Susan Brockmeier** (USA) and **Prof. Vahab Farzan** (CANADA)

8:30 - 9:00	**Keynote speaker – Dr. Astrid de Greeff** (THE **NETHERLANDS**) *Streptococcus suis* serotype 9 infections; novel animal models and diagnostics
9:00 - 9:20	**Zongfu Wu (CHINA)** Identification of six novel capsular polysaccharide loci (NCL21-26) from *S. suis* multidrug resistant non-typeable strains and genome analysis of NCL4 strain WUSS351
9:20 - 9:40	**Leann Denich (CANADA)** Investigation into the serotypes of *S. suis* isolates in nursery pigs in Ontario, Canada
9:40 - 10:00	**Han Zheng (CHINA)** Genomic characteristics of *S. suis* sequence type 7 sporadic strains isolated in Guangxi Zhuang Autonomous Region of China
10:00 - 10:45	**Coffee** break and **poster session** (45 min)
10:45 - 11:05	**Alessandra Morelli (ITALY)** Molecular typing and antimicrobial susceptibility profiles of *S. suis* isolated from diseased pigs in Italy
11:05 - 11:25	**Rujirat Hatrongjit (THAILAND)** Prediction of clonal complexes of *S. suis* by PCR
11:25 - 11:45	**Dr. Paul Lawrence:** Newport Laboratories, Boehringer-Ingelheim Animal Health USA Inc. *Streptococcus suis* serotyping based on whole genome sequencing and formulation of an inactivated vaccine capable of reducing mortality from a lethal challenge in young pigs **Gold sponsor**
11:45 - 13:00	**Lunch** and **poster session** (75 min) Registration for **CRIPA** members and veterinary practitioners

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DAY 2 - JUNE 4, 2019 | PM JOINT MEETING:
4th INTERNATIONAL WORKSHOP ON *STREPTOCOCCUS SUIS* AND 12th ANNUAL CRIPA SYMPOSIUM

13:00 - 13:15 Welcome talk (Prof. Carl Gagnon); Acknowledgements (CRIPA)

THEME 5 KNOWLEDGE EXCHANGE ACTIVITY FOR STAKEHOLDERS: PREVENTION/CONTROL OF *STREPTOCOCCUS SUIS* DISEASES

CHAIRS: **Prof. Peter Valentin-Weigand** (GERMANY) and **Prof. Maria Clavijo** (USA)

13:15 - 13:30 **Prof. Marcelo Gottschalk (CANADA)**
Summary of the problematic on the definition of *S. suis* virulence

13:30 - 14:00 **Keynote speaker – Dr. Susan Brockmeier** (USA)
The role of concurrent infections in predisposing to *Streptococcus suis* and other swine diseases

14:00 - 14:30 **Keynote speaker – Prof. Jeffrey J. Zimmerman** (USA)
Sorting out interactions in the field: challenges to understanding disease causality

14:30 - 14:45 Words from **Gold Sponsors**
Ceva Biovac & Newport Laboratories, Boehringer-Ingelheim Animal Health Inc.

14:45 - 15:20 **Coffee** break and **poster session** (35 min)

15:20 - 15:40 **Keynote speaker – Dr. Virginia Aragon** (SPAIN)
Perinatal antimicrobials: friend or foe?

15:40 - 15:55 **Prof. Mariela Segura (CANADA)**
Overview of *S. suis* vaccines

15:55 - 16:15 **Keynote speaker – Dr. Mariette Saléry** (FRANCE)
Recommendations for the manufacture, control and use of inactivated autogenous vaccines within the EEA

16:15 - 16:35 **Dr. Connie Gebhart and Dr. Douglas Marthaler (USA)**
Serotype and genotype of *S. suis* isolates from the United States serve as predictors of pathotype

16:35 - 17:00 Concluding remarks **Prof. Mariela Segura and Prof. Marcelo Gottschalk (CANADA)**
Announcement of the 5th International Workshop on *Streptococcus suis*

18:00 **Pint of science** (Students, post-docs and research assistants)

19:30 **Gala dinner** (Researchers)

Conferences in the continuous learning program for members of the OMVQ

DAY 3 - JUNE 5, 2019 |
12th CRIPA ANNUAL SYMPOSIUM

8:15 - 8:30	Registration (New participants)
NEW RESEARCH THEMES OF THE CRIPA: ONE HEALTH CHAIR: **Prof. Carl A. Gagnon** (CANADA)	
8:30 - 9:00	**New research axes of the CRIPA: Prof. Paul Thomassin** (CANADA) **Economics of *Salmonella* as food safety risk and the related use of whole genome sequencing**
9:00 - 9:45	**Keynote speaker – Prof. Shayan Shariff** (CANADA) **Theme: poultry vaccines**
9:45 - 10:00	**Creating a directed acyclic graph to identify confounders in cysticercosis research** Ellen Jackson (PhD student, UdeM)
10:00 - 10:45	**Coffee** break and **poster session** (45 min)
THEME 1 MICROBIOTA MANAGEMENT AND HOST HEALTH: AN ALTERNATIVE STRATEGY TO DAMPEN ANTIBIOTIC RESISTANCE	
10:45 - 11:15	**CRIPA New initiative talk– Prof. Alexandre Thibodeau** (CANADA) **Fecal microbiota transplantation in healthy suckling piglets: success parameters to consider**
11:15 -11:30	**Porcine enteric virome characterisation using high throughput sequencing** Nicolas Nantel-Fortier (PhD student, UdeM-AAC)
11:30 - 11:45	**Contribution of carcass surface microbiota in the contamination of pig carcasses in a slaughterhouse in Quebec** Charlotte Braley (PhD student, UdeM)
11:45 - 12:00	**Development of a protocol for colonization of the intestinal tract of chicks** Laura Franco (MSc student, UdeM)
12:00 - 13:15	**Lunch** and **poster session** (75 min)

THEME 2 PATHOGENESIS AND PATHOBIOMIC APPROACHES

CHAIR**: Prof. Charles Dozois** (CANADA)

13:15 - 13:30	**Characterization of the granulocyte-colony stimulating factor (G-CSF) response induced during *Streptococcus suis* infection** Marêva Bleuzé (PhD student, UdeM)
13:30 - 13:45	**Inactivation of yqhG in extraintestinal *Escherichia coli* reduces expression of type 1 fimbriae** Hicham Bessaiah (PhD student, INRS)
13:45 - 14:00	**The effect of *Streptococcus suis* on B lymphocytes and the development of the humoral response** Dominic Dolbec (PhD student, UdeM)
14:00 -14:15	**Characterization of two new SPATE autotransporters and cumulative role of SPATEs in pathogenesis of Extra-intestinal pathogenic *Escherichia coli*** Pravil Pokharel (PhD student, INRS)
14:15 - 14:30	**The increased permeability and inflammartory response of porcine intestinal epithelial cells caused by *Escherichia coli* and Salmonella enterica *Typhimurium* infections are decreased in presence of bovine colostrum** Michael Bouchard (MSc student, U Sherbrooke, AAC)

14:30 - 16:00 **Coffee** break and **poster session** (90 min)

THEME 3 BIOSECURITY FROM DIAGNOSTIC TO CONTROL MEASURES

CHAIR: **Prof. Marie-Odile Benoit-Biancamano** (CANADA)

16:00 - 16:15	**Field study on the immunological response induced by autogenous vaccines used in sows or in piglets to control post-weaned *Streptococcus suis* infections** Lorelei Corsaut (MSc student, UdeM)
16:15 - 16:30	**Determination of capture efficiency against viruses of mechanical and antimicrobial filters using artificially generated viral aerosols** Jonathan M Vyskocil (PhD student, U Laval)
16:30 - 16:45	**New member of the CRIPA: Prof. Neda Barjesteh** (UdeM, CANADA) **The effect of host genetic on pro-inflammatory responses in chickens against T cell-dependent and T cell-independent antigens**

16:45 - 17:00 12th Annual CRIPA Symposium - **Student awards**

CONFERENCE ABSTRACTS

DAY 1 - JUNE 3, 2019 | AM
4th INTERNATIONAL WORKSHOP ON *STREPTOCOCCUS SUIS*

Interactions of *Streptococcus suis* with the lympho-epithelial tissues of palatine tonsils

Jerry Wells (THE NETHERLANDS)

No abstract available

Viral co-infection replaces effects of suilysin on adherence and invasion of *Streptococcus suis* into respiratory epithelial cells grown under air-liquid interface conditions

Fandan Meng[1], Jie Tong[2], Désirée Vötsch[3], Maren Willenborg[3], Georg Herrler[2], Naihuei Wu[2], **Peter Valentin-Weigand**[3*]

[1] State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy, China
[2] Institute for Virology, University of Veterinary Medicine Hannover, Foundation, Germany
[3] Institute for Microbiology, University of Veterinary Medicine Hannover, Foundation, Germany

Streptococcus suis is an important zoonotic pathogen, which can infect humans and pigs worldwide, posing a potential risk to global public health. Suilysin, a pore-forming cholesterol-dependent cytolysin, is considered to play an important role in the pathogenesis of *S. suis* infections. It is known that infection with influenza A viruses may favor the susceptibility to secondary bacterial infection resulting in more severe disease and increased mortality. However, the molecular mechanisms underlying these co-infections are incompletely understood. Applying highly differentiated primary porcine respiratory epithelial cells grown under air-liquid interface conditions, we analyzed the contribution of swine influenza viruses (SIV) to the virulence of *S. suis* with a special focus on its cytolytic toxin suilysin. We found that during secondary bacterial infection, suilysin of *S. suis* contributes to the damage of well-differentiated respiratory epithelial cells in the early stage of infection, whereas cytotoxic effects induced by SIV became prominent at later stages of infection. Prior infection by SIV enhanced the adherence to and colonization of porcine airway epithelial cells by the wildtype *S. suis* strain (wt) and a suilysin-negative *S. suis* mutant in a sialic-acid dependent manner. A striking difference was observed with respect to bacterial invasion. After bacterial mono-infection, only wt *S. suis* showed an invasive phenotype whereas the mutant remained adherent. When the epithelial cells were pre-infected with SIV, also the suilysin-negative mutant showed invasion capacity. Therefore, we propose that co-infection with SIV may compensate for the lack of suilysin in the adherence and invasion process of suilysin-negative *S. suis*.

Replacement of the glutamate dehydrogenase gene of *Streptococcus suis* serotype 2 compromises growth and virulence

Ogi Okwumabua*

College of Veterinay Medicine, Midwestern University, USA

The glutamate dehydrogenase (GDH) of *Streptococcus suis* was thought to play a role in pathogenicity, but direct evidence has not been presented. In this study, a *gdh* isogenic mutant deficient in GDH activity (*Δgdh*) was constructed by gene replacement and subsequently characterized. Immunoblot analysis and enzyme activity assay confirmed that the mutant derivative was deficient in expression of enzymatically active GDH. Compared to the wild type (WT) strain, *Δgdh* displayed a longer lag phase during growth (10 h versus 3 h). When subjected to the stress of higher temperatures and varying pH, the *Δgdh* was significantly outgrown by more than 50% p (≤0.05) by the WT at 42oC and also outgrown at pH 8.5 and 9.0 respectively. To assess the ability of the *Δgdh* strain to cause disease, a swine infection model was used. Pigs inoculated with the WT strain exhibited fever, specific signs of disease and lesions and the strain could be re-isolated from the brain, lung, joint fluid and blood samples collected from the infected pigs. Pigs inoculated with the *Δgdh* strain did not exhibit any clinical signs of disease and the strain could not be re-isolated from any of the tissues or body fluid sampled. No histologic lesions were also observed. These results suggested that the observed defect in the cell growth and pathogenicity of the *Δgdh* could be attributed to the lack of *gdh* expression. The data demonstrated that the GDH is important in *S. suis* physiology with implication to colonize, disseminate and cause disease and that the GDH is critical in host-pathogen interactions.

The link between genome reduction and pathogenicity in *Streptococcus suis*

Gemma Murray[1*], Eric Miller[2], Hoa Ngo Thi[3], Nahuel Fittipaldi[4], Marcelo Gottschalk[5], Rui Zhou[6], Dan Tucker[1], Lucy Weinert[1], *et al.*

[1] University of Cambridge, UK
[2] Haverford College, USA
[3] Oxford University, UK
[4] Public Health Ontario, Canada
[5] University of Montreal, Canada
[6] Huazhong Agricultural University, China

Pathogenic bacteria tend to have smaller genomes and fewer genes than closely related non-pathogenic or less-pathogenic species. However, it is not known why this pattern holds, and what it can tell us about how and why bacteria become pathogens. Using a data set of whole genome sequences of *Streptococcus suis* strains, sampled from the UK, Canada, Vietnam and China, from pigs both with and without *S. suis* related disease, we tested for this association, and investigated what it can tell us about the evolution of pathogenicity within *S. suis*.
We found evidence of multiple transitions to pathogenicity within *S. suis*, and an association between these transitions and both a reduction in genome size and a loss of genes. We investigated several hypotheses about what might be driving this association. In particular, we looked for evidence of preferential loss of genes with functions that may be either harmful or unnecessary for pathogens, evidence of a faster rate of replication or mutation in pathogenic strains, and evidence of smaller population sizes or greater population isolation in pathogenic strains.

Switching of capsular type impacts on *Streptococcus suis* virulence in mice and pigs

Masatoshi Okura (JAPAN)[1*], Jean-Philippe Auger[2], Tomoyuki Shibahara[1], Guillaume Goyette-Desjardins[2], Marie-Rose Van Calsteren[3], Fumito Maruyama[4], Makoto Osaki[1], Mariela Segura[2], e*t al.*

[1] National Institute of Animal Health, Japan
[2] Faculty of Veterinary Medicine, University of Montreal, Canada
[3] Agriculture and Agrifood Canada, Canada
[4] Graduate School of Medicine, Kyoto University, Japan

Streptococcus suis is an important zoonotic pathogen causing various diseases in pigs and humans. Although strains of *S. suis* can be classified into different serotypes on the basis of antigenic differences in capsular polysaccharide (CPS), serotype 2 is the most frequently associated with clinical cases in both pigs and humans. CPS of *S. suis* is known to be a major virulence factor contributing to protection against phagocytosis by host cells. However, it is unknown whether differences in serotype themselves (i.e. differences in CPS structure) directly affect *S. suis* virulence. To answer this question, we experimentally generated four serotype switched mutants using the reference serotype 2 strain P1/7 (P1/7cps2to3, P1/7cps2to4, P1/7cps2to7 and P1/7cps2to8) by exchanging the CPS synthesis gene cluster for those of serotypes 3, 4, 7 and 8, respectively, and compared their virulence in mice (intraperitoneal infection) and piglets (intranasal infection). In mice, P1/7cps2to8, expressing serotype 8 CPS, was the most virulent and showed higher mortality and blood bacterial load than serotype 2 strain P1/7. The virulence of P1/7cps2to7 was equivalent to that of P1/7, whereas the mortality of mice inoculated with P1/7cps2to3 and P1/7cps2to4 was remarkably reduced compared with that of P1/7. In pigs, clinical symptoms such as lameness, shivering and dysstasia were observed only with P1/7, P1/7cps2to7, and P1/7cps2to8 strains. Inoculated strains were recovered from several major organs and blood from diseased pigs with P1/7 and P1/7cps2to8. In addition, P1/7cps2to8 was also recovered from several major organs of experimentally infected but clinically healthy pigs, suggesting increased ability of P1/7cps2to8 to evade the host's immune system. Our data suggest that CPS structure itself is an important factor in determining levels of *S. suis* virulence, although further studies are needed to elucidate the mechanisms behind the observed phenomena.

A human intestinal organoid model to study the translocation of *Streptococcus suis* in the human small intestine

Thomas Roodsant[1,2*], Marit Navis[3], Adithya Sridhar[2], Kees van der Ark[1,2], Vanesa Muncan[3], Constance Schultsz[1,2]

[1] Amsterdam UMC, Amsterdam Institute for Global Health and Development, University of Amsterdam, The Netherlands
[2] Amsterdam UMC, Department of Medical Microbiology, University of Amsterdam, The Netherlands
[3] Amsterdam UMC, Tytgat Institute for Liver and Intestinal Research, University of Amsterdam, The Netherlands

Streptococcus suis is a zoonotic pathogen that can cause septic shock and meningitis in pigs and humans. Human infections are mainly caused by *S. suis* serotype 2, while many other serotypes can be found in pigs. In humans, the consumption of undercooked or raw pig products contaminated with *S. suis* can lead to systemic infections, implying that the gastrointestinal tract is a potential entry point for the pathogen. We previously used Caco-2 cells as a model for the human gut epithelium and found that the ability to translocate through the Caco-2 cell monolayer correlated with the *S. suis* genotype, in which especially *S. suis* clonal complex 1 demonstrated high translocation. To further advance our research and gain novel insights in the host-pathogen interaction, we developed a two-dimensional human organoid derived small intestinal epithelium model. Our epithelial model represents the intestinal epithelium as it is polarized, has tight and adherent junctions and consist out of enterocytes, paneth cells, goblet cells, enteroendocrine cells and stem cells. As proof of concept, we tested the translocation of another intestinal pathogen causing meningitis. L. monocytogenes translocated at a frequency of 0.029% in proximal and 1.1% in distal small intestinal organoids and induced a 3-fold increase in monolayer permeability. Experiments with *S. suis* showed translocation of strains representing different genotypes at similar frequency as L monocytogenes. *S. suis* co-localized with the cell-cell border suggesting that *S. suis* translocates paracellular, similar to observations in Caco-2 cells. Taken together, we generated an intestinal epithelium model that appears suitable to unravel host-pathogen interactions leading to *S. suis* translocation in the human small intestine.

Growth of *Streptococcus suis* in normal porcine serum induces natural competence and increased expression of virulence factors and metabolic pathways predicted to promote survival in the blood and organs

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The transcriptome of *Streptococcus suis* strains during growth in porcine serum and laboratory medium was investigated by RNA-sequencing to identify differentially expressed genes which might promote survival in porcine serum.
Results: Two major North-European prevalent *S. suis* serotypes in pigs, *S. suis* serotype 2 (SS2) P1/7 and *S. suis* serotype 9 (SS9) strain 8067 strain, were incubated at 37 °C for 3.5 h in normal porcine serum. Both strains grew in porcine serum albeit with slightly different growth rates. Numerous genes were significantly differentially expressed in serum compared to laboratory medium. Genes involved in metabolism of carbohydrates such as galactose and starch and 13 phosphotransferase uptake systems for sugars (PTS) were identified to be contributing to both *S. suis* strains growth in porcine serum. Transcription of several genes involved in virulence and evasion of the host immune system were increased in serum in both strains despite some difference. In SS2 P1/7 genes encoding the DNA-uptake machinery (i.e. ComYC, ComYA, ComYF, ComYG, ComYB, ComYD) for natural competence were also more highly expressed in serum. To investigate whether serum promoted natural competence we added plasmid DNA carrying an antibiotic marker to SS2 strain P1/7 in absence of the competence-inducing peptide after 1, 2, and 3, h growth in normal porcine serum. After 2 h incubation transformants were recovered by plating and verified to contain the plasmid. Conclusions: Numerous genes encoding enzymes or transporters involved in carbohydrate metabolism and known or putative virulence factors of *S. suis* were more highly expressed in serum than in laboratory culture medium. These genes can serve as targets for the development of novel vaccines to prevent *S. suis* invasive disease. Serum induction of natural competence can be useful for genetically modifying strains which are not responsive to competence inducing peptide.

