Integration of new researchers into our organization

Since May 2010, the CRIP has welcomed seven new researchers. We are pleased and proud with the addition of the following experts:

**Julie Arsenault** is a university researcher in epidemiology at the Department of pathology and microbiology, Faculté de médecine vétérinaire, Université de Montréal. She works with Dr. Sylvie D’Allaire on projects related to the creation of a monitoring program and on the spatial, temporal and genetic distribution of the PRRS virus in Quebec, and with Dr. Ann Letellier on meat safety, julie.arsenault@umontreal.ca.

**Steve Charette** is a university researcher at the Department of biochemistry, microbiology and bioinformatics, Faculté des sciences et de génie of Université Laval, and a member of the Institut de biologie intégrative et des systèmes (IBIS). He studies the host-pathogen interactions that his laboratory analyzes using the Dictyostelium discoideum amoeba as an alternative host model. He collaborates with Dr. Daniel Grenier on a project dealing with the interaction between the amoeba and the S. suis pathogen, Steve.Charette@bcm.ulaval.ca.

**Martin Chénier**, assistant professor, Macdonald Campus, McGill University, studies the ecology of complex bacteria communities in agricultural ecosystems, martin.chenier@mcgill.ca.

**Martine Denicourt**, guest professor, Faculté de médecine vétérinaire, Université de Montréal, works on animal welfare and analyzes an electrocution euthanasia method that is acceptable for swine production and safe for workers, and contributes to the development of the on-farm biosafety program against the PRRS virus at the regional level, martine.denicourt@umontreal.ca.

**Christian Klopfenstein**, researcher, is responsible for the Swine Health Veterinary Program of the Centre de développement du porc du Québec inc. (CDPQ), cklopfenstein@sympatico.ca.

**Luke Masson**, researcher at the Biotechnology Research Institute, National Research Council of Canada, is working on the use of DNA biochips for the detection and identification of bacteria, virulence factors and antibiotic resistance genes, luke.masson@nrc-cnrc.gc.ca.

**Jean-Pierre Vaillancourt**, researcher and director of the Epidemiology of Zoonoses and Public Health Research Unit (GREZOSP), Université de Montréal, works in epidemiology and biosafety, jean-pierre.vaillancourt@umontreal.ca.

We bid them welcome!
News about CRIP members

Dr. Donald Niven, our colleague from the Department of Natural Resource Science, Macdonald Campus of McGill University, and distinguished CRIP member retired in September 2010. Dr. Niven is a long-time collaborator. From the very beginning, he was part of a country-wide group devoted to research on swine infectious disease called the Canadian Research Network on Bacterial Pathogens of Swine, later joined SIDNet “Swine Infectious Diseases Network” supported by the federal finding agency NSERC, and now the CRIP.

His research on animal pathogens lead the way to multidisciplinary research projects in microbiology and in animal science. His team was the first to demonstrate iron acquisition by an animal pathogen (Actinobacillus pleuropneumoniae) through the transferrin system of pigs (1989). His analyses then focused on the biochemistry and molecular biology aspects of the iron acquisition process used by a wide range of animal pathogens. These studies contributed significantly to our general understanding of the iron acquisition mechanisms that pathogenic bacteria employ. We wish to express our thanks to Dr. Niven for his contribution to the CRIP and his unwavering support. We wish him a happy and well-deserved retirement.

Dr. Charles Dozois, regular CRIP member, has succeeded Dr. Alain Fournier as director of the INRS–Institut Armand-Frappier research centre effective April 13, 2011. Congratulations on this promotion and best wishes.

Recognition of three members!

Dr. Mariela Segura received the 2011 Fisher Scientific Award which is given during the Annual Conference of the Canadian Society of Microbiologists to recognize young researchers for their outstanding contribution to microbiology. This year, the Conference was held on June 20-23, 2011 in St. John’s Newfoundland, Canada.