Novel insights in epidemiology and pathogenesis of zoonotic *Streptococcus suis* infections

Constance Schultsz (THE NETHERLANDS)

No abstract available

Situation of *Streptococcus suis* isolated from humans in Thailand

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A total of 850 *S. suis* isolates recovered from human infections in Thailand were characterized based on serotyping by multiplex PCR and co-agglutination, genotypic profiles by multilocus sequence typing, and PCR for virulence-associated genes, as well as review of medical records. Serotype 2 (93.7%) was predominant, followed by serotype 14 (4.9%), 24 (0.6%), 5 (0.3%), 9 (0.1%), 4 (0.1%), and 31 (0.1%). Multilocus sequence typing analyses revealed eight clonal complexes (CC): CC1 (58.2%), CC104 (29.8%), CC233/379 (4.2%), CC25 (5.1%), CC28 (1%), CC221/234 (0.8%), CC16 (0.1%), CC94 (0.1%), and three singletons (ST181, ST235, and ST236). The CC1 group contained serotype 2 and 14 isolates, while CC25, 28, 104, and 233/379 consisted of serotype 2 isolates only. CC221/234 contained serotype 5, 24, and 31 isolates, whereas the single serotype 4 and 9 isolates belonged to CC94 and CC16, respectively. Three singletons contained serotype 5 (ST181 and ST235) and 2 (ST236) isolates. Our data showed that ST1 isolates were more associated with meningitis than those of other STs ($p < 0.001$). The major route of infection was shown to be close contact with infected pigs or contaminated raw pork-derived products, including occupational exposure and recent consumption of raw pork products (64.2%). Cases fatality rate was 12.7%. Almost of human cases were adults, however, our study revealed one case was a child with serotype 24 infection. This study revealed a relatively large number of CCs of *S. suis* causing human infection in Thailand. Food safety campaigns and public health interventions would be important for controlling the *S. suis* infection in humans in Thailand.

The genomics of Australian *Streptococcus suis*: where do we fit in the global scene?

Mark O'Dea (AUSTRALIA)

No abstract available

Updated study of characterization of *Streptococcus suis* isolated from Spanish pigs with meningitis

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Introduction

Streptococcus suis is a worldwide swine pathogen frequently involved in septicemia, meningitis, arthritis, endocarditis and pneumonia. To date 29 different serotypes have been described showing different frequency distribution depending on the geographical area. Serotypes 2, 7 and 9 are predominant in Europe. Nonetheless, there is no updated and detailed information about the current situation in Spain. Diagnostic laboratories lack of affordable techniques to perform such investigations. Furthermore, although many virulence factors have been proposed for this bacteria, only SLY, MRP and EPF have been regularly associated with European diseased animals infected with particular serotypes.

Material and methods

A set of qPCR assays to detect individually serotypes 1-14, 2-1/2, 3, 4, 5, 7, 8 and 9 was developed targeting their respective *cps* locus. Moreover, 3 assays detecting virulence factors (VF) *sly*, *mrp* and *epf* were designed to run simultaneously in a multiplex qPCR reaction. Ninety-eight different strains of *S. suis* were recently isolated from brain of diseased animals showing nervous signs in Spain. Afterwards, isolates were analyzed by qPCR to determine their respective serotype. Furthermore, 56 of those isolates were characterized for the above mentioned virulence factors: *sly*, *mrp* and *epf*.

Results

The Table 1 shows the distribution of percentages found for the studied serotypes of *S. suis* and the respective virulence factors detected in each serotype.

Table 1

Serotype	(n) Isolates	%	(n) studied for VF	Virulence Factor pattern
1-14	11	11,2	4	sly+/epf+/mrp+
2-1/2	17	17,3	11	sly+/epf+/mrp+
3	1	1,0	1	sly-/epf-/mrp-
4	1	1,0	1	sly-/epf-/mrp-
5	0	0,0	0	
7	6	6,1	1	sly-/epf-/mrp-
8	3	3,1	3	sly-/epf-/mrp(+/-)
9	46	46,9	31	sly+/epf-/mrp+
nt	13	13,3	4	sly-/epf-/mrp-
Total	**98**		**56**	

Discussion and Conclusion

Serotype 2 was widely described as the most prevalent in Spain; however, our study shows that serotype 9 has replaced it and it was the most frequently detected by far (46.9%) and strains tested contain always sly and mrp as virulence factors. Serotypes 1-14 and 2-1/2 are also considerably present among Spanish diseased pigs and both groups show *sly*, *epf* and *mrp*. These findings endorse previous studies which pointed this particular pattern of VF as remarkable to determine the virulence of the strains of serotypes 2 and 9.

This set of qPCR assays is effective for serotyping *S. suis* in Spain. Over 75% of isolates are 1-14, 2-1/2 or 9 and just 13% of them remain as non typable (nt). This valuable technique is applicable for a direct analysis over the tissue due to its reliable sensitivity and specificity.

The genetic basis of antimicrobial resistance and virulence in *Streptococcus suis*

Lucy Weinert (UK)

No abstract available

Key role of chromosomal mobile genetic elements transferring by conjugation in the dissemination of antimicrobial resistance in *Streptococcus suis*

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Streptococcus suis is a zoonotic pathogen that leads to high economic losses in the pig industry and is responsible for serious infections in people working in close contact with infected animals or contaminated meat. Increasing reports of antimicrobial resistance (AMR) genes carried by mobile genetic elements in *S. suis* suggest that this species could be a reservoir of AMR genes spreading to other species by horizontal gene transfer. The thorough in silico analysis of 214 genomes of *S. suis* strains belonging to 27 serotypes revealed a huge number of chromosomal mobile genetic elements transferring by conjugation: 496 Integrative Conjugative Elements (ICEs; 233 complete and 263 defective or partial) and 457 Integrative Mobilizable Elements (IMEs; 406 complete and 51 defective or partial). A high diversity of integration sites was detected (7 different ones for ICEs and 13 for IMEs, including 5 newly described sites) as well as a high mosaicism of these elements. Their high plasticity results from an accretion in tandem with other ICEs, recombination between elements and module exchanges but also integration of other elements (ICE or IME) inside the ICE. Many of these genetic elements carry AMR genes thus indicating a key role of these chromosomal mobile genetic elements in the dissemination of antimicrobial resistance in *S. suis* and also likely towards other bacterial species.

Streptococcus suis serotype 9 infections; novel animal models and diagnostics

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The serotype distribution of *Streptococcus suis* isolates from clinical cases varies over time and differs between geographical region. Worldwide, serotype 2 is the main causative serotype of disease. However, in The Netherlands and other West-European countries, the previous predominance of serotype 2 has been replaced by serotype 9. One putative explanation for this phenomenon might be serotype replacement due to successful autovaccination against serotype 2. Although serotype 9 isolates are less virulent under experimental conditions, they are causing severe disease and outbreaks on farms and lead to financial losses. Here, we describe a study into animal models and diagnostic tools to facilitate research and control of serotype 9 infections. Previous studies have shown that within serotype 9, two populations of strains exist. One heterogeneous population is associated with healthy carrier pigs without clinical symptoms, whereas the other population is associated with clinical disease. We have shown that these population differ in virulence *in vivo* under experimental conditions. Using a clinical isolate, our consortium developed animal models to study serotype 9 infections in pigs. Different routes of infection were used, combining mucosal routes (oral, intranasal) with systemic routes (intra-tracheal). We present results of two different infection models: one non-clinical model to study colonization of porcine tonsils and intestine, and one clinical model that results in symptoms and colonization. The latter model resulted in lower colonization levels than the first model. Subsequently, a diagnostic quantitative PCR assay was developed to identify serotype 9 isolates associated with clinical disease, in isolates grown form clinical and tonsil swab samples. The PCR was tested on all reference isolates of *S. suis*, as well as on field isolates of *S. suis* of various serotypes. Sensitivity and specificity were determined. Application of the developed models and tools will be discussed.

Identification of six novel capsular polysaccharide loci (NCL21-26) from *Streptococcus suis* multidrug resistant non-typeable strains and genome analysis of NCL4 strain WUSS351

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Streptococcus suis is a major swine pathogen and an important zoonotic agent worldwide. At least nine serotypes can infect human so far. Although 29 serotypes (1–19, 21, 23–25, 27–31, and 1/2) strains are considered as authentic *S. suis*, a novel variant serotype Chz and strains carrying 20 novel capsular polysaccharide loci (NCL1-20) have been identified recently. However, information about pathogenic and antimicrobial resistance characteristics of strains carrying NCLs is still unavailable. In this study, we identified six new NCLs (designated as NCL21-26) from 35 non-typeable *S. suis* strains by agglutination test and whole genome sequencing analysis. Further analysis of the genetic context of NCL25 and NCL26 showed a mosaic structure of the capsular polysaccharide loci: NCL25 exhibited considerable similarity to that of serotypes 10 and 11; NCL26 shared similarity to that of serotype 9 and NCL4. Antimicrobial susceptibility testing demonstrated that strains carrying NCL21-26 were all resistant to

clindamycin, lincomycin, erythromycin, tilmicosin, and tetracycline. Animal infection experiments showed that the virulence of NCL26 strain NJ1112 isolated from a disease pig was similar to that of *S. suis* serotype 2 virulent strain SC070731 in both zebrafish and mouse infection models, highlighting the necessity for surveillance of strains belonging to NCL26. In addition, we also reported the complete genome of NCL4 strain WUSS351 which exhibits strong antibacterial activity against many *S. suis* isolates. Comparative genomic analysis showed that strain WUSS351 contains a novel type VII secretion system (T7SS), gene clusters of suicin 3908 and lactococcin 972, and several genes encoding different bacteriocin immunity proteins. The T7SS contains several effectors: EsxA, EsxB, and three different types of LXG domain toxins. Our findings expand the views of genetic diversity of *S. suis* capsular polysaccharide loci and *S. suis* pathogenic characteristic.

Investigation into the serotypes of *Streptococcus suis* isolates in nursery pigs in Ontario, Canada

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Streptococcus suis naturally inhabits the nasal cavities and tonsils of pigs. Some strains can cause systemic infection leading to a variety of disease conditions. This study describes *S. suis* serotypes isolated from sick pigs, analyses whether serotypes found in systemic sites are found in upper respiratory sites of the same pigs, and examines the serotypes found in upper respiratory sites of sick and healthy pigs. A case control study involving 4-8-week-old nursery pigs from Ontario farms was conducted. Cases with clinical signs of *S. suis* were selected and matched with equal numbers of healthy controls based on herd, time of visit and pen. An array of samples were cultured, and isolates were tested for glutamate dehydrogenase and recombination protein N genes by PCR. Proof of *S. suis* was concluded if both genes were present. Isolates were then serotyped using a two step-multiplex PCR. Twelve Ontario farms were visited, and 698 samples were collected from 128 pigs (451 from 64 cases and 247 from 64 controls). Serotypes commonly found in all pigs were 29 (8 farms), 16 (7 farms), 15 (6 farms) and 9 (6 farms). Serotypes commonly found in systemic sites were 29 (3 farms) and (2,1/2), 7 and 9 (2 farms each), as well as untypable (4 farms). In confirmed cases, serotypes 9, (2,1/2) and untypable were most commonly found. Cluster analysis suggested existence of four major groups of confirmed cases: i) serotype 9, ii) untypable, iii) mixed serotype and iv) serotype (2,1/2). Detection of serotype 9 ($p=0.03$) or (2,1/2) ($p=0.08$) in upper respiratory sites were associated with detection of the same serotype in systemic sites of cases; whilst no association was found in these sites between untypables; however, isolation of these serotypes was very low. There was also no association between presence of serotypes in upper respiratory sites amid cases and controls. This study provides a good understanding of which *S. suis* serotypes are most commonly found in clinical cases.

Genomic characteristics of *Streptococcus suis* sequence type 7 sporadic strains isolated in Guangxi Zhuang Autonomous Region of China

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Streptococcus suis is an important zoonotic pathogen. Serotype 2 and sequence type (ST)1 were the most frequently reported in both patients and diseased pigs. ST7 is only endemic to China and was responsible for the 1998 and 2005

Chinese outbreaks. In the present study, 38 sporadic ST7 *S.suis* strains which mostly caused sepsis were collected from patients in Guangxi Zhuang autonomous region (GX) between 2007 and 2018. Of 38 sporadic ST7 strains, serotype 14 is the most frequent (27 strains, 71.1 %), followed by serotype 2 (11 strains, 28.9 %). Phylogenetic structure of ST7 population including epidemic and sporadic ST7 strains was constructed using mutational SNPs. High diversity within ST7 population was revealed which were divided into 5 clades. Only one sporadic ST7 strain from streptococcal toxic-shock-like syndrome (STSLS) patient was clustered into same clade of epidemic strains. Sporadic ST7 strains of GX were mainly clustered into clade 5 which contained all serotype 14 strains and part of serotype 2 strains. The subtype of *mrp* in sporadic ST7 strains of clade 5 was NA2, obviously different from EU type in epidemic ST7 strains. All sporadic ST7 strains of GX origin harbored *tet(40)* and/or *tet(O)* gene which were prevalent tetracycline resistance gene in pigs, exclude GX14. Only tetracycline resistance gene in epidemic ST7 strains was *tet(M)*. Our data indicated that origins and evolutionary history between epidemic and sporadic ST7 strains were different. Sixteen sporadic ST7 strains (42.1%) of GX were resistant to at least four categories of antibiotics, while the most common resistance pattern was tetracycline-macrolide-lincosamide-aminoglycoside antibiotics. The intensive use of antibiotics, for prophylaxis or treatment in pig breeding in China already became a severe public health challenge.

Molecular typing and antimicrobial susceptibility profiles of *Streptococcus suis* isolated from diseased pigs in Italy

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The aim of this study was to investigate the distribution of virulence factors and antibiotic resistance profiles in 82 *S. suis* isolates from meningitis, endocarditis, septicemia and arthritis in piglets (2017-2019). The isolates were from six Italian regions and only one pathotype per farm was included.
All the isolates were tested for genes encoding for capsular types 2, 7, 9 and virulence factors *mrp*, *sly* and *epf* using PCR. Seventy of them were assessed for antimicrobial susceptibility using a MIC test, following CLSI guidelines (VET08).
Capsular types 9 and 2 were confirmed as the predominant ones in diseased pigs in Italy, accounting for approximately one third of cases each. Profiles *mrp+/sly+/epf+* and profile *mrp+/sly+/epf-* were detected in capsular type 2 and capsular type 9 isolates, respectively. Thirty-one isolates tested negative for capsular genes 2, 7, 9. Twenty- eight of them were confirmed as belonging to the species using a PCR test for *gdh*. Among them, 6 were *mrp+/sly+/epf+*, 4 *mrp+/sly+*, 1 *mrp+* and 1 *sly+*. Finally, fifteen tested negative for all the virulence genes.
All isolates were resistant to tetracycline and susceptible to ceftiofur. Twenty-three (32.8%) were susceptible to enrofloxacin, 68 (97.1%) to florfenicol and 58 (82.9%) to penicillin. Twelve isolates (17%) were fully resistant to penicillin and two of them were intermediate to ampicillin. Nine penicillin-resistant isolates belonged to capsular type 9.
In conclusion, our study confirms the importance of capsular types 2 and 9 in cases of invasive infections in pigs in Italy. Differently from other EU countries, capsular type 7 was never detected. Interestingly, a third of isolates were non-typeable, suggesting the presence of other capsular types and/or non-typable isolates in our collection. Finally, the presence of capsular 9 isolates with a reduced susceptibility to beta-lactams underlines the need of a responsible use of antibiotics in the pig sector.

Prediction of clonal complexes of *Streptococcus suis* by polymerase chain reaction

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Multilocus sequence typing (MLST) is considered a reliable method for providing insight into the *Streptococcus suis* population structure, clonal complexes (CCs) and the potential of particular clones to cause disease. However, the method is costly, time-consuming and difficult to use for screening large numbers of isolates. Here we described multiplex PCR (mPCR) and random amplified polymorphism DNA (RAPD) assays to predict *Streptococcus suis* CCs that are relevant to human infections. The mPCR assay was capable of simultaneously distinguishing CC1, CC25, CC28, CC104, CC221/234 and CC233/379, in a single reaction. Nonetheless, the mPCR assay could not be applied to CC16. RAPD assay could distinguish CC16, CC1, CC25, CC28, CC104, CC221/234, and CC233/379. Combination between mPCR and RAPD will be useful for precise screening or predicting major CCs relevant to human and pig *S. suis* clinical isolates and for low-cost screening of large numbers of isolates with rapid analytical capacity and could be utilized in most laboratories.

CONFERENCE Gold Sponsor Newport Laboratories, Boehringer Ingelheim Animal Health

Streptococcus suis serotyping based on whole genome sequencing and formulation of an inactivated vaccine capable of protecting pigs from a lethal challenge

Paul Lawrence

Newport Laboratories, Boehringer Ingelheim Animal Health USA Inc., USA

Streptococcus suis is a Gram positive bacterium, a natural commensal of the upper respiratory tract and associated with meningitis, arthritis, and septicemia in young pigs. The serotypes 1, ½ and 2 are most commonly associated with the disease in the U.S. Many of the field isolates are non-typeable or ambiguous using the current methods. We used whole genome sequencing (WGS) to identify the serotypes of several field isolates. This method appears to be robust and could correctly assign a serotype to all isolates tested. Isolates selected based on WGS, were used for vaccination-challenge studies demonstrating that the *S. suis* capsule is the primary target of protective immune responses. Briefly, the genomic DNA from *S. suis* isolates were extracted, libraries constructed and sequenced on a MiSeq instrument. Reads were mapped to reference genomes to identify the serotype of each isolate. Vaccination challenge studies were performed on commercial weaned pigs. Inactivated whole cell vaccines were formulated in proprietary adjuvant or mock vaccine without antigen. Animals were challenged 1 week after their second vaccination. Clinical symptoms and mortality were evaluated post-challenge. The serotyping method described here can be used to rapidly establish a serotype for *S. suis* isolates and more reliable than standard serotyping methods such as PCR and serum agglutination. In addition, this method allows a more holistic examination of differences present in the capsular locus of strains from different serotypes. Our data indicate that the field isolates of *S. suis* have a complex and plastic genome. The capsular locus is amenable for extensive recombination. Thus, creating a complex mosaic structure. Our vaccination/challenge study demonstrates the importance of the capsule against protective immune response. When used in conjunction with a robust serotyping method, inactivated vaccines can provide protection against a lethal *S. suis* challenge.

Summary of the problematic on the definition of *Streptococcus suis* virulence
Marcelo Gottschalk (CANADA)

No abstract available

The role of concurrent infections in predisposing to *Streptococcus suis* and other swine diseases
Susan Brockmeier (USA)

No abstract available

Sorting out interactions in the field: challenges to understanding disease causality
Jeffrey J. Zimmerman (USA)[1]

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In the arena of animal health, the Holy Grail of basic research is the achievement of a break-through discovery that translates into truly meaningful improvements in the field: healthier, more productive pigs. It is generally accepted that such discoveries are derived from a fundamental understanding of disease causality. In the larger sense, this is our inheritance from Pasteur and Koch. However, given the power of our current "tools of discovery", why are impactful discoveries so rare, and how can we increase the frequency of their occurrence?

In 1982, Dr. Calvin Schwabe in a visionary essay published in the inaugural issue of Preventive Veterinary Medicine noted that the veterinarians of the time were struggling to adapt to the disease challenges associated with changes in animal production. In particular, livestock production at that time was evolving into larger, intensive systems characterized by 1) spatial separation of animals by age, production stage, and/or function; 2) rapid turnover of populations over the course of the production cycle; and 3) the continual introduction of new, immunologically susceptible subpopulations. Of course, this change continues apace, in part spurred on my global disease challenges, such as African swine fever virus, against which traditional outdoor production systems have little means of protection.

An unexpected outcome of this evolution in animal husbandry was the appearance of "production diseases" (Schwabe, 1982). Unlike the single etiology upon which the work of Koch and Pasteur was based, "production diseases" were/are characterized by multiple interacting infectious and non-infectious components. They are often difficult to understand, difficult to model in the research setting, and are no less difficult to deal with today than they were when they first appeared 50 years ago.

Schwabe's 1982 solution for "production diseases" was for practitioners to implement "*on-going on-farm research based upon surveillance*" in order to establish patterns of disease and against which to measure the effects of specific interventions. This approach was never implemented because routine surveillance was impractical and excessively

expensive. However, the development of surveillance methods based on aggregate samples has changed this calculation. In this presentation, we describe successes and challenges in on-going research in this area.

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Perinatal antimicrobials: friend or foe?

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Streptococcus suis is a zoonotic bacterium and a major porcine pathogen. *S. suis* causes meningitis and polyserositis and it is considered one of the main bacterial causes of economic losses in nursery piglets. *S. suis* is an early colonizer of the upper respiratory tract of piglets, which are commonly colonized by *S. suis* from birth. Virulence of the *S. suis* strains and immunity of the animals influence the outcome of infection. Infections in humans have been associated mainly with occupations with contact with swine.

S. suis infections are one of the main causes of antimicrobial usage in piglets. Due to the young age of the piglets affected, many farms use metaphylactic perinatal antibiotics for control of this disease. Nowadays, public and private institutions are urging for a significant reduction in antibiotic use, since the emergence of drug resistances has become a major health problem. An additional problem of antimicrobials is that these treatments also affect the beneficial bacteria of the microbiota. Microbiota is the community of microorganisms that live on different tissues on all multicellular organisms. Pathogen exclusion is an important role of the microbiota, and it can be achieved by competition or by induction of the correct maturation of the immune system. Thus, the microbiota is essential for health and this has change our current view on antimicrobials, since they can have deleterious effects on favourable bacterial populations. However, the use of metaphylactic antimicrobials at early stages of life is still in practice in porcine production.

Clinical observations indicate that overuse of antimicrobials at early age can have a harmful effect later in the pigs' health. In an attempt to understand this phenomenon, the outcome of perinatal antimicrobial treatment on the nasal microbiota at weaning was studied. The nasal microbiota was determined when antimicrobial treatment was used early in life and with no antimicrobial treatment during the lactation period. Elimination of perinatal antimicrobials resulted in an increase in bacterial diversity in the nasal microbiota at weaning. Elimination of antimicrobials produced an increase in the relative abundance of *Prevotella* and *Lactobacillus*, and a decrease in *Moraxella* and *Bergeyella*. Importantly, these changes in microbiota composition were accompanied by an improvement of the piglets' health and a higher productivity in the nursery phase. The use of perinatal antimicrobials has to be carefully considered since antimicrobials interfere with the establishment of the microbiota and, in consequence, with immune maturation and other microbiota functions.

Overview of *S. suis* vaccines

Mariela Segura (CANADA)

No abstract available

Recommendations for the manufacture, control and use of inactivated autogenous vaccines within the EEA

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The manufacture and use of autogenous vaccines are currently under national competence of Member States in European Union. Face to the increasing wide use of autogenous vaccines and free movement of vaccinated animals with autogenous vaccines, recommendations have been drawn by the CMDv (coordination group for Mutual Recognition and Decentralised Procedures - Veterinary) two years ago as a starting point for an harmonised approach of these autogenous vaccines in Europe. The recommendations paper gives advices concerning the use, the manufacture and the control of those autogenous vaccines. In particular, the definition of autogenous vaccines as given by the Directive EU 2001/82 is completed by providing clarifications for the terms “same locality”, “same rearing site/same farm” and “epidemiological link”. Also Good Manufacturing Production-like requirements that should be followed by manufacturers are set. As well, conditions for use and authorisations to be delivered are recommended. The CMDv recommendations paper was a first step for a future where use and manufacture of autogenous vaccines will be harmonised over Europe. The increasing use of autogenous vaccines reflects the need for alternative therapeutics to antibiotics and guidance for manufacturers will certainly encourage them to develop high quality autogenous vaccines. In this sense, the new EU 2019/6 Regulation on Veterinary Medicinal Products, adopted in December 2018 and implemented in 2022, will change the situation as some requirements, especially GMP production, will become mandatory. Then autogenous vaccines will partially fall under the European regimen. The oral presentation will develop the requirements from the recommendation paper and from the new regulation.

Serotype and genotype of *Streptococcus suis* isolates from the United States serve as predictors of pathotype

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Streptococcus suis, an increasingly important primary pathogen of swine in the United States, is composed of both pathogenic and commensal strains. Serotyping and multilocus sequence typing (MLST) are widely used methods for subtyping strains, but little is known about the current subtype distribution of *S. suis* strains in the United States. Furthermore, subtyping methods cannot be reliably used to predict the virulence potential of *S. suis* isolates. The objective of this study was to characterize the diversity of 208 *S. suis* isolates collected between 2014 to 2017 across North America (mainly the United States) by serotyping and MLST. Isolates were also characterized by pathotype, based on clinical information and site of isolation, as pathogenic (from neurologic or systemic tissues), possibly opportunistic (from lung samples), or commensal (from healthy pigs). We further investigated associations between subtype (serotype and MLST) and pathotype classifications using odds ratio and phylogenetic analyses. Nineteen serotypes were identified in this sample set and the predominant serotype was 1/2, with 80.4% of this serotype classified as the pathogenic pathotype. Fifty-eight sequence types (ST) were identified and the predominant ST was ST28, with 80.8% of ST28 isolates classified as the pathogenic pathotype. All of ST1 isolates were also classified as the pathogenic pathotype. These results demonstrate the use of serotyping and MLST to differentiate pathogenic from commensal isolates and establish links between pathotype and subtype, thus increasing the knowledge on *S. suis* strains circulating in the United States.

Immune-based strategies for control of avian influenza

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The Canadian poultry industry has suffered significant losses due to outbreaks of avian influenza viruses. In addition to biosecurity, which is the primary strategy for control of AIV infections, other approaches such as vaccination may also be envisaged. Although vaccines have been used with some success, they suffer from a few drawbacks, including their inability to completely curtail virus replication and its shedding into the environment. Hence, developing novel and rational vaccines is warranted which can curtail virus shedding. AIV replicates in mucosal tissues, including the respiratory and gastrointestinal systems. Therefore, it is logical to develop vaccines or other immune-based strategies that can induce mucosal immune responses against this virus. To this end, have developed immune-based strategies, including vaccines and antiviral compounds to reduce avian influenza virus shedding from infected chickens. Further, we have attempted to optimize formulations and routes of administration of these vaccines and prophylactic antiviral compounds. In this talk, I will provide an overview of these strategies and their mechanisms of action.

Creating a directed acyclic graph to identify confounders in cysticercosis research

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Cysticercosis is a neglected tropical disease caused by the zoonotic tapeworm *Taenia solium*. Although it does not cause significant health problems in the pig intermediate host, it may cause economic losses because infected pigs and their meat could be difficult to sell. Additionally, it can cause severe neurologic disease in humans if they become accidental hosts to the intermediate stage. Transmission of *T. solium* could be interrupted by targeting factors that affect contact between pigs and humans. However, to maximise the effectiveness of potential interventions, it is paramount to estimate the magnitude of the association between these factors and the outcome while considering potential confounders and colliders. Directed acyclic graphs (DAG) were developed to model the causative network for cysticercosis and determine minimally sufficient sets for adjustment for four key behavioural factors (handwashing, latrine availability, pig roaming, and pork cooking method). The DAGs were constructed based on a systematic literature search identifying studies investigating factors associated with *T. solium*'s epidemiology in humans and/or pigs published before June 2018. A total of 1,713 publications were identified, 356 were reviewed in full, and 130 were included to determine the minimum sufficient set for each key behavioural factor. Effect estimates could not be combined in meta-analyses due to high levels of heterogeneity in diagnostic tests, methods, settings and study quality. The DAGs are constructed on a framework of one infectious/life cycle modeled as a causal network that begins and ends with parasitic eggs in the environment at different timepoints, with a dead-end branch for human cysticercosis (the DAG outcome). This project is innovative in its use of DAGs to identify minimal sufficient sets for adjustments of an infectious outcome.

Porcine enteric virome characterisation using high throughput sequencing

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Our understanding of the enteric porcine microbiota has improved in recent years, thanks mostly to the characterization of its bacterial components. However, the viral constituents (the virome) remains poorly characterized. Nonetheless, specific viruses, such as rotavirus A, astroviruses, caliciviruses, circoviruses and adenoviruses have been extensively studied. These are common in pigs where they are generally thought to be related, or highly suspected to cause swine diarrhea. Apart from these viral pathogens, we know little about the virome evolution in piglets from farrowing to the end of the fattening period, and if diarrhea affects the viromes' diversity. Unlike the 16S ribosomal RNA sequence analyses commonly used to characterise bacteria and archaea, viruses lack conserved or common sequences. To overcome this limitation, a shotgun metagenomics approach was conducted on the porcine feces' samples collected in this study. A total of 12 piglets, 5 of which had diarrhea symptoms at farrowing, were monitored at 4 different life stages: farrowing, nursing and twice during the fattening period. Viral nucleic material was extracted, purified, amplified and then sequenced on an Illumina MiSeq platform. Reads were then assigned a taxonomic identifier using Kraken 2.0 and the "nt" database from NCBI. The beta-diversity between the four life stages were significantly different, except during the fattening period. Viral diversity between diarrheic and healthy individuals in the farrowing farms was similar. Caliciviridae and picornaviridae were predominantly found in the farrowing and nursing life stages. Picobirnaviridae, circoviridae and parvoviridae were found in all life stage apart from farrowing. Bacteriophages families, such as siphoviridae, microviridae and myoviridae were found in all 4 life stages. These results indicate that the pig`s enteric virome varies with age, despite the fact that specific viral families were found at multiple life stages.

Contribution of carcass surface microbiota in the contamination of pig carcasses in a slaughterhouse in Quebec

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Microbial communities on pork carcasses during slaughter are far from being fully described and, when done, it is based on more or less specific bacterial counts (indicators). The aim of this project was to compare the microbiota of different pork carcass batches according to sampling site (the top or the bottom part of carcass), sampling time (morning/afternoon) and before/after a crucial step of the slaughtering process (chilling) in a single facility. This was achieved by enumerating bacterial indicators (mesophilic aerobic bacteria, *enterobacteria*, *Escherichia coli*, *Pseudomonas* and lactic bacteria), by detecting Salmonella and by characterizing the surface microbiota using 16S rRNA gene amplicon sequencing (V4 region) for 26 carcasses. Results showed that bacterial counts of indicators were significantly higher in the lower carcass part and decreased after chilling (with the exception of *Pseudomonas*) and there was no influence of the sampling time during slaughtering. Salmonella was also more detected in the lower carcass part and was no longer present after the chilling. Microbiota analyses demonstrated that *Proteobacteria*, *Firmicutes*, *Bacterioidetes* and *Actinobacteria* were the most abundant phyla on the sampled carcasses. Interestingly, richness/diversity and the microbiota structure were not significantly different between the top and bottom of carcasses. Moreover, surfaces microbial communities did not significantly differ according to the carcass batches. Taken together these results suggested that despite the quantitative differences observed, the slaughter process seems to

homogenize the carcass surface microbiota. Considering the well-established inter-batches diversity of the pork intestinal microbiota, we can suggest that the contribution of intestinal content to overall carcass spoilage is well controlled by the slaughter practice in place in this facility.

Development of a protocol for colonization of the intestinal tract of chicks

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Introduction: The gastrointestinal tract of chickens is colonized by bacteria that are important for health. First colonisers can influence immune system immunity. To date, microbiota manipulation in chicks had limited success. Objective: To develop a protocol of colonization of newly hatched chicks using different profiles of cecal microbiota. Methods: 240 Cobb eggs were dived into 4 groups: Conventional: cecal content of broilers raised under conventional production system.
Organic: cecal content of organically raised layer hens with open access to pasture. Autoclaved: autoclaved cecal content of organic layer hens. Control no cecal content. The cecal content of 10 donors was diluted in 1L of saline, which was spread on eggs the day before hatching, given by gavage (1 drop) after hatching and mixed with the drinking water. 10 birds per group were euthanized at day 2, 7, 14, 28 and 42. Next generation sequencing was used to characterize cecal bacterial communities. Results: Cecal microbiota composition at D7, D14, D28 and D42 was statistically different between treatments ($P<0.001$) and more similar to the donor's bacteria. Time of sampling (age) also impacted the cecal microbiota ($P<0.001$). Conclusions: This protocol was efficient to induce colonization with the donors' microbiota. Age and source (donor) influenced the cecal microbiota of chickens during the production cycle. Initial colonization had long term impact on the cecal microbiota. The data generated in this study can be used for the development of microbiome-based interventions to enhance performance and to prevent diseases in commercial flocks.

Characterization of the granulocyte-colony stimulating factor (G-CSF) response induced during the *Streptococcus suis* infection

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Streptococcus suis serotype 2 is an important porcine bacterial pathogen and emerging zoonotic agent. Infections induce an exacerbated inflammation that can result in sudden death, septic shock and meningitis. Though neutrophilic leukocytosis characterizes *S. suis* infection, the mediators involved are poorly understood. Among them, granulocyte-colony stimulating factor (G-CSF), a pro-inflammatory cytokine, triggers neutrophil proliferation and mobilization. However, the systemic production of G-CSF induced during *S. suis* serotype 2 infection, the cell types involved, and the underlying mechanisms remain unknown.
In a *S. suis* serotype 2 mouse model of systemic infection (septic shock), plasma levels of G-CSF rapidly increased after infection. Studies reports endothelial cells and neutrophils as sources of G-CSF in various immunological contexts, but little is known regarding the capacity of dendritic cells (DCs) and macrophages to produce G-CSF, yet these cell types are central to the *S. suis*-induced inflammation. We demonstrated herein that *S. suis* infection of DCs and macrophages results in important and comparable production levels of G-CSF, as measured by ELISA. Based on these results, we

evaluated the role of certain *S. suis* virulence factors in G-CSF production and their interactions with the Toll-like receptor (TLR) pathway, that is known to be involved in *S. suis* recognition. Our results showed that G-CSF production occurs via TLR2 activation by subcapsular lipoproteins and *S. suis* DNA internalization. Then, these signals activate NF-κB and MAPK pathways.
In conclusion, this study demonstrated for the first time that *S. suis* induces G-CSF production in vivo and in vitro by DCs and macrophages via the binding of lipoproteins to TLR2. The implication of other virulence factors and TLRs, the role of the G-CSF in vivo and particularly its influence on neutrophils during the course of *S. suis* infection are currently under evaluation.

Inactivation of yqhG in extraintestinal *Escherichia coli* reduces expression of type 1 fimbriae

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Les *E. coli* pathogènes extra-intestinaux sont responsables d'un large éventail d'infections invasives chez l'homme et les animaux, conduisant souvent à une septicémie. Les fimbriae de type 1, codés par les gènes *fim*, sont l'un des facteurs de virulence les plus importants impliqués dans la pathogenèse des *E. coli* pathogènes aviaires et des E. coli uropathogènes. Nous nous intéressons à comprendre davantage la régulation des fimbriae type 1 chez les ExPEC. Pour déterminer les facteurs impliqués dans l'expression des fimbriae type 1, nous avons développé un système rapporteur luciférase (système lux) à simple copie fusionnée à la région promotrice de l'opéron *fim*, qui est situé sur un élément inversible, *fimS*. L'utilisation du système lux permet de mesurer l'activité des promoteurs et d'autres éléments régulateurs de la transcription, ainsi que les effets des activateurs et des inhibiteurs. Dans ce contexte, nous avons généré trois banques de mutants par transposition chez la souche CFT073, le mutant pst et la souche sauvage avec une fusion transcriptionnelle du promoteur *fimS* verrouillé en phase ON. Nous avons par la suite criblé les mutants générés par le transposon Tn10 pour le niveau de production des fimbriae type 1 suivi de séquençage à haut débit afin d'identifier des gènes qui, lorsqu'ils sont altérés, affectaient l'expression des fimbriae de type 1. Nous avons montré que *yqhG*, codant pour une protéine de fonction inconnue, est l'un des médiateurs importants contribuant à une diminution de l'expression des fimbriae de type 1 chez la souche CFT073. Nos résultats démontrent que la délétion de *yqhG* altère l'expression des fimbriae de type 1 et diminue significativement la capacité du mutant à coloniser le tractus urinaire murin. De plus, l'atténuation de la virulence du mutant *yqhG* est concomitante avec la répression de l'expression des fimbriae de type 1. Nous avons démontré aussi que le mutant *yqhG* est plus sensible au stress oxydatif.

The effect of *Streptococcus suis* on B lymphocytes and the development of the humoral response

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Streptococcus suis is an important swine pathogen for which no effective vaccine exists. Previous reports showed that infected animals produce low antibody levels against the bacteria, suggesting an impaired immune response. *S. suis* is known to interfere with antigen-presenting cells and downstream primary and memory responses of T cells. No studies evaluated the interactions between *S. suis* and B cells, key players of the humoral response. Our objective was to determine if *S. suis* modulates the antibody response by interfering with B cell activation. To this aim, mice were infected with successive doses of *S. suis*, and blood samples were taken to monitor bacteremia and antibody production. Infection with two bacterial doses is required to induce maximal production of antibodies which can reduce, but not

completely clear, the infection. A memory IgM response along with a mixed Th1 (IgG2b, IgG2c) and Th2 (IgG1) response is observed in the serum of mice infected multiple times. However, the avidity of the produced IgG isotypes targeting *S. suis* antigens does not increase between successive infections, suggesting an impaired affinity maturation. To evaluate the interactions between *S. suis* and immune cells, mouse splenocytes were isolated 1-week post-infection and activated *ex vivo* for seven days. Production of IL-6, a mediator of B cell activation is not increased in splenocytes from infected mice, independently of the number of bacterial doses. However, production of the regulatory cytokine IL-10 is enhanced in cells from infected mice. Only IFN-gamma and IgG production were increasingly higher following repeated infections. These results suggest that B cell activation and germinal center reactions might be partially impaired or delayed in the cases of *S. suis* infections. Further *in vivo* studies are ongoing to dissect the inner workings of the germinal center reactions following *S. suis* infections.

Characterization of two new SPATE autotransporters and cumulative role of SPATEs in pathogenesis of Extra-intestinal pathogenic *Escherichia coli*

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Serine protease autotransporters of *Enterobacteriaceae* (SPATE) are associated with various pathogenic extraintestinal pathogenic *Escherichia coli* (ExPEC) including avian pathogenic E. coli (APEC). We have identified two new genes encoding SPATE proteins, located adjacent to each other on a genomic island in an APEC strain (5 SPATES in total), and we refer to them as "tandem autotransporter genes, *tagB*, and *tagC*". Interestingly, these autotransporter genes are present in some APEC and also some human uropathogenic *E. coli* (UPEC). The possible function and roles of these new SPATES in the pathogenesis of ExPEC were investigated.
Clones of these proteins were tested for various phenotypes including adherence to human renal and bladder cell lines, biofilm formation, autoaggregation, cytotoxicity and hemagglutination, which represent possible mechanisms of colonization of the host. Results showed that these SPATEs are autoaggregating and can promote adherence to the HEK 293 renal and 5637 bladder cell lines, but did not contribute significantly to biofilm production or hemagglutination. TagB and TagC exhibited cytopathic effects on the bladder epithelial cell line. Following transurethral infection of CBA/J mice with a *tagBC* mutant, no significant difference in colonization was observed. However, the competitive fitness of a mutant derivative lacking all 5 SPATEs was significantly lower in the kidney. This underlines the potential cumulative role of SPATEs for survival and competitive fitness during extra-intestinal infection.