In 2011, our colleague, Dr. Marie Archambault, received a teaching excellence award from Université de Montréal. Having also received the best teacher award attributed by second-year students from the Faculté de médecine vétérinaire the year before, Dr. Archambault’s teaching ability was once more recognized by the Université.

On November 19, 2010, Dr. Marcelo Gottschalk was awarded the title of Honorary Professor of Veterinary Medicine by the University of Buenos Aires, Argentina, the same institution where he first studied veterinary medicine. This honour was presented to him by the dean and the assistant dean of the Faculty of Veterinary Sciences, Drs. Marcelo Miguez and Humberto Cisale, during the commencement ceremony, in recognition of Dr. Gottschalk’s outstanding contribution throughout his career.

Swine Immunology Tool Bank

You have a new project in swine immunology? You are looking to quantify cytokines in pigs? Visit the Web site of the Swine Immunology Tool Bank, or SITB for short. The SITB proposes a list of databases, tools and protocols in swine immunology that have been developed by CRIP members and their collaborators. You will find there among other things: reagents for swine immunology; a list of failed cross-reactions; techniques for studying infection pathogenesis. Click on this link: http://www.medvet.umontreal.ca/SITB/.
4th CRIP Symposium

The 4th CRIP Symposium was held on May 30-31, 2011 at the Faculté de médecine vétérinaire (FMV) of Université de Montréal (UdeM). The Symposium enjoyed a record attendance (108 participants) a bright and sunny sky. Participants were treated to high-level lectures by Drs. Janet Hill, from University of Saskatchewan, Paul R. Langford, from the Imperial College London, UK, and John Prescott, from the Ontario Veterinary College of the University of Guelph. From the FMV, Drs. Nadia Bergeron and Carl A. Gagnon presented the latest advances on Salmonella typhimurium and the H3N2 influenza virus. These two stimulating days highlighted the contribution of students and postdoctoral researchers. We were impressed by the quality of the presentations and the depth of the research. In fact, 13 lectures were presented by students, three by postdoctoral researchers and five by researchers. There were also a total of 22 poster presentations that rivalled in quality and innovation. During the afternoon of the second day, our participants had the opportunity to attend a training session on RSS given by Ms. Huguette Mallet, librarian at UdeM.

With the support of our sponsors, FMV, Pfizer and Life technologies, four students were rewarded for the quality of their oral or poster presentations. The Assistant Dean of Research presented Sébastien Sabbagh, of the Faculté de médecine of UdeM, with 1st Prize for the best lecture. A doctoral student under the direction of France Daigle, Sébastien gave a presentation entitled: “Identification et caractérisation de gènes chez Salmonella impliqués dans l’interaction avec les macrophages.” Another student of the FMV, Alexandre Thibodeau, under the joint direction of Drs. Ann Letellier, Sylvain Quessy and Évelyne Guèvremont, was awarded 2nd Prize for his lecture entitled: “Microarray characterization of Campylobacter jejuni genes involved in colonization and antimicrobial resistance of broiler chickens”.

First Prize in the best poster category was awarded to Jason Létourneau (FMV), under the direction of Michaël Mourez. Jason presented his results on: “Escherichia coli expressing AIDA-I binds to apolipoprotein AI, a novel interaction.” Second Prize in the best poster category went to Jean-Philippe Brousseau, student at Agriculture and Agri-Food Canada and Université Laval, under the direction of Martin Lessard and Denis Roy. Jean-Philippe’s presentation dealt with: “Effets de Pediococcus acidilactici et Saccharomyces cerevisiae boulardii sur le microbiote de l’iléon et du côlon chez le porcelet sevré”.

To paraphrase Dr. Guy Breton, Dean of Université de Montréal, in his email of thanks addressed to the academic community (June 1, 2011): “That is what the CRIP does fine and well”.

Congratulations to prize recipients and to all students for the quality of their presentations and of course. Thanks to all participants!
Café CRIP: Swine Health Diagnosis

For the first edition of this event, the CRIP partnered with the Diagnostic Service (DS) of the Faculté de médecine vétérinaire to organize this joint happening. Five members of the organization with Drs. Estela Cornaglia (DS), Younès Chorfi and a guest lecturer, Dr. Kyoung-Jin Yoon, College of Veterinary Medicine, Ames, Iowa State University, presented the results of their work in diagnosis research in the swine sector.

Topics that were addressed: the tests available as well as the most recent epidemiological results and the new clinical cases in the fields of virology, bacteriology, immunology, meat safety, antibiotic resistance and the detection of mycotoxins. Nine oral presentations were given and further discussed with the speakers in porcine health at a round table held in the afternoon. More than 65 participants enjoyed the friendly atmosphere of this first Café CRIP event, which was a great success.

CReSA: 6th International Symposium on Emerging and Re-emerging Pig Diseases

The 6th International Symposium on Emerging and Re-emerging Pig Diseases was held from June 12-15, 2011 in Barcelona, Spain. Dr. Sylvie D’Allaire gave a lecture on the genetic and spatial correlations for the PRRS virus, while Dr. Laura Batista’s presentation dealt with the regional control and elimination of that virus.

5e Colloque international francophone de microbiologie animale

The fifth CIFMA convention was held on April 3-5, 2011 in Marrakech, Morocco, under the theme of: “Apport des biotechnologies en vaccinologie”. A large delegation of CRIP members were in attendance, including Drs. Marie Archambault, Daniel Dubreuil, John M. Fairbrother, Marcelo Gottschalk, Josée Harel, Mario Jacques, Ann Letellier, Sylvain Quessy and Mariela Segura, who presented their latest work.

Briefing in swine health: new strategies to fight against the PRRS virus

Among the speakers, Drs. Martine Denicourt (Maelstrom, UdeM), Laura Batista (Boehringer Ingelheim) and Christian Klopfenstein (CDPQ) presented an overview of Quebec pork production and the most relevant methods and protocols. From May 31 to June 2, 2011, the Maelstrom group, in association with Boehringer Ingelheim and the CDPQ, held a series of regional evening conferences intended for producers and stakeholders interested in swine health designed to provide information about the latest strategies in the fight against the PRRS virus adapted to the Quebec setting.
**Advances in research: a few accounts**

**Outreach of CRIP members**

François Malouin participated to one of the top 10 best discoveries of 2010 selected by Québec Science magazine

Photos from left to right: Daniel Fontaine, François Malouin, Louis-Charles Fortier

The collaboration struck between the laboratories of Drs. Daniel Lafontaine, François Malouin and Louis-Charles Fortier, from the Department of biology and the Department of microbiology and infectious diseases of Université de Sherbrooke, lead to the discovery of a new class of antibiotics capable of stopping a mammary gland infection caused by Staphylococcus aureus in a mouse model of mastitis. This type of antibiotic acts on the riboswitch. 

By controlling the expression of a gene, a riboswitch acts as a switch that regulates the quantity of certain substances that are essential to proper cell functioning. Under the supervision of researchers, post-doctoral researcher Jérôme Mulhbacher identified and tested PC1 as the molecule having a structure similar to that of guanine and that is capable of binding to the guaA gene riboswitch. PC1 can then inhibit the expression of guaA and therefore GMP synthesis by Staphylococcus. Bacterial growth is then inhibited both *in vitro* and *in vivo*, as doctoral student Marianne Allard effectively demonstrated the importance of this gene for the bacteria under experimental infection conditions. Researchers have also shown that PC1 can act against all bacteria that have the riboswitch-controlled guaA gene, which means that it also acts against MRSA that is frequently found in swine and Clostridium difficile. This joint discovery was selected by Québec Science magazine as one of the top ten best discoveries of the year 2010. Congratulations to the authors of this breakthrough!