The increased permeability and inflammartory response of porcine intestinal epithelial cells caused by *Escherichia coli* and Salmonella enterica *Typhimurium* infections are decreased in presence of bovine colostrum

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Gastroenteric infections caused by *Escherichia coli* and Salmonella enterica *Typhimurium* are the source of important losses in porcine industry. The use of antibiotics in piglets' food to prevent these infections is raising many concerns, leading the government to call for finding alternatives that could allow the control of these infections. Bovine colostrum is loaded with bioactive pro-immune components that could mediate anti-microbial activities. Therefore, we tested the

effect of complete bovine colostrum (BC) and bovine colostrum sero or casein purified fractions (SC and CAS respectively) on porcine intestinal epithelial cell (IPEC J2) response to the two pathogens. Both pathogens increased the cell monolayer permeability 4h following the infection, as determined by TEER assays. After 2h, the expression of IL8, IL6, TNFA, CCL20, CXCL2 and CXCL10 was increased by both pathogens, while IL1B, CCL5 and SAA2 were only induced by Salmonella, as measured by Q-PCR. BC treatment prevented the monolayer disruption caused by both pathogens, while SC only prevented the one caused by Salmonella. BC and SC reduced the induction of IL8, CCL20 and CXCL2 genes by *E. coli* ($p<0,05$). Both BC and SC reduced the expression of IL6 and CCL20 genes by Salmonella ($p<0,05$), while only BC reduced the induction of IL8, IL6, CCL20, IL1B, SAA2 ($p<0,05$) and TNFA ($p<0,1$). Altogether, these results show that the complete form of bovine colostrum can reduce both *Escherichia coli* and Salmonella enterica *Typhimurium* infections of porcine intestinal epithelial cells and could be a good alternative to antibiotics in order to control these infections.

Field study on the immunological response induced by autogenous vaccines used in sows or in piglets to control post-weaned *Streptococcus suis* infections

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Streptococcus suis, one of the most important bacterial pathogen in weaned piglets, is responsible for serious economic losses in the pork industry. Today, only autogenous vaccines composed of killed bacteria (bacterins) are available. Their cost is low but the ability to protect is controversial and field studies are missing. We report for the first time a scientifically sound and comparative field study on the immunological response induced by an autogenous vaccine in piglets or sows. Using a sow herd with recurrent *S. suis* problems, the study was divided in two parts: I) Piglets from non-vaccinated sows received an autogenous bacterin at 1 and 3 weeks of age and sera were collected at 1, 3, 5 and 8 week-old. II) Sows received the same vaccine at 5 and 3 weeks pre-farrowing, and sera collected from sows and from their piglets as above. Antibody responses (and isotypes) were analyzed by ELISA and antibody-protective effect evaluated by an opsonophagocytosis assay (OPA). Vaccination of piglets failed to induce an active immune response even after two vaccine doses. In the 2nd part of the study, high levels of antibodies (mainly maternal-derived) with marked OPA activity were observed in piglets at 1st week of age, independently of the presence or absence of sow vaccination. Indeed, only a slight increase of antibodies was noticed in vaccinated sows. Maternal immunity rapidly dropped by 3 weeks of age and stayed negative until 8-week-old, indicating possible absence of antibodies in the post-weaning high-risk period. Vaccine formulation used in this study promoted IgG1 production, while IgG2 is known to be more protective against *S. suis*. Clinical data from these trials is currently under analysis. Overall, an active or a passive piglet vaccination program with the autogenous bacterin used herein mostly failed to induce lasting protection in post-weaned piglets. To be able to protect piglets at this age-window, an improvement of vaccine formulation may be required.

Determination of capture efficiency against viruses of mechanical and antimicrobial filters using artificially generated viral aerosols

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Air transmission of pathogenic agents is responsible for epidemic episodes in swine herds and, complementary to biosecurity measures already in place, filtration may reduce particles potentially transporting animal and/or human pathogens. The minimum efficiency reporting value (MERV) system classifies filters for their capture efficiency against inorganic salt particles but information regarding a commercial filter's ability to reduce bioaerosols is still exploratory. The objectives of the present study were to: 1) develop a protocol to prepare phage-spiked dust with properties similar to standard dust 2) artificially generate viral aerosols similar in size to those in swine buildings as bioaerosols exist in farms as aggregates of microorganisms and other organic material and 3) use artificial viral aerosols to evaluate the capture efficiency of different filters. Bacteriophages (MS2 or PhiX174) were combined with cryoprotectants (sucrose, glycerol, or milk) and standard dust then lyophilized. Higher concentrations of MS2 were obtained and sucrose provided the highest infectious rate after lyophilization. A size distribution of particles similar to those in swine buildings was obtained using A3 medium test dust (ISO-12103-1). MERV 16-rated and 15 layer antimicrobial (AM) filters were challenged inside an ASHRAE standard 52.2 test duct. Up- and downstream aerosols were collected isokinetically (AGI-30 and OPS). Results indicate a difference in reduction efficiency of particles between the two filter types (96% MERV 16 and 49% AM). Less difference was observed in efficiencies of removal for MS2 genomes, 99% and 83%, and little difference when examining efficiencies for removing infectious MS2, 99% and 98% for MERV 16 and AM, respectively. Transmission Electron Microscopy of MS2-dust revealed that the bacteriophages were primarily associated with the test dust particles.

The effect of host genetic on pro-inflammatory responses in chickens against T cell-dependent and T cell-independent antigens

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Poultry industry incurs an enormous cost for challenging with outbreaks of infectious diseases despite the vaccination and biosecurity protocols. In addition, new regulations to reduce the use of antibiotics is also encouraging the industry to seek alternative approaches. Improving host defense through direct selection for resistance to pathogens or immunocompetence is an alternative approach. Comparing the immune response profile of chickens with distinct genetic architecture can shed light on the genetic regulation of immunocompetence and molecular mechanisms that lead to variation. Previously, we demonstrated the effect of the genetic background on the magnitude of antibody and cellular immune response in chickens. To understand the mechanisms that are associated with variation of immune responses in chickens, the expression of interleukin (IL)-4, IL-6, IL-18, IFN-γ and iNOS in spleen after exposure to sheep Red Blood Cells (sRBC) and *Brucella abortus* (BA) was investigated in the current

study. sRBC and *B. abortus* are not natural pathogens of chickens, which would eliminate the effect of previous exposure. The cytokine expression was measured by TaqMan real-time PCR in five populations (Ross, Cobb and three Iranian indigenous strains) of chicken (n = 7 per experimental unit (2 levels in exposure time* 3 levels in sample collection time* 2 levels in treatment), in total 84 chickens per population). The expression of IL-6, IL-18, iNOS, and IFN-γ was higher in Ross. The kinetics of expression of iNOS and IFN-γ was similar with a peak at 6 hours post challenge while the expression of IL-6 and IL-18 peaked at 2 hours with gradual decrease up to 10 hours post-challenge, in Ross. The overall profile of the expression was associated with each population genetic structure. However, our data did not demonstrate a distinct difference between indigenous versus commercial populations.

POSTER ABTSRACTS
4th INTERNATIONAL WORKSHOP ON *STREPTOCOCCUS SUIS*

1. Antimicrobial resistance of *Streptococcus suis* isolates recovered from healthy pigs at different stages of production

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The objective of this study was to investigate antimicrobial resistance in *S. suis* isolates recovered from healthy pigs. Three hundred and four *S. suis* isolates recovered on 28 Ontario swine farms were tested for antimicrobial susceptibility by agar disk diffusion method. The isolates were recovered from tonsil, nasal cavities, and genital tracts of 194 healthy pigs at different stages of production. Overall, 94.4% of isolates demonstrated resistance to one or more antimicrobials. Low prevalence of resistance (< 0.1%) was found to ampicillin, ceftiofur, and florfenicol, while high to moderate resistance seen against tetracycline (82.9%), tiamulin (63.8%), spectinomycin (38.5%), and trimethoprim/sulfa (14.8%). Isolates from nursery piglets were 5.4 times more likely to have resistance to tetracycline in comparison to suckling piglets ($P<0.05$). There was no significant difference between other stages of production. Isolates from nursery piglets were 6.8 times more likely to have resistance to tiamulin than isolates from finisher pigs ($P<0.05$). There was no significant difference found for resistance to spectinomycin between production stages. The most common resistance profile seen among all isolates was tetracycline-tiamulin (23.7%). The most prevalent serotypes with their most common resistance profiles were as follows: serotype 31 (tetracycline-tiamulin), serotype 5 (tetracycline-tiamulin), and serotype 21 (tetracycline). There was a high to moderate prevalence of resistance to antibiotics commonly used in nursery pig diets such as tetracycline, tiamulin, and spectinomycin. On the other hand, there was a low prevalence of resistance to antimicrobials that are only used as parenteral treatments and not added to feed (e.g., ceftiofur, florfenicol). These findings should help practitioners in choosing appropriate drugs for use on Ontario swine farms.

2. Antigen I/II participates in the interactions of *Streptococcus suis* serotype 9 with phagocytes and the development of systemic disease

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Streptococcus suis is an important porcine bacterial pathogen and a zoonotic agent causing a variety of pathologies including sudden death, septic shock, and meningitis. Though serotype 2 is the most studied serotype due to its presence worldwide, serotype 9 is responsible for the greatest number of porcine cases in Spain, the Netherlands, and Germany. Regardless of its increasing importance, very few studies have investigated *S. suis* serotype 9 virulence factors and pathogenesis. Antigens I/II (AgI/II) are multimodal adhesion proteins implicated in host respiratory tract and oral cavity persistence of various pathogenic human streptococci. We recently demonstrated that AgI/II is involved in various bacterial functions for serotype 9, participating in the initial steps of the pathogenesis of the infection. However, its contribution to the systemic infection remains unknown. As such, we evaluated herein the role of the *S. suis* serotype 9 AgI/II in the interactions with phagocytes and the development of systemic disease in a mouse model of infection. Results demonstrated that the presence of AgI/II is important for the development of clinical systemic disease by promoting bacterial survival in blood possibly due to its effect on *S. suis* phagocytosis,

as shown with macrophages and dendritic cells. Furthermore, AgI/II directly participates in dendritic cell activation and pro-inflammatory mediator production following recognition by the Toll-like receptor pathway, which may contribute to the exacerbated systemic inflammation responsible for host death. Taken together, this study demonstrates that the *S. suis* serotype 9 AgI/II is important for virulence during systemic infection and development of disease. In fact, this is the first study to describe a role of an AgI/II family member in systemic bacterial disease.

3. Characterization of the role of monocytes and neutrophils during *Streptococcus suis* systemic and central nervous system infections

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Streptococcus suis is an important porcine bacterial pathogen and a zoonotic agent responsible for causing sudden death, septic shock, and meningitis. These pathologies are the consequence of elevated bacterial replication leading to exacerbated inflammation, which is a hallmark of the *S. suis* systemic and central nervous system (CNS) infections. Though monocytes and neutrophils are amongst the most important innate immune blood cells and that they massively infiltrate the CNS during *S. suis*-induced meningitis, their role during infection caused by this pathogen remains unknown. As such, the role of monocytes was determined using CCR2-/- and Nr4a1-/- mice, which lack Ly6Chigh inflammatory and Ly6Clow circulating monocytes, respectively, while neutrophils were depleted using anti-Ly6G neutralizing antibodies. Results demonstrated that neutrophils, and to a lesser extent inflammatory monocytes, but not circulating monocytes, participate in *S. suis*-induced systemic disease via their role in inflammation required for controlling bacterial burden control. Meanwhile, monocytes partially contributed to the exacerbation of *S. suis*-induced CNS inflammation while neutrophils participated in CNS bacterial burden control. However, development of clinical CNS disease was independent of both cell types, suggesting that resident immune cells are mostly responsible for *S. suis*-induced CNS inflammation leading to clinical signs. Consequently, this study demonstrates that though monocytes and neutrophils contribute to the *S. suis* systemic infection, they play differential roles during the CNS disease.

4. *Streptococcus suis* suilysin disrupts integrity of the pig tracheal epithelial barrier

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Streptococcus suis is a bacterial pathogen that colonizes the upper respiratory tract of pigs. It is known to cause severe infections such as septicemia, meningitis, arthritis, and endocarditis in pigs and is responsible for major economic losses in the swine industry worldwide. To better understand the interactions between *S. suis* and the porcine respiratory epithelium, we investigated the ability of this pathogen to damage the tracheal epithelial barrier. We showed that *S. suis* can compromise the integrity of the tracheal epithelial barrier as determined by measuring transepithelial electrical resistance and FITC-dextran transport. As a consequence of this breakdown, *S. suis* can translocate across an epithelial cell monolayer. On the other hand, a *S. suis* mutant deficient in the production of suilysin, a cholesterol-dependent cytolysin, did not cause any damage to the epithelial barrier. In addition, a recombinant suilysin disrupted the integrity

of the tracheal epithelial barrier. Immunofluorescence staining suggested that suilysin affects two major tight junction proteins (occludin and zonula occludens-1). In summary, *S. suis* is able to compromise the function of the porcine respiratory epithelial barrier through the action of suilysin. This enhanced understanding of the interactions between *S. suis* and tracheal epithelial cells may help in the development of novel strategies to prevent the invasion of the epithelium by this and other swine respiratory pathogens.
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5. Effect of ceftiofur treatment in pigs on the nasal microbiota and *Streptococcus suis* carriage

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Streptococcus suis (Ss) is one of the most important bacterial pathogens in swine production. Due to the lack of effective vaccines, antimicrobial, and especially beta-lactams, are commonly used to prevent or decrease the losses caused by this microorganism. The goal of this study was to characterize the effect of the use of antimicrobials in sows or piglets on the microbiota and the carriage of Ss in the nasal cavity.
A longitudinal study with 363 piglets from birth to 8 weeks of age was conducted. Sows were housed in 3 different rooms: 1) TS-NTP, 11 sows treated with ceftiofur (CEFT) 3-6 days before farrowing and non-treated piglets; 2) NTS-TP, 9 non-treated sows and piglets treated with CEFT at birth ; and 3) NTS-NTP, control group of 10 non-treated sows and non-treated piglets. Nasal swabs from 6 piglets per litter were collected at birth, 7, 15, 21 and 49 days of age. Swabs from 1 piglet per litter was processed for Ss isolation. When Ss-like colonies were observed, up to 4 colonies were selected for identification and characterization. Swabs from the other 5 piglets per litter were processed for DNA extraction. DNA was used in Ss, ser2 and ser9 PCR assays. In addition, DNA from five animals per group at each time-point was subjected for NGS 16S rDNA gene sequencing.
Microbiota analysis showed that the CEFT treatment, when administer in sows or piglets, produced a temporal increase in microbial diversity at day 7, which was not observed at the time of weaning. In NTS-NTP control group, the microbiota diversity at day 7 was lower than in TS-NTS and NTS-TS, but increased at weaning. Detection of *S. suis* in nasal samples at birth was 30% lower in the piglets from TS. Although Ss was detected by PCR in almost all samples, Ss isolation was higher at day 15. Ss-ser9 was more prevalent in the piglets than ser2. Strain characterization showed up to 11 different strains before weaning, with a different predominant strain in each group and time-point.

6. Characterization of the granulocyte-colony stimulating factor (G-CSF) response induced during the *Streptococcus suis* infection

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Streptococcus suis serotype 2 is an important porcine bacterial pathogen and emerging zoonotic agent. Infections induce an exacerbated inflammation that can result in sudden death, septic shock and meningitis. Though neutrophilic leukocytosis characterize *S. suis* infection, the mediators involved are poorly understood. Among them, granulocyte-colony stimulating factor

(G-CSF), a pro-inflammatory cytokine, triggers neutrophil proliferation and mobilization. However, the systemic production of G-CSF induced during *S. suis* serotype 2 infection, the cell types involved, and the underlying mechanisms remain unknown.
In a *S. suis* serotype 2 mouse model of systemic infection (septic shock), plasma levels of G-CSF rapidly increased after infection. Studies reports endothelial cells and neutrophils as sources of G-CSF in various immunological contexts, but little is known regarding the capacity of dendritic cells (DCs) and macrophages to produce G-CSF, yet these cell types are central to the *S. suis*-induced inflammation. We demonstrated herein that *S. suis* infection of DCs and macrophages results in important and comparable production levels of G-CSF, as measured by ELISA. Based on these results, we evaluated the role of certain *S. suis* virulence factors in G-CSF production and their interactions with the Toll-like receptor (TLR) pathway, that is known to be involved in *S. suis* recognition. Our results showed that G-CSF production occurs via TLR2 activation by subcapsular lipoproteins and *S. suis* DNA internalization. Then, these signals activate NF-κB and MAPK pathways.
In conclusion, this study demonstrated for the first time that *S. suis* induces G-CSF production in vivo and in vitro by DCs and macrophages via the binding of lipoproteins to TLR2. The implication of other virulence factors and TLRs, the role of the G-CSF in vivo and particularly its influence on neutrophils during the course of *S. suis* infection are currently under evaluation.

7. Resistance and virulence profiling of *Streptococcus suis* isolated from diseased pigs in Brazil

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Introduction The S. suis is one of most important pathogens in the swine industry worldwide that causes systemic infections in swine and humans. The objective of this study was to characterize the antimicrobial resistance profiles and virulence markers of Brazilian S. suis strains isolated from 2001 to 2016.
Materials and Methods A total of 215 S. suis strains were isolated from pigs with different clinical signs, from nine different Brazilian states during 2001 to 2016. The identification was performed by PCR and MALDI-TOF MS analysis. The virulence profiles were identified by PCR screening of four virulence genes (sly, arcA, epf, mrp). The antimicrobial resistance profile was characterized by broth microdilution and the minimum inhibitory concentrations (MIC) were assessed for ampicillin, ceftiofur, penicillin, doxycycline, oxytetracycline, marbofloxacin, enrofloxacin, florfenicol, spectinomycin, gentamicin, neomycin, sulfadimethoxine, trimethoprim sulfamethoxazole, clindamycin, tylosin, tilmicosin, tulatromycin, tiamulin.
Results Among the 215 strains, 78.1% were positive for the detection of gene sly, 50.2% for epf, 84.2% for mrp, including three variants of this gene, and 98.1% for arcA. Four strains were negative for all genes. The combination of these genes resulted in 11 virulence profiles. High rates of resistance to tetracyclines, macrolides, clindamycin and sulfadimethoxine were observed and 30% of the strains were resistant to aminoglycosides, while the most effective antimicrobials were the β-lactams, fluoroquinolones, tiamulin and florfenicol. A total of 72% of the samples presented multiresistance (resistance to three or more antimicrobials classes).
Conclusion These results are relevant to understand the behavior of S. suis in Brazilian swine industry. The high genetic variability seen in virulence patterns, as well as the multiresistance rate identified indicates the importance of better approaches to treating sick animals and prevents outbreaks.

8. Co-transfer of the phenicols-oxazolidinones (PhO) resistance gene optrA and macrolide-lincosamide-streptogramin B (MLSB) resistance gene erm(B) between *Streptococcus suis* serotypes

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optrA, a novel transferable oxazolidinones resistance gene, has been detected together with MLSB resistance gene *erm (B)* in *Streptococcus suis* with an increasing rate in China. The aim of this study is to evaluate the co-transfer of *optrA* and *erm(B)* among *S. suis* strains with different serotypes. The *erm(B)*/*optrA*-carried strains were investigated by PCR and sequencing. Filter conjugating assays were applied to evaluate the interserotypic transferability of *erm(B)* and *optrA* from donor strain SH0918 with serotype 5 to recipient *S. suis* serotype 2 P1/7RF. The conjugant was further confirmed by PCR, sequencing and resistance phenotype to oxazolidinones and MLSB. Mating frequency was calculated by dividing the number of transconjugants by that of recipient. Among 320 tested isolates, a total of 75 isolates were detected to possess both *optrA* and *erm(B)* genes. The donor strain *S. suis* SH0918 carrying *erm(B)*/*optrA* can undergo conjugative transfer from *S. suis* serotype 5 to serotype 2 strain (SScSH0918) with the frequency of 1.77×10-10. The transconjugants exhibited the resistance phenotype to erythromycin and florfenicol. The sequencing results showed an ICE also transferred into recipient cells indicating that it mediated the transfer as a carrier of *optrA* and *erm(B)*. The above results suggested that the co-transfer of *erm(B)*/*optrA* might have contributed to the wide spread of MLSB-PhO resistance among *S. suis*. The extensive use of MLSB antibiotics in veterinary clinic might be a selector for oxazolidinones resistance, which need to pay much attention to.