Josée Harel contributed to an article selected by Nature Reviews Microbiology 2010

Photos from left to right: Christine Martin, Josée Harel

The results of an international research collaboration directed by Dr. Christine Martin, of France’s Institut national de la recherche agronomique (INRA) with Dr. Josée Harel, of Université de Montréal, have shown for the first time how the *E. coli* bacteria responsible for Hamburger disease can survive in the cow’s intestine by tapping into a specific and exclusive nutrition source: ethanolamine. Published in the October issue of Environmental Microbiology (Bertin et al., 2010) and highlighted in Nature Reviews Microbiology, the results of this study could lead to the development of non-medical interventions to eradicate this bacteria.


Research highlights in Nature Reviews Microbiology: [http://www.nature.com/nrmicro/index.html](http://www.nature.com/nrmicro/index.html).
Advances in research

The Council of Canadian Academies published on September 22nd, 2011, a report entitled: "Healthy animals, healthy Canada". In 2009, the Minister of Agriculture and Agri-Food, on behalf of the Canadian Food Inspection Agency, asked the Council of Canadian Academies to assemble an Expert Panel to assess the state and comprehensiveness of risk assessment techniques in animal health science, specifically pertaining to risks which may impact human health. The Panel was chaired by Dr. Alastair Cribb, Professor and Dean of the Faculty of Veterinary Medicine at the University of Calgary. Our colleague, Dr. John M. Fairbrother, sited on among the twelve members of the panel, as the director of the reference laboratory for Escherichia coli of the World Organisation for Animal Health (OIE). Link to access the complete report: http://www.sciencepourlepublic.ca/.

For more information, please contact:
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PRRS : 3 programs to improve on farm biosecurity

Thanks to the initiative of CRIP members and swine health stakeholders, three programs will help pork producers to reduce their production costs by improving on-farm biosafety. These projects have been made possible by the financial support of the Canadian Swine Health Board. First, Dr. Christian Klopfenstein (CDPQ and CRIP) and his colleagues have put together a four-hour biosafety training session that will be offered throughout Quebec. The sessions have begun in August and should be completed by the end of December. Second, 50 producers will be given an opportunity to get an action plan that will combine some technical, financial and sanitary components, also from August to December 2011. This program will be conducted in two steps: 1. Summary diagnosis by the veterinarian who is responsible for the herd health; 2. Assessment by the management advisor of the costs associated with the implementation of the actions suggested by the veterinarian. And third, the last program deals with the Local Control of Sanitary Stabilization and Eradication (C.L.E. in French). It is a pilot project on the local control of PRRS. The porcine reproductive and respiratory syndrome (PRRS) is the costliest disease in the pork industry. Three or four areas will be targeted, each with a maximum of 40 producers. The objective is to prompt producers in these areas to consult one another and to collaborate as a group to identify the main risks of spreading the PRRS virus within the area and identify potential control measures to prevent the virus from spreading in the area. Participating producers commit to implementing the necessary measures to stabilize the sanitary status of their herd in order to protect all the producers in their area.

Links to organizations mentioned in this article: Centre de développement du porc du Québec inc., Canadian Swine Health Board, Fédération des producteurs de porcs du Québec.


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Bacterial biofilms

Bacterial biofilms are structured aggregates of bacterial cells encased in a polymeric matrix and attached to a surface. The ability to form a biofilm is now recognized as a property of all microorganisms. In fact, it is estimated that biofilm accounts for 80% of the microbial biomass on our planet. Bacterial biofilms isolated from various environments share common characteristics: (i) bacterial cells are held together by a polymeric matrix composed of exopolysaccharides, proteins and nucleic acids; (ii) the development of biofilm occurs as a response to extracellular signals, either present in the environment (e.g. concentration of nutrients) or produced by bacterial cells (e.g. quorum sensing); (iii) the biofilm protects bacteria against the host immune system, desiccation and antimicrobial substances (e.g. antibiotics and disinfectants).

Although there is abundant literature about biofilms associated with infection in humans or with industrial processes, surprisingly enough, little research has been devoted to the formation of biofilms in pathogenic bacteria found in animals and zoonotic bacteria. We have recently published a review paper (1) and written a BioTrends* brief (2) on the subject in order to raise the awareness of agri-food industry stakeholders about the importance of biofilms.