9. The ICE/IME Finder approach and its application to ICE/IME annotation in *Streptococcus*

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Mobile genetic elements play a key role in bacterial genome evolution by enabling gene acquisition through horizontal gene transfer. Among these elements, Integrative Conjugative Elements (ICEs) and Integrative Mobilizable Elements (IMEs) are integrated in the chromosome of their hosts and transferred by the conjugation machinery. Transfer and maintenance into the recipient cell are the two biological functions indispensable for ICEs and IMEs. Genes and sequences involved in these functions are physically close on the DNA molecule and organized as functional “modules”. Two characteristics of ICEs/IMEs and related elements can be enlightened: (i) they evolve rapidly mainly through acquisition, loss and exchange of modules and (ii) they are frequently integrated in tandem arrays or can be nested, resulting to fuzzy bounds. Consequently, the detection and accurate annotation of ICE/IME bounds is a difficult task that requires dedicated bioinformatics approaches. So far, two bioinformatics approaches allow to automatically detect and to annotate ICEs in bacterial genomes [Cury *et al.* 2017 ; Liu *et al.* 2019) but with imprecise bounds. None of them can detect nested or tandem elements. Moreover, none of these methods can accurately detect IMEs. Thus, we have designed a new approach for ICE/IME detection and annotation named “ICE/IME Finder”(Ambroset *et al.* 2016 ; Coluzzi *et al.*, 2017).
In this communication, we will present the ICE/IME Finder methods and will demonstrate its efficiency to annotate ICEs and IMEs within streptococcal genomes. Information collected in this work reveals the abundance and huge diversity of

ICE, IMEs, nested elements, and tandem arrays of elements within streptococcal genomes. Moreover, the analysis of genes they carry demonstrates that many ICEs and IMEs encode antimicrobial resistance. Therefore, the accurate annotation and delineation of these elements is essential to fully understand the spreading of antibiotic resistance genes.

10. Field study on the immunological response induced by autogenous vaccines used in sows or in piglets to control post-weaned *Streptococcus suis* infections

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Streptococcus suis, one of the most important bacterial pathogen in weaned piglets, is responsible for serious economic losses in the pork industry. Today, only autogenous vaccines composed of killed bacteria (bacterins) are available. Their cost is low but the ability to protect is controversial and field studies are missing. We report for the first time a scientifically sound and comparative field study on the immunological response induced by an autogenous vaccine in piglets or sows. Using a sow herd with recurrent *S. suis* problems, the study was divided in two parts: I) Piglets from non-vaccinated sows received an autogenous bacterin at 1 and 3 weeks of age and sera were collected at 1, 3, 5 and 8 week-old. II) Sows received the same vaccine at 5 and 3 weeks pre-farrowing, and sera collected from sows and from their piglets as above. Antibody responses (and isotypes) were analyzed by ELISA and antibody-protective effect evaluated by an opsonophagocytosis assay (OPA). Vaccination of piglets failed to induce an active immune response even after two vaccine doses. In the 2nd part of the study, high levels of antibodies (mainly maternal-derived) with marked OPA activity were observed in piglets at 1st week of age, independently of the presence or absence of sow vaccination. Indeed, only a slight increase of antibodies was noticed in vaccinated sows. Maternal immunity rapidly dropped by 3 weeks of age and stayed negative until 8-week-old, indicating possible absence of antibodies in the post-weaning high-risk period. Vaccine formulation used in this study promoted IgG1 production, while IgG2 is known to be more protective against *S. suis*. Clinical data from these trials is currently under analysis. Overall, an active or a passive piglet vaccination program with the autogenous bacterin used herein mostly failed to induce lasting protection in post-weaned piglets. To be able to protect piglets at this age-window, an improvement of vaccine formulation may be required.

11. Genome comparison of the capsule-negative *Streptococcus suis* isolates from endocarditis

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Streptococcus suis produces capsule (cap) which confers an ability to resist phagocytosis during the development of diseases. Therefore, cap-negative cells can easily be killed by phagocytes and are believed to be avirulent. However, we have found that some of the *S. suis* isolates from porcine endocarditis did not possess cap. The cap-negative cells showed a high degree of ability to adhere to porcine and human platelets. Furthermore, both cap-positive and -negative cells were proved to coexist in the same lesion of endocarditis by examination of multiple isolates from the same sample and by immunohistochemically staining. Comparisons of the nucleotide sequences of capsular polysaccharide synthesis (cps) genes between pairs of a cap-positive and a cap-negative isolates indicated that a pair of isolates from the same samples were the closest to one another compared with those isolated from other pigs, indicating that the cap-negative

mutants arose independently in each farm or pig body by means of spontaneous mutations in cps genes. Since multiple numbers of cap-negative isolates could be collected from some of the endocarditis lesions, we further compared those cap-negative isolates from the same pigs by whole genome sequencing. Although some cap-negative isolates from the same origin showed the same genome sequences, different mutations in cps genes and/or different SNPs in whole genomes were also found, indicating that multiple events of mutations occurred not only in cps genes but also in whole genomes. Since the capsule-negative isolates can rarely be found in lesions of other diseases such as meningitis, these results suggest that the endocarditis lesions are the niches to preserve various mutants that generally be eliminated under the selective pressures of host defense system. An ability of cap-negative mutants to adhere platelets could accelerate accumulation of fibrin that protected the mutants, leading to the appearance of them in the niches.

12. Genetic characterization and pathogenicity of vancomycin- and linezolid- resistant *Streptococcus suis* harboring *vanG* operon and *optrA*

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Vancomycin and linezolid are among the last-resort antimicrobial agents to treat infections caused by multidrug-resistant Gram-positive bacteria. Linezolid- and vancomycin-resistant (LVR) Gram-positive bacteria may pose severe threats to public health. In this study, three *vanG*- and *optrA*-positive *S. suis* were isolated from different farms. Antimicrobial resistance (AMR) profiles showed they also exhibited resistance or elevated MICs to 13 other antibiotics. Mobilome analysis revealed the presence of *erm(B)*, *aadE-apt-sat4-aphA3* cluster, *tet(O/W/32/O),* and *vanG* on an integrative and conjugative element, ICESsuYSJ17, and *erm(B)*, *aphA3*, *aac(6')-aph(2'')*, catpC194, and optrA on a prophage, ΦSsuYSJ17-3. ICESsuYSJ17 is mosaic ICE of ICESsuBSB6 and ICESsuHA681 and belongs to a highly prevalent and transferable ICESa2603 family in Streptococcus species. ΦSsuYSJ17-3 shared conserved backbone to a transferable prophage Φm46.1. A 6,568-bp composite transposon organized in IS1216E-*araC-optrA-hp-catp*C194-IS1216E structure was present on ΦSsuYSJ17-3. And translocatable unit (TU) verification PCR confirmed the formation of TU of IS1216E-*araC-optrA*-hp-*catpC*194 from this composite transposon. Vancomycin resistance phenotype and *vanG* transcription assays revealed the *vanG* operon was inducible. The virulence of the LVR strain YSJ17 was less virulent to that of *S. suis* serotype 2 virulent strain SC070731 in zebrafish infection model. To our knowledge, this is the first report of the co-existence of *optrA* and *vanG* operon in Gram-positive bacteria. Since it has been suggested that *S. sui*s may act as an AMR reservoir contributing to the spread of resistance genes to major streptococcal pathogens, the potential dissemination of these resistance genes among Gram-positive bacteria is worrisome and routine surveillance should be strengthened.

13. Enolase and dipeptidyl peptidase IV protein subunit vaccines are not protective against a lethal challenge with *Streptococcus suis* serotype 2 in a mouse model of infection

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Streptococcus suis is a major swine pathogen, causing arthritis, meningitis and sudden death in post-weaning piglets, but is also a zoonotic agent, mainly in South East Asia. *S. suis* comprises 35 different serotypes of which the serotype 2 is the most prevalent. Since the application of new policies regarding antibiotic use in pigs, vaccination has become the main tool used to reduce *S. suis* clinical cases. In the absence of commercial vaccines, autogenous bacterins are used in the field, with controversial results. In the past years, the focus has turned toward the development of subunit vaccine candidates, mostly surface proteins that are ideally present in most *S. suis* strains belonging to different serotypes. However, published results are sometimes contradictory concerning the protective effect of a same candidate, and the adjuvant used may significantly influence the protective capacity of a given antigen. This study focused on the dipeptidyl peptidase IV (DPPIV) and the enolase (Eno), which are both involved in *S. suis* pathogenesis. While contradictory protection results have been obtained with Eno, no data on the protective capacity of DPPIV was available. In this study, results showed that 86 % and 88 % of the 359 strains tested were positive for the expression of Eno and DPPIV, respectively, suggesting that they are widely expressed by strains of different serotypes. However, no protection was obtained after vaccination in a mouse model of infection, regardless of different adjuvants tested. Even though no protection was obtained, significant amounts of antibodies were produced against both antigens, regardless of the adjuvant used. These results showed that *S. suis* DPPIV and Eno do not seem to be good vaccine candidates, at least not under the conditions evaluated in this study. Further studies in the natural host should still be carried out. Moreover, this work highlights the importance of confirming results obtained by different research groups.

14. The effect of *Streptococcus suis* on B lymphocytes and the development of the humoral response

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Streptococcus suis is an important swine pathogen for which no effective vaccine exists. Previous reports showed that infected animals produce low antibody levels against the bacteria, suggesting an impaired immune response. *S. suis* is known to interfere with antigen-presenting cells and downstream primary and memory responses of T cells. No studies evaluated the interactions between *S. suis* and B cells, key players of the humoral response. Our objective was to determine if *S. suis* modulates the antibody response by interfering with B cell activation. To this aim, mice were infected with successive doses of *S. suis*, and blood samples were taken to monitor bacteremia and antibody production. Infection with two bacterial doses is required to induce maximal production of antibodies which can reduce, but not completely clear, the infection. A memory IgM response along with a mixed Th1 (IgG2b, IgG2c) and Th2 (IgG1) response is observed in the serum of mice infected multiple times. However, the avidity of the produced IgG isotypes targeting *S. suis* antigens does not increase between successive infections, suggesting an impaired affinity maturation. To evaluate the interactions between *S. suis* and immune cells, mouse splenocytes were isolated 1-week post-infection and activated *ex vivo* for seven days. Production of IL-6, a mediator of B cell activation is not increased in splenocytes from

infected mice, independently of the number of bacterial doses. However, production of the regulatory cytokine IL-10 is enhanced in cells from infected mice. Only IFN-gamma and IgG production were increasingly higher following repeated infections. These results suggest that B cell activation and germinal center reactions might be partially impaired or delayed in the cases of *S. suis* infections. Further *in vivo* studies are ongoing to dissect the inner workings of the germinal center reactions following *S. suis* infections.

15. Virulence-associated gene profiling of *Streptococcus suis* in the United States

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Identification of the virulence factors of *Streptococcus suis* is important for differentiating pathogenic from commensal strains and understanding the pathogenesis of *S. suis* infection. This enables the targeting of potential virulence factors for the prevention of *S. suis*. Currently, there are over 100 putative virulence factors, many of which have yet to be confirmed in experimental models. Much has been published on *S. suis* virulence factors worldwide, but information on the distribution of these factors in U.S. isolates is limited. In this study, we utilized a genomic approach to investigate the distribution of 66 virulence-associated genes (VAGs) in 208 U.S. isolates. The isolates were assigned into pathotypes (pathogenic, possibly opportunistic, and commensal) based on clinical information and site of isolation, and serotype and multilocus sequence type data was generated for each isolate. The predominant serotypes in the U.S. were serotypes 1/2, 3, and 7, and the predominant sequence types (STs) were ST1, ST28, and ST94. Clustering of isolates by VAG profiling generated four main clades, three of which appeared to be associated with pathotype. Clade A was mostly composed of pathogenic isolates subtyped as serotype 1/2 ST28, indicating a novel association for U.S. *S. suis* strains. Clade B was composed of pathogenic isolates characterized as serotypes 1 and 2 and ST1. Clade C was mostly composed of commensal and possibly opportunistic isolates belonging to numerous serotypes and STs. Our study expands the knowledge on *S. suis* strains in the U.S. and demonstrates the use of VAG profiling as a typing method for *S. suis*. This approach elucidated various genes capable of differentiating among pathotypes.

16. Identification of bacitracin resistant and tolerant genes in *Streptococcus suis* using a transposon library

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Antimicrobial resistance (AMR) and tolerance (AMT) of bacterial pathogens becomes a prominent problem in human and animal health in the world. *Streptococcus suis* is an important zoonotic bacterium threatening human health and pig industry. Bacitracin was ever widely used as feed additives in livestock, and resulted in AMR and AMT. To understand their mechanisms, a *S. suis* transposon library was constructed, and Tn insertion sites were mapped by DNA sequencing. Totally 417 genes were functionally inactivated by Tn insertion among 1665 mutants, and these genes involve in 54 KEGG pathways. Through transposon mutant pool screening, 27 genes were associated with AMT, and 2 of them also contributed to AMR. These genes were grouped into five categories: transcription regulator (3/27), transporter (5/27), peptidase (2/27), DNA repair and other metabolism (8/27), and hypothetical proteins (9/27). We found that the mutant with inserted gene encoding ABC-type exporter exhibited double increase of tolerance, and the exporter is responsible for pumping bacteriocin and lantibiotic out of cells. Growth rate is considered as the main factor influencing AMT, but

this mutant did not show dramatic decline. We speculated that the inactivation of the gene influenced the tolerance through other ways, for instance altered substrate of the inserted transporter or decrease of bactericidal activity of bacitracin. Gene *sntC* encodes bifunctional metallophosphatase/5'-nucleotidase in *S. suis*, but the function of SntC has not been confirmed. Research has found that the other 5'-nucleotidase SntA of *S. suis* was a surface-exposed protein and contributed to virulence and complement evasion in *S. suis*. In our study, the mutant with inserted gene *sntC* not only exhibited increased tolerance to bacitracin, but also exhibited declined susceptibility. The inactivation of these genes was first discovered contributing to AMR and AMT, which needs further research to clarify their mechanism.

17. TREM-1 signal plays protective and pathogenic roles on STSLS caused by *Streptococcus suis*

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Streptococcus suis infections can cause septic shock, which is referred to as streptococcal toxic-shock-like syndrome (STSLS). However, the mechanism underlying STSLS is different and remains to be elucidated. Triggering receptor expressed on myeloid cells-1 (TREM-1) is an activating receptor expressed on myeloid cells, and has been recognized as a critical immune modulator in several inflammatory diseases of both infectious and non-infectious etiologies. Here, we identified the role of TREM-1 on STSLS. In the resting state, TREM-1 signaling could not be activated due to the absent expression of TREM-1. At the early stage of *S. suis* infection, TREM-1 expression was induced through various pattern-recognition receptors, which was further activated by HMGB1 and PGLYRP1, secreted by activated host cells. In addition, the activated neutrophils could interact with platelets which could further provide surface actin for TREM-1 activation. The activation of TREM-1 signaling was essential for further activation of neutrophils and monocytes, which were important for bacterial clearance. If *S. suis* could be significantly killed by these innate immune cells, the infection would be under control. However, severe infection would occur if the bacterial could resist the clearance. The highly virulent *S. suis* had developed many strategies to resist the early killings, and the quick propagation of bacteria would provide more ligands for TLR activation to induce a significantly high level of TREM-1 expression. In addition, necrosis of host cells due to the infection would provide much more ligands (such as actin and HMGB1) to activate TREM-1 signaling to cause severe inflammation. Ultimately, a TREM-1-mediated severe inflammatory response may further resulted in STSLS. Therefore, TREM-1 signal plays protective and pathogenic roles on STSLS caused by *S. suis.*

18. Model assessment of time-discrete batch management systems on *Streptococcus suis* disease incidence and persistence in nursery pigs

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Streptococcus suis is a significant swine pathogen that causes disease and mortality in nursery pigs and requires the use of antimicrobials. In Canada, the use of a weekly-farrowing systems remains common; however, high recruitment rates that introduce a continuous surge of young and immunologically naïve piglets can change the contact-structure between pigs and facilitate disease spread. Changes in recruitment rates through the use of time-discrete batch management systems (BMS) are tangible approaches for reducing transmission and spread of disease; yet the effects have not been modelled. Therefore, the aim of this study is: 1) to develop a mathematical model that describes *S. suis* transmission in

the nursery and 2) use the model to evaluate the effects of BMS as a potential management-driven alternative for disease control. Using retrospective nursery outbreak data, we derived realistic epidemiological parameters to inform our model state transitions. We developed a compartment susceptible-exposed-infectious (SEI) model with typical swineherd dynamics and used it to examine patterns of on-farm disease persistence and the probability of fade-out using R open-source software. Using our model, we will simulate a range of commonly found BMS used in swine production and assess them as an effective strategy for disease control using a 1-week BMS as the base case scenario. Estimations of the 'critical community size' will also be calculated, which will help us to better understand the behaviour of *S. suis* disease persistence on-farm with increasing herd sizes. We propose a simple, yet robust modelling framework that uses empirical outbreak data to capture the transmission dynamics of this disease, while enabling direct quantitative assessment of interventions strategies performed *in silico*. Our model will help to enable producers to initiate management-driven intervention as alternative forms of disease control while also minimizing the use of antimicrobials.

19. Understanding the relationship between tonsil microbiota and clinical *Streptococcus suis* infection in nursery pigs

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Streptococcus suis naturally resides in the swine upper respiratory tract. However, the majority of swine do not develop the disease. The aim of this study was to address whether the tonsil microbiome is altered in nursery pigs with clinical signs of *S. suis* disease compared to healthy pigs. The pigs were classified into 3 categories: confirmed (with clinical signs of infection and presence of *S. suis* in systemic sites), probable (with clinical signs but absence of *S. suis* in systemic sites), and control (without clinical signs and absence of *S. suis* in systemic sites). In total, 62 (19 confirmed, 23 probable, and 20 controls) were collected from 7 farms. DNA was extracted from the tonsils of the soft palate of the selected pigs. Illumina MiSeq sequencing of the 16S V3-V4 hypervariable region was done to assess the composition of the microbiota and the mothur pipeline was used for clustering and taxonomy assignment. Using a linear regression method, it has been demonstrated that the observed number of OTUs was higher in the confirmed than the control group ($P < 0.001$). A higher species diversity was also observed in the confirmed than the control group ($P<0.001$). The probable group had a higher OTU count than both control and confirmed group, however they had a lower species diversity than the confirmed group but higher than the control group ($P<0.001$). The top 5 phyla identified were *Proteobacteria*, *Firmicutes*, *Fusobacteria*, *Bacteroidetes* and *Tenericutes*. The relative abundance of streptococcus was higher in the control group than confirmed but it was borderline significant ($P=0.05$). In conclusion, it has been demonstrated that there is a statistically significant difference in tonsil microbiota of both healthy and clinical *S. suis* pigs. For a more conclusive investigation, statistical analysis by applying more farm and pig level factors is needed.

20. Determination of serovars and virulence genes distribution of *Streptococcus suis* strains isolated from diseased pigs in Taiwan

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Streptococcus suis (S. suis) is an important zoonotic pathogen in porcine industry worldwide, causing meningitis, septicemia, arthritis, pneumonia, and even acute death. It has been classified into 29 serovars based on the differentiation of capsule polysaccharide structures, and specific serovars have higher pathogenicity, such as serovar 2. Some proteins translated from specific genes may be the virulence determinants, such as *sly, mrp, epf*. Thus, both of these important indicators are meaningful for case screening in disease diagnosis. In this study, total 293 isolates had been collected from diseased pigs submitted to Animal Diseases Diagnosis Center of National Pingtung University of Science and Technology from 2015 to 2018. The multiplex PCR assay were performed for serovars and virulence genes identification. Serovar 3, 2 or 1/2, and 8 were dominant with 57 (19%), 41 (14%), and 31 (11%) isolates, respectively. Serovars 4 and 8 isolates obviously increased in 2018 that reached 16.7% and 15.6% of the year comparing to 6.8% and 8.0% in 2017. The most prevalent virulence genotype profiles were *sly −/mrp −/epf −, sly −/mrp +/epf −*, and *sly + /mrp +/epf −* that occupied 114 (39%), 97 (33%), and 51 (17%) isolates, respectively. Some isolates show specific serovar versus virulence patterns, e.g. serovar 8 with *sly −/mrp −/epf −* (10%) and serovar 9 with *sly −/mrp −/epf −* (8%). Collectively, the main serovars such as 3, 2 or 1/2 in Taiwan are similar to researches in other Asia countries. Moreover, these predominant serovars may shift by times, so continuous surveillance is necessary to obtain up-to-date information.

21. Cribriform plate of ethmoid: a possible pathway for *Streptococcus suis* infection to reach the pig brain?

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Meningitis is one of the main clinical signs for *Streptococcus suis (Ss)* in swine and in zoonotic infections. In a few reported cases of human bacterial meningitis, the bacterial pathogens reached the meninges through the cribriform plate of the ethmoid bone, however this has not been previously reported in pigs. We performed immunohistochemistry (IHC) analyses in a pig animal model to compare infection patterns of two different strains. *Ss* infection was performed by intranasal inoculation in 16 cesarean-derived colostrum-deprived piglets at 29 days of age. Two serotype 2 strains of *Ss* were used in the infection: S10, a highly virulent strain and T15, a low virulent strain. A control group of 4 piglets was not inoculated. After 1 and 3 days post-inoculation, 4 piglets from each group were euthanised. Samples for bacterial detection were taken and tissues were collected, fixed in formalin and embedded in paraffin for IHC examination with a rabbit monoclonal anti-*Ss* serotype 2 antibody. Clinical signs other than high rectal temperature were not observed in any animal after *Ss* inoculation. At necropsy, only mild fibrin accumulation was observed in the abdomen of some animals in both infected groups. *Ss* was detected in 14 out of 16 nasal samples from inoculated animals by specific PCR, whereas was not detected in control animals. *Ss* was observed by IHC in the cribriform plate of ethmoid epithelium in 7/8 piglets inoculated with T15 strain and 6/8 with S10 strain, and in the lamina propria

in 2/8 piglets inoculated with T15 strain and 4/8 with S10 strain. At day 3 post-inoculation, some *Ss* were detected next to the cartilage in 2/4 piglets inoculated with S10 strain. In summary, IHC results revealed the colonization of the cribriform plate of the ethmoid by both *Ss* strains. Moreover, the highly virulent strain S10 was located closer to the cartilage in the ethmoid than strain T15, suggesting this may be an infection route by which *Ss* can access the meninges.

22. Study of the involvement of pilus G in the virulence of *Streptococcus suis* serotype 2

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Streptococcus suis is one of the most important swine bacterial pathogen that can be transmitted to humans by contact with diseased animals or contaminated raw pork products. It causes mainly septicemia, meningitis, arthritis, endocarditis in pigs and meningitis and septic shock in humans. Of the 35 serotypes described, serotype 2 is the one most frequently isolated from diseased pigs in most parts of the world. Pili-like structures have been shown to contribute to the virulence of different Gram-positive bacteria. For example, pili may play a key role in adherence to host cells and to proteins of the extracellular matrix, biofilm formation, self-auto-aggregation and virulence. Genomic studies revealed the presence of at least four distinct putative pilus gene clusters in *S. suis* (*srtBCD*, *srtE*, *srtF*, and *srtG* clusters). The *srtG* gene cluster has precisely been described in the representative North American *S. suis* serotype 2 strain 89/1591. It consists of one sortase gene (*srtG*) and two putative pilin subunit genes (*sgp1* and *sgp2*). Isogenic mutants defective in the production of Sgp1, Sgp2 and *srtG* (Δ *sgp1*, Δ *sgp2* and Δ *srtG*) were obtained by splicing-by-overlap-extension PCR. The *PCR products were then cloned into the temperature-sensitive S. suis-E. coli* shuttle vector pSET4s and the plasmids were introduced into *S. suis* 89/1591 strain by electroporation. Using these mutants, we demonstrated that the *srtG* pilus is not involved in the self-aggregation phenomenon and in the biofilm formation of strain 89/1591. Further tests to evaluate the involvement of the *srtG* pilus in the pathogenesis of the infection are presently being evaluated by studies of invasion of and adhesion to porcine tracheal epithelial and porcine brain microvascular endothelial cells, using the wild-type and mutant strains. The role of the *srtG* pilus in virulence using a mouse model of infection will also be evaluated.

23. Identification and characterization of Tyrosine phosphorylation systems in *Streptococcus suis*

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Stretotococcuis suis serotype 2 (*S. suis* 2) is an important swine pathogen and an emerging zoonotic agent that causes severe infections in both animal and human. In the present study, two different kinds of Tyrosine phosphorylation systems were identified frim the *S. suis* 2 genome. The one located within a polysaccharide synthesis gene cluster is composed of three annotated genes termed Cps2B, Cps2C and Cps2D. The other is composed of a single gene, which encodes a low molecular weight protein tyrosine phosphatase (LMW-PTP), termed as SS-PTP. Our study revealed that the annotated Tyrosine kinase Cps2C is capable of autophosphorylation at tyrosine, serine, and threonine residues and its function is fully dependent on the Cps2B protein. However, we only detected Tyrosine phosphatase activity for the SS-PTP, but not for the annotated Tyrosine phosphatase Cps2D. mutation of the protein at the Cyst-33 and Arg-39

completely abolished the activity of SS-PTP, suggesting functional indispensable of the two residues. To further explore the function of *ss-ptp* in *S. suis* 2, a *ss-ptp* knockout strain was constructed. Our data revealed that the *ss-ptp* knockout strain shows no significant differences with the wild strain in morphological phenotype, hemolytic activity, *in vitro* cell experiments, and mouse virulence test, excepting for its lower growth rate.

24. Role of the capsular polysaccharide in the interactions of *Streptococcus suis* serotype 9 with host cells and virulence: Comparison with serotypes 2 and 14

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Streptococcus suis is a natural inhabitant of the upper respiratory tract of pigs (tonsils, nasal cavities, and saliva) and an important bacterial pathogen responsible for meningitis, arthritis, and septicemia. Current knowledge of the *S. suis* pathogenesis and virulence is mostly based on serotype 2, even though serotype 9 is responsible for the greatest number of porcine cases in Spain, the Netherlands, and Germany. Of the different virulence factors, capsular polysaccharide (CPS) is required for *S. suis* virulence as it promotes resistance to phagocytosis and killing and masks surface components responsible for host cell activation. However, these roles have been described for serotypes 2 and 14, whose CPSs are structurally and compositionally similar. Consequently, we evaluated the interactions of serotype 9 with host cells and the role of its CPS, which structure and sugar composition greatly differs from those of serotypes 2 and 14, using an isogenic non-encapsulated mutant. Results demonstrated that serotype 9 adhesion to but not invasion of respiratory epithelial cells was greater than that of serotypes 2 and 14. Furthermore serotype 9 was more internalized by macrophages but equally resistant to whole blood killing. Though recognition of serotypes 2, 9, and 14 by dendritic cells required MyD88-dependent signaling, in vitro pro-inflammatory mediator production induced by serotype 9 was much lower. In vivo, however, serotype 9 causes an exacerbated inflammatory response responsible for host death during its systemic infection. Meanwhile, presence of the serotype 9 CPS differentially modulated interactions with host cells in comparison to serotypes 2 and 14 by masking surface components less efficiently. However, its CPS remains a critical virulence factor required for bacterial survival in blood and development of clinical disease regardless of its unique composition and structure.

25. ParB-GFP fusion reporter as a tool for tracking nucleoid segregation in *Streptococcus suis* and antimicrobial screening application.

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Nucleoid segregation is efficient process which ensures the bacterial daughter cells inherit genetic material and it is also driven by DNA-interacting proteins such as ParABs system. ParA is a walker type ATPase and ParB, partitioning protein that bind to the specific DNA sequences, *parS*-sites, located proximal to *ori* region. For *ovococci* bacteria, nucleoid segregation and cell division mechanisms have been previously study only in *Streptococcus pneumoniae*. *S. suis* is a Gram-positive *ovococci* bacteria which causes severe systemic infection in human and weaning piglets. To date very little is known about *S. suis* nucleoid segregation. The present study aims to elucidate the process of nucleoid segregation and cell division. Recombinant *S. suis* carrying ParB-GFP fusion reporter strain was successfully constructed. Time-lapse fluorescence microscopy indicated that the nucleoid duplication of recombinant ParB-GFP *S. suis* was clearly visualizes as two bright green foci. By tracking of ParB-GFP fusion protein in *S. suis* cells during cell division cycle, it is demonstrated that *S. suis* cell division cycle spanned 60 mins equivalent to that of *S. pneumoniae*. Following the completed segregation, the septum formation was initiated and completed within 15 mins to generate the two daughter cells of which carry a single nucleoid serving as a final checkpoint of cell division. Similar, to those *pneumococi*, *S. suis* nucleoid segregation was covered a majority of cell division duration. These data suggesting all cocci genera carrying ParBs may share the same duration and kinetic of cell division cycle. Moreover, the arbitrary intensity value of GFP signal which were gradually increased during chromosome replication and segregation corresponding to the number of the ParB-GFP foci were significantly decreased following Rifampicin and Nalidixic acid treatments. These data indicated the potential of recombinant ParB-GFP *S. suis* as antimicrobial screening tool.

26. The subtilisin of *Streptococcus suis* cleaves kininogen with production of pro-inflammatory fragments

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Streptococcus suis produces several proteases including a subtilisin for which a number of evidence suggest a role as virulence factor. High molecular weight kininogen is a plasma glycoprotein with a large variety of biological properties. This study aimed to i) investigate the ability of the *S. suis* subtilisin to cleave kininogen, and ii) assess the pro-inflammatory activity of the degradation products in a model of human brain microvascular endothelial cells. To obtain the recombinant subtilisin, the *sspA* gene was cloned and expressed in *Escherichia coli*. The recombinant subtilisin was found to cleave kininogen as determined by SDS-PAGE and Western immunoblotting analysis. The pattern of cleavage appeared to be different from that observed with kallikrein and plasmin. It was found that the kininogen degradation products generated by the subtilisin did not contain bradikinins, as determined by ELISA analysis. However, the degradation products of kininogen induced a significant production of both interleukin-6 and interleukin-8 by human brain microvascular endothelial cells. In summary, the subtilisin of *S. suis* cleaves plasma kininogen with production of fragments possessing pro-inflammatory activity towards endothelial cells. This may contribute the disrupt and increase the permeability of the blood-brain barrier thus allowing bacteria to migrate to the central nervous system.

27. Phase variation is common in *Streptococcus suis* and is regulated by multiple mechanisms

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Streptococcus suis is a commensal bacterium in pigs and a pathogen in both pigs and human. Recent studies show the presence of multiple phase variable systems in *S. suis*, but the associated phenotypes have never been described. We observed heterogeneity in wild type *S. suis* single colony morphologies after 48h incubation on THY agar plates but not on blood agar plates. The proportion of colonies showing variation in morphology varied per isolate, correlating with genotype but not with serotype. For example, the zoonotic MLST CC1 serotype 2 isolate *S. suis* BM407 shows only minor heterogeneity in colony morphology, whereas CC20 serotype 2 isolate S. suis 861160 shows heterogeneity in nearly all colonies on THY. DNA recognition domains of type I restriction modification (RM) systems, which control the methylation pattern in bacterial genomes are potential phase variable genes. We studied phase variation of a type I RM system present in SsuCC20p of *S. suis* strain 861160 and other CC20 serotype 2 isolates, but absent in CC1 isolates, and its potential role in colony morphology heterogeneity. First, we confirmed that SSuCC20p is indeed phase variable and can rearrange into 16 different phases. One out of 16 phases appears enriched when *S. suis* 861160 is cultured on blood agar when compared to growth on THY agar and one phase vice versa, while two out of 16 phases are dominant when cultured on both THY and blood agar. We then created mutants with locked phases in SSuCC20p, to study the influence of SsuCC20p on phenotypes. Colonies of the locked mutants still showed heterogeneity in morphology after growth on THY, but in different ratios for each of the mutants compared to wildtype. This indicates that whilst the SSuCC20p system may contribute to colony morphology heterogeneity, this phenotype is likely to be also regulated by additional (phase variable) systems.

28. Factors contributing to suilysin-induced damage of different epithelial cell types: membrane binding, cholesterol content and resealing capacity

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Suilysin (SLY) is a secreted toxin and an important virulence-associated factor of *Streptococcus (S.) suis*. It belongs to the family of cholesterol-dependent pore-forming cytolysins (CDC) and damages different cell types by lytic pore-formation. Furthermore, it has been demonstrated to play an important role in the pathogenesis of *S. suis* infection and host-cell interaction *in vitro* and *in vivo*.

In the present study, we investigated the susceptibility of different respiratory epithelial cells, either immortalized cell lines (HEp-2 and NPTr) or cells of primary porcine origin (tracheal and bronchial epithelial cells), towards the cytotoxic effects of SLY. The different cell types were infected with a virulent *S. suis* serotype 2 strain, its isogenic SLY-deficient mutant strain or were treated with the recombinant SLY (rSLY). Then, cytotoxicity was determined by LDH-release assay. HEp-2 cells were more susceptible towards the toxin as compared to all other cell types tested. Thus, we investigated the binding capacity of SLY using flow cytometry analysis. Since binding and pore-formation of CDC is dependent on the membrane composition, we also determined the cellular cholesterol content of the different cell types using TLC and HPLC, respectively. Finally, we examined the ability of the tested epithelial cells to reseal SLY-induced pores using flow cytometry analysis.

Our results indicated that the amount of membrane-bound SLY, the cholesterol content of the cells as well as their resealing capacity affected the susceptibility of the different cells towards the cytotoxic effects of SLY.

In conclusion, though the contribution of the three factors to the suilysin susceptibility of the epithelial cells differed between cell types, the ability to reseal SLY-induced cell damage seems to be crucial.

29. Antimicrobial susceptibility of *Streptococcus suis* isolated from diseased pigs, asymptomatic pigs, and human patients in Thailand

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Prophylaxis and treatment of emerging zoonotic *Streptococcus suis* infection in agricultural and healthcare settings mainly rely on antibiotics. However, continued use of antibiotics contributing to emergence and widespread of antibiotic resistant *S. suis* becomes a significant challenge in many endemic countries, including Thailand. In this study, antibiotic susceptibility of Thai-isolated *S. suis* strains to different antibiotic classes was investigated by disk diffusion method to gain an insight into the distribution of antibiotic-resistant patterns of *S. suis* strains in different regions of Thailand. The result revealed the antimicrobial resistance and multidrug resistance of Thai-isolated *S. suis* strains. Susceptibility testing indicated widespread resistance to macrolides and tetracyclines of Thai-isolated *S. suis* strains and beta-lactam antibiotic drugs (including cefotaxime and ceftiofur), vancomycin, chloramphenicol, as well as florfenicol were potentially the most effective therapeutic drugs for the treatment of *S. suis* infection in both pigs and humans. High prevalence of intermediate susceptibility of *S. suis* isolated from asymptomatic pigs for penicillin G, gentamicin, enrofloxacin, and norfloxacin could be the premise of the emergence of *S. suis* antibiotic resistance. Resistance was also found in *S. suis* strains isolated from asymptomatic pigs indicating that they could act as reservoirs of antibiotic resistance genes. The knowledge gained from this study raises an awareness and encourage best practices of appropriate antibiotic drug prescribing and use among human health and agriculture sectors.

30. *Streptococcus suis* two component systems involved in virulence and in acid and oxidative stress survival

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Streptococcus suis is an important swine pathogen worldwide, but relatively little is known about virulence mechanisms promoting survival in the host. Many bacterial pathogens use two-component systems (TCS) to regulate expression of genes required for virulence, pathogenesis, antibiotic resistance and adaptation to environmental conditions, including those encountered in the host. Bacterial two-component systems are therefore considered attractive drug targets for prevention and treatment of bacterial infections. We investigated the effects of deleting of two poorly studied TCS (SSU1930/31 and SSU0827/28) on *S. suis* virulence and their capacity to respond to adverse conditions. Deletion of either TCS, reduced survival of *S. suis* in acidic or oxidative environments. Transcriptomics was used to predict the regulon controlled by each TCS and the genes involved that promote survival under physiological stress. Both TCS deletion mutants showed a lower survival rate than the wild-type strain when combined with neutrophils *in vitro* and reduced pathogenicity in zebrafish larvae. We conclude that these TCSs contribute to the survival of *S. suis* serotype 2 strain S10 under different stress conditions and are likely to play a crucial role in the survival and persistence of *S. suis* in the bloodstream and host tissues.

31. *In vitro* characterization of the role of a Type I Restriction-Modification protein (HsdS) in *Streptococcus suis* survival and virulence

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Recent studies in *Streptococcus suis* have led to the discovery of three Type I Restriction-Modification (RM) systems in the clinical isolate P1/7. Type I RM systems are large pentameric protein complexes composed of two methyltransferase subunits (HsdM), two restriction subunits (HsdR), and one specificity subunit (HsdS). RM systems are best known for their role in selectively degrading incoming foreign DNA elements. Recently, RM systems were shown to regulate gene expression through phase variation of the *hdsS* gene, generating fitness advantages to subsets of the population. In a previous study, a variant of the *hsdS* gene (SSU1589) was identified and evaluated as a novel genetic marker of invasive *S. suis* disease in pigs on farms in the UK. In clinical isolates, *hsdS* has a duplicated organization of two target recognition domains (TRDs) in tandem. In contrast, non-diseased associated isolates contain a truncated version of this gene with only one TRD. To investigate the potential role of the *hsdS* gene and its truncated version in *S. suis* we used CRISPR-Cas9 technology to create a P1/7 strain lacking the second TRD which is absent in non-diseased associated isolates. The *hsdS* variant exhibited a reduced ability to survive in acidic conditions, but its capacity to survive under oxidative stress and macrophage internalization remained comparable to the parental strain. Transcriptome analyses of the wild-type strain revealed significant upregulation of the *hsdS* gene in serum, which could point to the involvement of this gene in invasive disease. To examine if truncation of this gene leads to differential gene expression that could result in reduced virulence when compared the wild-type strain, analogous transcriptome analyses of the *hsdS* variant were carried out in serum. Understanding the molecular mechanisms underlying this RM system may bring the scientific community one step further in unraveling the mechanisms that regulate *S. suis* virulence.

32. Over 70% florfenicol resistant *Streptococcus suis* isolates from clinically healthy pig herds carry a novel resistance gene *optrA* on the chromosome

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Streptococcus suis, an important zoonotic pathogen, not only affects the global pig industry but also threatens public health. Florfenicol is one of the most widely used antimicrobials in pig farms and was reported as an effective therapeutic drug for the treatment of *S. suis* infection, although it's use in Europe is now restricted. However, in our investigation, 50.0% (112/224) of the *S. suis* isolates from the tonsil swabs collected from clinically healthy pig herds of ten Chinese pig farms were resistant to florfenicol (MIC ≥ 8 μg/ml). The prevalence and distribution of antimicrobial resistance genes (ARGs) in these isolates were investigated based on their whole genome sequences. The chromosomes of 71.4% (80/112) florfenicol-resistant *S. suis* isolates carried the *optrA* gene, a novel plasmid-borne ABC transporter gene that functions as a ribosomal protection protein, conferring resistance to oxazolidinones and phenicols in enterococci, *staphylococci* and *streptococci.* The other reported ARGs responsible for the phenicols resistance, such as *fexA*, *cfr* and *poxtA* were not detected in our *S. suis* collection. Although *the optrA* gene had been identified in *S. suis* genomes in a retrospective analysis, the distribution of *optrA* in *S. suis* populations in pig herds and the corresponding

resistant phenotype were not described. In terms of the genetic context of the *optrA* harboring chromosomes, 78.75% (63/80) were the potential integrated regions relevant to the enterococcal plasmids and 13.75% (11/80) were located in *S. suis* integrative and conjugative elements. The antimicrobial susceptibility test results of linezolid and tedizolid, two main oxazolidinone antibiotics used to treat infections caused by vancomycin-resistant enterococcus and antimicrobial-resistant staphylococci and streptococci, showed that out of 87 *optrA* harboring florfenicol-resistant *S. suis* isolates, to linezolid only 6 isolates (MIC = 4 µg/ml) and to tedizolid only 5 isolates (MIC = 1 µg/ml) had higher…(truncated).

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POSTER ABTSRACTS
12th CRIPA ANNUAL SYMPOSIUM

PhD COMPETITION

PhD1. Role of the capsular polysaccharide in the interactions of *Streptococcus suis* serotype 9 with host cells and virulence: Comparison with serotypes 2 and 14

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Streptococcus suis is a natural inhabitant of the upper respiratory tract of pigs (tonsils, nasal cavities, and saliva) and an important bacterial pathogen responsible for meningitis, arthritis, and septicemia. Current knowledge of the *S. suis* pathogenesis and virulence is mostly based on serotype 2, even though serotype 9 is responsible for the greatest number of porcine cases in Spain, the Netherlands, and Germany. Of the different virulence factors, capsular polysaccharide (CPS) is required for *S. suis* virulence as it promotes resistance to phagocytosis and killing and masks surface components responsible for host cell activation. However, these roles have been described for serotypes 2 and 14, whose CPSs are structurally and compositionally similar. Consequently, we evaluated the interactions of serotype 9 with host cells and the role of its CPS, which structure and sugar composition greatly differs from those of serotypes 2 and 14, using an isogenic non-encapsulated mutant. Results demonstrated that serotype 9 adhesion to but not invasion of respiratory epithelial cells was greater than that of serotypes 2 and 14. Furthermore serotype 9 was more internalized by macrophages but equally resistant to whole blood killing. Though recognition of serotypes 2, 9, and 14 by dendritic cells required MyD88-dependent signaling, in vitro pro-inflammatory mediator production induced by serotype 9 was much lower. In vivo, however, serotype 9 causes an exacerbated inflammatory response responsible for host death during its systemic infection. Meanwhile, presence of the serotype 9 CPS differentially modulated interactions with host cells in comparison to serotypes 2 and 14 by masking surface components less efficiently. However, its CPS remains a critical virulence factor required for bacterial survival in blood and development of clinical disease regardless of its unique composition and structure.

PhD2. Characterization of the microbiota variability on cutting room surfaces in a pig slaughterhouse during production

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The pork industry is a pillar in the agri-food industry in the province of Quebec. It is the second largest market after milk and the biggest in terms of exports. In pork slaughterhouses, the microbiota of the production environment is recognized as a source of microorganisms that potentially affect the quality and the safety of the meat. During the processing, the associated microbiota can become detached from the carcasses and lead to the contamination of the products. Technological advances in the field of high throughput sequencing offer the opportunity to quickly and comprehensively study the microbiota of various environments. The aim of this study was to characterize, for the first time to our knowledge, the microbiota of pig slaughterhouse cutting room surfaces during production. A total of 294 conveyor belt samples of 1800cm2 in contact with the meat products were collected with wipes during six visits. The samples were equally distributed between six conveyors that were each associated with a type of meat cut. The total DNA of the microorganisms harvested from each sample was extracted using a phenol-chloroform protocol and the sequencing of the V4 region of the gene coding for the 16S RNA was carried out using the Illumina MiSeq platform. The processing of the sequencing data was achieved using the software Mothur 1.39.5 and RStudio 3.5.1. The results shown significant differences in both alpha and beta diversities between the conveyors. Differences have been described in terms of presence/absence of specific OTUs as well as in terms of relative abundance. The potential impact of the visits has been taken into consideration. The results highlighted the presence of specific microbial environments related to conserved component of the microbiota in the same meat production room. This "pattern" of representative species on conveyors can be used as a tool to accurately target association of the geographical environment with potential harmful microorganisms.

PhD3. The influence of trehalose metabolism on production of type 1 fimbriae in avian pathogenic *Escherichia coli* strain MT78

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Avian Pathogenic *Escherichia coli* (APEC) cause infections in poultry that may evolve into septicemia. Among the factors that can contribute to APEC infection, type 1 fimbriae (T1F) can mediate adherence to and invasion of host cells. Previously, we determined that loss of the periplasmic trehalase, encoded by *treA*, contributed to a decrease in T1F production and decreased virulence in a mouse urinary tract model, reduced adherence and invasion of avian fibroblasts. Trehalase degrades trehalose in the periplasm; whereas *otsA* and *otsB* genes encode cytoplasmic enzymes required for trehalose biosynthesis. In *E. coli* K-12, the pathways of trehalose biosynthesis and degradation are involved in the response to osmotic stress. To elucidate whether it is the excess of trehalose in the periplasm that affects T1F production, we generated isogenic mutants, MT78Δ*otsBA* and MT78Δ*treA*Δ*otsBA* using the lambda red recombinase technique. Further, we performed yeast agglutination and osmotic resistance stress tests to observe T1F. The most diluted yeast agglutination titers were: 3 (wild-type strain), 2 (MT78Δ*treA* and MT78Δ*otsBA*) and 1 (MT78Δ*treAotsBA*). Titer of 3 was restored to MT78Δ*treA*Δ*otsBA* upon complementation with the *treAotsAB* genes. Resistance to osmotic stress was determined by growth diluted cultures on LB plates with or without 0,6 M of urea. The WT, Δ*otsBA* and Δ*trea*Δ*otsab* reduced their growth 50-fold when compared to control growth.The Δ*trea* strain and the Δ*trea*Δ*otsab* complemented mutant were reduced by 40 and 30-fold, respectively. Altogether, our results suggest that deletion of

otsBA and *treA* affect T1F. However, because the production T1F in the *treA* mutant is not regained by the loss of the enzymes required to synthesize trehalose, the excess of trehalose in the bacterial periplasm from loss of TreA as well as the loss of synthesis of trehalose itself may both influence the production of T1F,which may alter virulence by other mechanisms that have yet to be elucidated.

PhD4. Culture independent study on the co-occurrence of *L. monocytogenes* with other bacterial genus and diversity description in pig slaughterhouse conveyor surfaces

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Resistance to disinfection and persistence on working surfaces are important attributes allowing bacterial pathogens, such as *Listeria monocytogenes*, to survive and contaminate food products. Persistence is a complex phenomenon involving interactions between many bacterial genera within biofilm and modulated by in situ characteristics such as the type of matrix and presence of remnant chemical compounds that might be present in case of disinfection context. Using new molecular tools for characterization of microbial communities may allow to better appreciate the relative contribution of different bacteria to this phenomenon as well as in the contamination risk by *L. monocytogenes*. In this work we sought to describe *in silico* the bacterial communities identified on meat conveyor surfaces after sanitation procedures through four sampling periods and to study co-occurence between *Listeria monocytogenes* and other bacteria. From the background microflora, a total of twenty-five genera, such as *Sphingomonas*, were found to interact negatively with *Listeria spp*, suggesting a possible *Listeria* growth inhibition or an absence of shared habitat. Through these results, a complete scenario of interactions of *Listeria* and *L. monocytogenes* was established which may contribute in identifying avenues that could prevent the growth and persistence of *L. monocytogenes* on working surfaces.

PhD5. Effect of co- infections of Porcine circovirus 2b and Swine Influenza virus H1N1 in epithelial cells and swine alveolar macrophages

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Swine flu is a worldwide distributed respiratory disease of pigs caused by Influenza virus type A. The H1N1 sub-type is one of the most prevalent swine influenza virus (SIV) sub-types associated to respiratory disease outbreaks in pig farms. Porcine circovirus genotype 2b (PCV2b) is the causative agent of postweaning multisystemic wasting syndrome (PMWS) and it is frequently identified in co-infection with other swine pathogens, including influenza virus.
The objective of this study was to evaluate the effects of the co-infection between PCV2b and SIV sub-type H1N1 in newborn porcine tracheal epithelial cells (NPTr) and immortalized porcine alveolar macrophages (iPAM 3D4/21). The replication kinetics of H1N1 and PCV2b in single or co-infected cells was determined at different times post-infection. Cell viability was evaluated at 24, 48 and 72 hrs post-infection using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). The mRNA levels of IL-1α, IL-6, IL-8, IL-10, IFN-α/β, IFN-γ, TGFb and TNF-α were determined at 24h post-infection by quantitative reverse transcription-polymerase chain reaction (RT-PCR).
The results showed that PCV2b differentially modulated H1N1 replication kinetics at 24 hours post-infection in both cell lines. The co-infection slightly reduced H1N1 replication in NPTr whereas it was significantly increased in iPAM 3D4/21. PCV 2b replication was not affected during the co-infection in neither of the two cell lines. No significant difference was

observed in regards to the viability of PCV2b/SIV H1N1 co-infected cells compared to PCV2b infected cells. The mRNA expression level of almost all cytokines tested was up-regulated in single and co-infected iPAM 3D4 / 21 cells, but not in NPTr cells, where only IL-6, Il-8 and IL-10 mRNA expression level was up-regulated. In conclusion, the effects observed during PCV2b/SIV H1N1 co-infection depend on the infected cell line.

OUTSIDE THE COMPETITION

OC1. The subtilisin of *Streptococcus suis* cleaves kininogen with production of pro-inflammatory fragments

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Streptococcus suis produces several proteases including a subtilisin for which a number of evidence suggest a role as virulence factor. High molecular weight kininogen is a plasma glycoprotein with a large variety of biological properties. This study aimed to i) investigate the ability of the *S. suis* subtilisin to cleave kininogen, and ii) assess the pro-inflammatory activity of the degradation products in a model of human brain microvascular endothelial cells. To obtain the recombinant subtilisin, the *sspA* gene was cloned and expressed in *Escherichia coli*. The recombinant subtilisin was found to cleave kininogen as determined by SDS-PAGE and Western immunoblotting analysis. The pattern of cleavage appeared to be different from that observed with kallikrein and plasmin. It was found that the kininogen degradation products generated by the subtilisin did not contain bradikinins, as determined by ELISA analysis. However, the degradation products of kininogen induced a significant production of both interleukin-6 and interleukin-8 by human brain microvascular endothelial cells. In summary, the subtilisin of *S. suis* cleaves plasma kininogen with production of fragments possessing pro-inflammatory activity towards endothelial cells. This may contribute the disrupt and increase the permeability of the blood-brain barrier thus allowing bacteria to migrate to the central nervous system.
This study was funded by Discovery grants to DG from the Natural Sciences and Engineering Research Council of Canada (NSERC).

OC2. Antigen I/II participates in the interactions of *Streptococcus suis* serotype 9 with phagocytes and the development of systemic disease

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Streptococcus suis is an important porcine bacterial pathogen and a zoonotic agent causing a variety of pathologies including sudden death, septic shock, and meningitis. Though serotype 2 is the most studied serotype due to its presence worldwide, serotype 9 is responsible for the greatest number of porcine cases in Spain, the Netherlands, and Germany. Regardless of its increasing importance, very few studies have investigated *S. suis* serotype 9 virulence factors and pathogenesis. Antigens I/II (AgI/II) are multimodal adhesion proteins implicated in host respiratory tract and oral cavity persistence of various pathogenic human streptococci. We recently demonstrated that AgI/II is involved in various bacterial functions for serotype 9, participating in the initial steps of the pathogenesis of the infection. However, its contribution to the systemic infection remains unknown. As such, we evaluated herein the role of the *S. suis* serotype 9 AgI/II in the interactions with phagocytes and the development of systemic disease in a mouse model of infection. Results demonstrated that the presence of AgI/II is important for the development of clinical systemic disease by

promoting bacterial survival in blood possibly due to its effect on *S. suis* phagocytosis, as shown with macrophages and dendritic cells. Furthermore, AgI/II directly participates in dendritic cell activation and pro-inflammatory mediator production following recognition by the Toll-like receptor pathway, which may contribute to the exacerbated systemic inflammation responsible for host death. Taken together, this study demonstrates that the *S. suis* serotype 9 AgI/II is important for virulence during systemic infection and development of disease. In fact, this is the first study to describe a role of an AgI/II family member in systemic bacterial disease.

OC3. Characterization of the role of monocytes and neutrophils during *Streptococcus suis* systemic and central nervous system infections

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Streptococcus suis is an important porcine bacterial pathogen and a zoonotic agent responsible for causing sudden death, septic shock, and meningitis. These pathologies are the consequence of elevated bacterial replication leading to exacerbated inflammation, which is a hallmark of the *S. suis* systemic and central nervous system (CNS) infections. Though monocytes and neutrophils are amongst the most important innate immune blood cells and that they massively infiltrate the CNS during *S. suis*-induced meningitis, their role during infection caused by this pathogen remains unknown. As such, the role of monocytes was determined using CCR2-/- and Nr4a1-/- mice, which lack Ly6Chigh inflammatory and Ly6Clow circulating monocytes, respectively, while neutrophils were depleted using anti-Ly6G neutralizing antibodies. Results demonstrated that neutrophils, and to a lesser extent inflammatory monocytes, but not circulating monocytes, participate in *S. suis*-induced systemic disease via their role in inflammation required for controlling bacterial burden control. Meanwhile, monocytes partially contributed to the exacerbation of *S. suis*-induced CNS inflammation while neutrophils participated in CNS bacterial burden control. However, development of clinical CNS disease was independent of both cell types, suggesting that resident immune cells are mostly responsible for *S. suis*-induced CNS inflammation leading to clinical signs. Consequently, this study demonstrates that though monocytes and neutrophils contribute to the *S. suis* systemic infection, they play differential roles during the CNS disease.

OC4. Enolase and dipeptidyl peptidase IV protein subunit vaccines are not protective against a lethal challenge with *Streptococcus suis* serotype 2 in a mouse model of infection

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Streptococcus suis is a major swine pathogen, causing arthritis, meningitis and sudden death in post-weaning piglets, but is also a zoonotic agent, mainly in South East Asia. *S. suis* comprises 35 different serotypes of which the serotype 2 is the most prevalent. Since the application of new policies regarding antibiotic use in pigs, vaccination has become the main tool used to reduce *S. suis*

clinical cases. In the absence of commercial vaccines, autogenous bacterins are used in the field, with controversial results. In the past years, the focus has turned toward the development of subunit vaccine candidates, mostly surface proteins that are ideally present in most *S. suis* strains belonging to different serotypes. However, published results are sometimes contradictory concerning the protective effect of a same candidate, and the adjuvant used may significantly influence the protective capacity of a given antigen. This study focused on the dipeptidyl peptidase IV (DPPIV) and the enolase (Eno), which are both involved in *S. suis* pathogenesis. While contradictory protection results have been obtained with Eno, no data on the protective capacity of DPPIV was available. In this study, results showed that 86 % and 88 % of the 359 strains tested were positive for the expression of Eno and DPPIV, respectively, suggesting that they are widely expressed by strains of different serotypes. However, no protection was obtained after vaccination in a mouse model of infection, regardless of different adjuvants tested. Even though no protection was obtained, significant amounts of antibodies were produced against both antigens, regardless of the adjuvant used. These results showed that *S. suis* DPPIV and Eno do not seem to be good vaccine candidates, at least not under the conditions evaluated in this study. Further studies in the natural host should still be carried out. Moreover, this work highlights the importance of confirming results obtained by different research groups.

OC5. Early antiretroviral (ART) initiation does not affect the frequencies and homing of regulatory T-cells (Tregs) within mesenteric lymph nodes during acute SIV infection of Chinese Rhesus Macaques

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Increased frequencies of regulatory T-cells (Tregs) is associated with the gut mucosal fibrosis and dysfunction and disease progression in chronic HIV/SIV infections. However, the dynamics of Treg cells in mesenteric lymp nodes (MLNs) is less known due to the lack of accessibility to these tissues in HIV-infected individuals. Here, we assessed the dynamics of Tregs following early ART initiation during acute SIV infection. 26 female Rhesus macaques of Chinese origin were enrolled in this study. 21 animals were infected intravenously with SIVmac251 virus. Seven monkeys were early ART treated at day 4 post-infection and ART was interrupted in three of them 8 weeks after the ART initiation. Non-treated animals in both acute (n=13) and chronic (n=4) phases were also analyzed. Peripheral blood and isolated cells from mesenteric lymph nodes (MLNs) were analyzed by flow cytometry and markers of lymphoid fibrosis were assessed by qPCR. Early ART initiation reduced T-cell immune activation assessed by HLA-DR/CD39 expression and prevented the depletion of memory CCR6+Th17 cells in both blood and MLNs. Untreated animals showed higher frequencies of Treg, CD39+Tregs, thymic Tregs and new memory CD4 populations sharing phenotypic characteristics with Treg cells as CTLA4+PD1- and CTLA4+PD1-FoxP3+ T-cells. Despite early ART, the frequencies of these Treg sub-sets remained unchanged within the MLNs and, in contrary to blood, the Th17/Treg ratio remained disturbed in MLNs. Furthermore, our results highlighted that the expressions of IDO-1 enzyme and fibrosis markers TGFβ1 and collagen-1 mRNA remained unchanged in MLN of ART-treated RMs. ART interruption didn`t affect T-cell immune activation and Th17/Treg ratio in MLNs. Our data demonstrated that early ART initiation within the first few days of SIV infection is unable to reduce the frequencies and homing of various subsets of Tregs within the MLNs, which may result in fibrosis, impairment in MLNs function and HIV persistence.

OC6. Determination of microbiological contamination of recycled manure solid bedding in dairies farms

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Recycled manure solids (RMS) bedding is used in Quebec dairy farms. It is obtained by extracting the solids from the manure produced by cows, sometimes followed by composting. However, the microbiological characteristics of RMS are poorly documented. The hypothesis of this project is that RMS will contain more problematic microorganisms for cattle than straw bedding (the most commonly used bedding on Quebec's dairies). The bedding before use (clean) and after use (dirty) of 27 RMS farms and 65 straw farms was sampled in parallel, a survey was administrated about health on the farms. Bacterial counts were enumerated using MacConkey, Edwards, VJ Agar, and blood agar media targeting respectively *Escherichia coli /Klebsiella spp, Streptococcus spp, Staphylococcus spp* and total mesophilic aerobic bacteria. Analyses showed that clean RMS bacterial counts were higher than those of the clean straw, except for total counts on MacConkey medium that clean straw counts were higher than clean LFR. Dirty RMS contained an average of 1 log CFU/g more E. coli and total mesophilic bacteria than dirty straw bedding. On the contrary, streptococci counts were, on average, 1 log CFU/g fewer in dirty RMS compared to dirty straw. There was no significant difference between dirty RMS and dirty straw for staphylococci and counts on MacConkey medium. Differences between RMS and straw were most significant before use and became ambiguous after utilisation. The microbial populations of both beddings evolved with the use (clean to dirty) but differentially depending on the type of bedding. RMS bedding seems have more problematic microorganisms as *E. coli* and *Klebsiella spp* which could cause grade 3 mastitis than straw bedding and this seems to confirm our hypothesis.

OC7. New exosome-based biomarkers for the rapid diagnosis of drug-resistant parasites

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Leishmaniasis is a complex of zoonotic vector-borne diseases transmitted by sand flies in more than 80 countries. Infected dogs develop the disease and serve as a major reservoir of transmission for humans. Leishmaniasis is a neglected disease so the resources available to eradicate it are limited. In the absence of an effective vaccine, leishmaniasis control is mainly based on chemotherapy, but with a limited number of licensed molecules available. Therefore, the same drugs are used to treat dogs and humans, which has stimulated the emergence and propagation of drug-resistant (DR) strains, a global veterinary and public health problem. There is an urgent need for rapid and accurate methods to diagnose the disease, as well as the drug-resistant nature of certain populations in order to propose appropriate and more effective treatments. Recent studies in *Leishmania* have shown the occurrence of extracellular vesicles, so-called exosomes, and their diverse roles in intercellular communication. We have characterized leishmanial exosomes to identify signature patterns, which could be implemented as biomarkers for the rapid diagnosis of resistant parasite strains. To this end we have analyzed different *L. infantum* strains resistant to either antimonial drugs or miltefosine. Exosomes were isolated and characterized in terms of size and morphology, and analyzed their protein content. Briefly, exosomes were isolated by ultracentrifugation and exclusion chromatography. Size, numbers and morphology were determined by nanoparticle-tracking analysis and TEM. Specific protein signatures were determined by LC-MS/MS analysis and some confirmed by Western blot. Our first results revealed the enrichment of unique proteins

in the exosomes of DR parasites; a key point that could help to better understand antimicrobial resistance and their use in the development of new biomarkers for rapid diagnosis DR parasites. This project is supported by CIHR and NSERC grants to MO and CFP.

OC8. Use of probiotic strains against porcine rotavirus OSU infection evaluated by an *in vitro* model

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Enteric infections affecting the intestinal health of pigs, including peri-weaning diarrhea, are recognized to have a negative impact on the value chain due to the economic losses caused by the death of thousands of piglets each year and the increase in production costs associated with growth delays. Among the microbial agents responsible for diarrhea, viruses such as rotavirus, caliciviruses and astroviruses found in Canadian livestock can play a key role in the establishment of enteric infections as well as promote secondary bacterial infections related to the weakening of the host's immune defences. However, recent data have shown that the use of probiotic strains can reduce the symptoms of rotavirus infections in children and gnotobiotic pigs. The objective of this project was to evaluate the efficacy of probiotic strains in preventing and/or reducing porcine rotavirus infection in an *in vitro* model with IPEC-J2 cells. The cells were pre-incubated 24 hours with each selected probiotic bacterial strains (*Lactobacillus rhamnosus* R011, *L. rhamnosus* GG, *L. casei* A234, *L. gasseri* A237, *Bifidobacterium animali lactis* A026, *B. longum* R0175, L. plantarum 299V) at different concentrations. Cells pre-incubated with probiotics were then infected with OSU rotavirus for 5 hours. The cells were fixed, permeabilized, labelled with fluorescent antibodies and the infection rate was analyzed by flow cytometry. Significant results of reduction of OSU rotavirus infection have been obtained in the presence of the bacterial strains *B. animali lactis* A026 (13.6% when inoculated at 107 cfu/ml and 16.2% with a 106 cfu/ml), *B. longum* R175 (16% with 106 cfu/ml and 19.6% with 105 cfu/ml), *L. plantarum* 299V (12.5% with 106 cfu/ml and 15.1% with 105 cfu/ml). Some slight reductions were observed with other probiotic strains but they were not significant. The results obtained advance knowledge, pave the way for new avenues and therapeutic applications in animal production.

OC9. Adhesion and cytotoxicity studies of *C. perfringens pilA2* and *pliA3* mutants and their corresponding wild-type using a chicken hepatocellular carcinoma cell line, LMH

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Necrotic enteritis (NE) is a severe clostridial enteric disease of poultry caused by *Clostridium perfringens* type A. The disease is characterized by damage to the intestinal mucosa by toxins. Type IV pilus and NetB toxin were recently determined to have important roles in the pathogenesis of NE but little is known on the adhesion process using chicken leghorn male hepatoma (LMH) epithelial cell line. The objective of this study was to evaluate the adhesion and cytotoxicity of an epithelial cell line infected with *C. perfringens pilA2* (CPpA2) and *piliA3* (*CPpA3*) mutants and their corresponding wild-type (*CP1*). *CPpA2*, *CPpA3* and *CP1* were selected for the bacterial adherence assay that we developped using LMH epithelial cell line. An initial concentration of 10^6 CFU/ml of bacteria was added to LMH epithelial cells and adhesion was allowed for 1 h to 5 h post-infections (P-I). To study cytotoxicity, the supernatant was tested using a colorimetric assay, based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells. At 1 h and 2 h P-I, the highest levels, 1.5x10^5 CFU/ml and 1.4x10^6 CFU/ml, of cell adhesion

were observed, respectively, for CP1. In contrast, low levels of cell adhesion were observed for *CPpA2* mutant (3,6x10^4 CFU/ml and 7,2x10^3 CFU/ml), and *CPpA3* mutant (2,3x10^4 CFU/ml and 8,5x10^3 CFU/ml). No cytotoxicity was observed at 1 h and 2 h P-I. At 3h P-I adhesion decreased in all strains, and cytotoxicity started to increase. At 4 h, cytotoxicity was high,reaching 77,6%; 82,9% and 44% for *CP1*, *CPpA2* and *CPpA3*, respectively. Data showed that *CP pilA2* and *pilA3* mutants demonstrated lower adhesion capabilities compared to the wild-type strain, indicating PilA as a factor facilitating adherence to LMH epithelial cell. Also, cytotoxicity was observed to be associated with prior higher levels of attachment, indicating a possible adhesion-dependent cytotoxicity process.

OC10. Identification of genes Involved in autoagglutination and biofilm formation in *E. coli* Strains O157: H7

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Enterohemorrhagic *E. coli* (EHEC) O157:H7 strains are foodborne pathogens capable of forming biofilms, allowing their persistence in different environments. Some EHEC seropathotype A strains including Sakai, have a greater capacity for autoagglutination and biofilm formation. The aim of the work is to identify genes involved in this phenotype. In Sakai reference strain a transposon mutant library was created. The insertion sites were identified by high-throughput sequencing of a pool of 22 non-agglutinating and weak biofilm forming mutants. *csgB* and *csgG* genes involved in the curli formation, were highly represented. Individual mutants were identified and complementation was done using pTrc99a expression plasmid. Mutants and complements were characterized for biofilm formation, colony morphotype and autoagglutination as well as yeast mannose-independent agglutination. *csgB* and *csgG* mutants formed less biofilm, displayed non-autoagglutinating and mannose-independent non-agglutination phenotypes. Mutants showed a SAW (smooth and white) colony morphotype, contrasting with the RDAR (red, dry and rough) Sakai morphotype. The complementation of *csgG* mutant restored the wild type phenotype i.e. strong autoagglutination and biofilm formation, RDAR morphotype, and mannose-independent yeast agglutination. Also, the expression of curli genes was evaluated by qRT-PCR in Sakai 24h biofilm compared to that of weaker biofilm producer strain EDL933. *csgA*, *csgD* and *csgG* were significantly over-expressed in Sakai biofilm. Moreover, 11 isolates O157:H7 were tested for the curli formation, and Sakai-like strains were high curli producers, while EDL933-like strains were low curli producers. Results suggest that the production of curli amyloid extracellular fibers is involved in the autoagglutination and strong biofilm formation phenotype in certain O157: H7 strains and could be an interesting target to counteract the persistence of these bacteria in natural and industrial environments.

MSc COMPETITION

M1. Protection of water sources: Design of a biomolecular tool for detection of *Cryptosporidium sp*. from environmental water samples

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The parasitic protozoan *Cryptosporidium* is a major veterinary and public health issue as some species of this genus are pathogenic for humans and animals. Furthermore, it possesses the capability to adopt a rigid dissemination configuration that grants it an increased resistance to environmental stresses. However, this conformation makes it also practically insensitive to chlorine-based water treatments applied to water destined to consumption. Other means of treatment can be used whose concentration/intensity must be adapted according to the input of *Cryptosporidium* in water entering the water treatment plant. But in order to do so, a reliable, sensitive and specific method must be able to quantify this input. Therefore, the goal of the present project is to elaborate a tool to detect and quantify this microorganism with the use of molecular biology. This requires to standardize the realization of each step of the process: sampling, filtration, DNA extraction and the biomolecular method itself. To do so, living intact cells were used to determine the best parameters to use, for example, the pore size of the filters, the best way to lyse cells to collect DNA, etc. Also, as a positive control for the biomolecular part of the project, synthetic DNA of a known sequence (known as Gene Fragment gBlock) was produced and used as a template for DNA amplifications. So far, it has been determined that the capture of Cryptosporidium on filters is better with a pore size of 0,22 µm. Primers specific to the genus *Cryptosporidium* were designed targeting the 18S rRNA gene and the detection limit of the method as it actually is was determined. There is still work needed to complete the elaboration of this tool but once done, it will be possible to put it to the test with environmental samples to compare its reliability to other protocols exposed in the literature.

M2. Characterizing the profile of released exosomes from chicken tracheal cells treated with Toll-like receptor (TLR) ligands

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Innate responses provide the first line of defence against pathogens, such as viruses at mucosal surfaces aiming to block the entry of the virus and viral replication. The effective elimination of the virus depends on the coordinated communication between different cells of the innate system and the epithelial cells in the mucosal surfaces. Immune responses are coordinated between different cells through several mechanisms including the release of exosomes from host cells. The exosome contents can be affected by different stimuli. It is critical to characterize the composition of exosome cargo released from different cells which provides a platform for subsequent studies to identify immunoregulatory functions of exosomes. Also, exosomes may contain viral particles which affect recipient cells. In this study, we hypothesized that chicken tracheal cells secret exosomes whose contents are affected by different TLR ligands and inactivated avian influenza virus. In the current study, tracheal organ culture (TOC) has been prepared from 20-day-

old-chicken-embryo. Exosomes released from tracheal rings will be isolated and characterized following stimulation with TLR ligands, UV inactivated H9N2 low pathogenic AIV. The profile of RNA types, expression of viral mRNA and host-derived miRNAs and antiviral genes and cytokines mRNA in exosomes released from cells will be identified. We will identify which miRNA is induced by TLR ligand or influenza virus stimulation. The results of this study will provide insight into the complex interplay of the immune system and the induction of antiviral responses in chickens.

M3. Analysis of the immunomodulatory/adjuvant effect of the P97c recombinant protein for the development of a vaccine against the avian influenza virus

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The use of an appropriate adjuvant remains an important strategy for the development of effective subunit vaccines. The P97 protein of *Mycoplasma hyopneumoniae*, the etiologic agent of porcine enzootic pneumonia, is involved in microbial adhesion to the epithelial cells of the respiratory tract. We previously showed that the C-terminal portion of P97 (P97c), when coupled to immunogenic proteins and delivered in vivo with adenovectors, increases the antibody response to these immunogens, suggesting an adjuvant effect of P97c (Roques et al. 2013, Vaccine 31:2698-2704) In this study, the P97c protein was fused to a viral epitope and analyzed for its adjuvant effect. The selected viral epitope is the ectodomain of the matrix 2 protein (M2e) derived from the avian influenza virus. The M2e epitope is believed to be a promising candidate for the development of a vaccine strategy against various sub-types of the virus. The nucleic acid sequence encoding P97c was inserted into the pGex-4T1 expression vector upstream of three repeats of the sequence coding for the M2e epitope. The resulting construct was used to produce the GST-P97c-3M2e fusion protein in Rosetta *E. coli* bacteria (DE3). The P97c-3M2e portion of the expressed protein was obtained and purified by using a glutathione agarose column and a protein thrombin cleavage procedure. In vitro experiments demonstrated that both P97c and P97c-3M2e activated TLR5 by using HEK-Blue TLR5 reporter cells, thus triggering an innate immune response. Circular dichroism spectrum analysis of P97c showed a predominantly α-helix conformation, similar to the structure of flagellin which is also known to activate TLR5. These results confirm the immunomodulatory effect of P97c on the innate immune system and further are in support of an adjuvant effect of the protein. In vivo experiments are currently carried out to evaluate the adjuvant effect of P97c for the development of a vaccine against avian influenza virus.

M4. Global picture of nidovirus-host cell interactions revealed by comparative proteomics

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The porcine reproductive and respiratory syndrome virus (PRRSV) and the porcine epidemic diarrhea virus (PEDV) are responsible for severe economic losses. Earlier, we and others showed that the composition of virus-host multiprotein complexes is controlled by viruses through a direct recruitment of the host proteins. Growing evidence indicates that extracellular microvesicles (EMV) play an important role in viral pathogenesis and modulation of host immune responses to infection. Consequently, the characterization of the molecular composition of the EMV of virus-infected cells and identification of the host proteins that are specifically encapsidated into or bound to virions are important for our further understanding of virus-host interactions. To accomplish this objective, we produced and purified PRRSV and

PEDV virions and EMV. We hypothesized that alterations in the proteomic profiles of PRRSV and PEDV virions and EMV will reflect changes in the environmental conditions. Furthermore, we hypothesized that the tight interactions between host and viral proteins defines the fate of infection and pathogenesis. We examined the composition of progeny virions identifying cellular proteins that are associated with virions or EMV using mass spectrometry (MS) strategies. Here we demonstrated the incorporation of cellular proteins into PRRSV and PEDV virions. We found that the both viruses infections affected the abundance levels of numerous host proteins associated with EMV. More specifically, the abundance of proteins involved in immune responses and metabolic processes was dramatically affected by PRRSV infection. Proteins involved in immune responses were also changed in PEDV infected cells. Interestingly, in PEDV infected cells, host proteins involved in cell cycle regulation and cytoskeletal system were affected in abundance. Further investigations are needed to evaluate the role of individual cellular proteins in the nidoviral replication, assembly, and pathogenesis.

M5. Effect of naturally contaminated diet with deoxynivalenol (DON) on vaccine response against Newcastle disease and infectious bronchitis virus in broiler chicken

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Chickens are susceptible to mycotoxins. Deoxynivalenol (DON), a type B trichothecene mycotoxin, produced by *Fusarium* species, causes adverse effects on birth health and performances. This study was conducted to investigate the impacts of naturally contaminated diet with DON on vaccine response against Newcastle (NDV) and infectious brochities (IBV) diseases in boilers and to evaluate the ability of commercial feed additive (Alfatrol) to prevent these impacts. A total of 600 1-d-old broiler chickens (Ross 308 males) were randomly divided into 6 dietary treatments (100 birds per group); (1) control group fed with noncontaminated diet; (2) diet contaminated with 1.5 mg DON/kg feed; (3) diet contaminated with 3 mg DON/kg feed; (4) diet contaminated with 0.5 mg DON + 250 g of feed additive/kg feed; (5) diet contaminated with 1.5 mg DON + 250 g of feed additive/kg feed; (6) diet contaminated with 3 mg DON + 250 g of feed additive/kg feed for five weeks. Zootechnical parameters and sera were collected weekly. At 35 days of age, birds were scarified. Tissues from intestine (duodenum, jejunum, ileum, caecum), bursa of Fabricius and thymus were collected for histological examination. Organs (heart, liver, spleen, bursa of Fabricius) were also weighted. DON and deepoxy-DON (DOM-1) were analyzed in the sera with HPLC method. Contaminated diets with DON at 1.5 and 3 mg/kg had no effect on body-weight gain, live body weight, feed consumption, feed conversion ratio and mortality. They also did not affect serum antibodies against NDV and IBV, biochemistry parameters, intestinal morphology or organ weights. However, serum concentrations of DON and DOM-1 were higher in experimental groups. DON at the concentration used in this study 1.5 and 3 mg/kg feed had no impact on birth performances and did not affect birth immune response against NDV and IBV. Because there were no noticeable effects of the contaminated diets, adding feed additive had no mitigating impacts on the births.

M6. Setup and tuning of a continuous feeding *in vitro* bioreactor to mimic a pig intestinal microbiota

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Modulation of the pig's intestinal microbiota is being explored to prevent the presence of foodborne pathogens at the farm. Microbiota studies classically require the use of animals which is limiting the quantity of experimental conditions that can be tested simultaneously. The goal of this project was to create a continuous feeding system that mimics and is able to maintain the intestinal microbiota, in order to lower the use of live animals. This system is composed of a mother reactor that is used to feed 7 daughter reactors, allowing different conditions to be tested in each of them such as unique pH profiles, unique culture media or even introduction of different selected bacteria. The reactors are in a closed environment, allowing control over oxygen levels in the system. The bioreactor can use synthetic media that represent ileal content or use an in vitro digested standard feed as for culture media, a digestion equivalent to ileal content. Theses preliminaries studies allowed the determination of the ileal nutriment media to be used. Digested feed from the IViDiS and PBS in a ratio 1:1 was selected after incubating fecal matter in different digested fee/PBS ratios for 24h in aerobic and anaerobic conditions at 37°C. Total aerobic and anaerobic bacterial count on blood agar, at 37°C for 24h, showed no difference between 100% and 50% digested feed with PBS (1:1 ratio). When using 10% digested feed, significantly less anaerobic total bacteria and significantly more total bacteria counts were observed. For the first experiments with the system, 5 reactors will be used, where 2 of them will be used as controls, 2 will be supplemented with 10 CFU/ml of Salmonella and one of them will not be feed fresh media. The system will run for 48 hours, with sample taken at 0, 6, 12, 24 and 48h. DNA will then be extracted for sample, and quantitative PCR for total bacteria, *lactobacillaceae* and Salmonella will be made to compare each condition.

M7. *Streptococcus suis* suilysin disrupts integrity of the pig tracheal epithelial barrier

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Streptococcus suis is a bacterial pathogen that colonizes the upper respiratory tract of pigs. It is known to cause severe infections such as septicemia, meningitis, arthritis, and endocarditis in pigs and is responsible for major economic losses in the swine industry worldwide. To better understand the interactions between *S. suis* and the porcine respiratory epithelium, we investigated the ability of this pathogen to damage the tracheal epithelial barrier. We showed that *S. suis* can compromise the integrity of the tracheal epithelial barrier as determined by measuring transepithelial electrical resistance and FITC-dextran transport. As a consequence of this breakdown, *S. suis* can translocate across an epithelial cell monolayer. On the other hand, a *S. suis* mutant deficient in the production of suilysin, a cholesterol-dependent cytolysin, did not cause any damage to the epithelial barrier. In addition, a recombinant suilysin disrupted the integrity of the tracheal epithelial barrier. Immunofluorescence staining suggested that suilysin affects two major tight junction proteins (occludin and zonula occludens-1). In summary, *S. suis* is able to compromise the function of the porcine respiratory epithelial barrier through the action of suilysin. This enhanced understanding of the interactions between *S. suis* and tracheal epithelial cells may help in the development of novel strategies to prevent the invasion of the epithelium by this and other swine respiratory pathogens.
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M8. Study of the involvement of pilus G in the virulence of *Streptococcus suis* serotype 2

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Streptococcus suis is one of the most important swine bacterial pathogen that can be transmitted to humans by contact with diseased animals or contaminated raw pork products. It causes mainly septicemia, meningitis, arthritis, endocarditis in pigs and meningitis and septic shock in humans. Of the 35 serotypes described, serotype 2 is the one most frequently isolated from diseased pigs in most parts of the world. Pili-like structures have been shown to contribute to the virulence of different Gram-positive bacteria. For example, pili may play a key role in adherence to host cells and to proteins of the extracellular matrix, biofilm formation, self-auto-aggregation and virulence. Genomic studies revealed the presence of at least four distinct putative pilus gene clusters in *S. suis* (*srtBCD*, *srtE*, *srtF*, and *srtG* clusters). The *srtG* gene cluster has precisely been described in the representative North American *S. suis* serotype 2 strain 89/1591. It consists of one sortase gene (*srtG*) and two putative pilin subunit genes (*sgp1* and *sgp2*). Isogenic mutants defective in the production of Sgp1, Sgp2 and *srtG* (Δ *sgp1*, Δ *sgp2* and Δ *srtG*) were obtained by splicing-by-overlap-extension PCR. The *PCR products were then cloned into the temperature-sensitive S. suis-E. coli* shuttle vector pSET4s and the plasmids were introduced into *S. suis* 89/1591 strain by electroporation. Using these mutants, we demonstrated that the *srtG* pilus is not involved in the self-aggregation phenomenon and in the biofilm formation of strain 89/1591. Further tests to evaluate the involvement of the *srtG* pilus in the pathogenesis of the infection are presently being evaluated by studies of invasion of and adhesion to porcine tracheal epithelial and porcine brain microvascular endothelial cells, using the wild-type and mutant strains. The role of the *srtG* pilus in virulence using a mouse model of infection will also be evaluated.