Our laboratory studies the formation of biofilms with different pathogenic bacteria found in swine, including *Actinobacillus pleuropneumoniae*. We routinely use a 96 well microplate system in static mode with crystal violet solution to colour the biofilm. We have observed that *A. pleuropneumoniae* could form a very significant biofilm in just a few hours, which would suggest a possible role during acute infections (3). The use of a mutagenesis method (4) and the analysis of transcriptomes enabled us to identify several new genes involved in the formation of biofilms with this bacteria.

Recent results show that *A. pleuropneumoniae* strains in the form a biofilm are 100 to 30,000 times more resistant to antibiotics than the same strains grown as planktonic bacteria in a liquid medium. Indeed, the concentration required to eradicate a biofilm is much higher than the minimum inhibitory concentration (MIC), which unfortunately remains the reference value to determine the sensitivity of bacteria to various antimicrobial agents. Interestingly, we have observed that a low concentration of zinc could inhibit biofilm formation by *A. pleuropneumoniae* (3).

A research project funded as part of the CRIP New Initiatives is currently ongoing to assess the ability of zinc to inhibit the formation of biofilm in many other pathogenic bacteria found in swine.

Finally, we have developed a continuous flow model used to study the formation of biofilms at the air-liquid interface. This model has the advantage of being representative of the conditions found in the lung. We expect to use it to study the formation of biofilms by various pathogenic bacteria found in the respiratory tract of pigs.

Figure 1. Stages of the formation and dispersal of a bacterial biofilm (2).

Figure 2. Biofilm produced by *A. pleuropneumoniae* in a microplate system (3).
The formation of biofilm represents a significant animal health and public health issue. Additional research is required to develop strategies for the prevention and treatment of animal infections that will take into account biofilm characteristics. Further research is also required to develop disinfection procedures that can eliminate biofilms at the farm, slaughterhouse or processing plant, since these biofilms represent potential reservoirs of infectious agents.


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The effect of antimicrobials as growth promotants in conventional status growing-finishing pigs

The use of antibiotic substances as growth promotants is a practice that is challenged by public health authorities and consumer groups. It is considered to be an improper use of antibiotics. The objectives of this project were to: 1) quantify growth performance (average daily gain – ADG, feed conversion index – FCI, weight variability and carcass characteristics) associated with the use of two antibiotics (tylosin phosphate, salinomycin) as growth promotants in pigs with a conventional health status kept in conditions similar to those found in the field. 2) describe the temporal variation of the antibiotic resistance in commensal bacteria (Enterococcus, Campylobacter, generic Escherichia coli) and pathogens (Salmonella) isolated in the feces of pigs from the three different treatment groups.

The study was conducted at the Centre de recherche en sciences animales de Deschambault (CRSAD) from November 2007 to February 2008 in a facility called the swine feeding testing and experimentation unit (Unité de testage et d’expérimentation en alimentation porcine). Three hundred and sixty-four commercial pigs (162 males and 162 females) from a cross between hybrid sows (Yorkshire X Large-White) and a Duroc boar were divided at random among 36 lots and assigned to one of three treatments (control, salinomycin (25 ppm) and tylosin (22 ppm)). The animals selected for this study had a conventional health status (minimally contaminated with the PRRS virus, Mycoplasma hyopneumoniae, Streptococcus suis and Haemophylus parasuis). The area available for each finishing pig was reduced to a minimum (0.69 m²/pig or 7.5 sq. ft.) in order to ensure that it was representative of the conditions observed in commercial farms.
Overall, in pigs from 26 to 113 kg, enriching feed with antibiotics resulted in beneficial effects below 2% on the average daily gain and feed conversion (insignificant effects p > 0.05). Beneficial effects were a little higher (2-3%) but still insignificant (p > 0.05) during finishing (82 to 113 kg). In addition, no measurable effect was observed on either carcass characteristics or weight variability of pigs sent to slaughter.

A total of 70 bacterial isolates of Enterococcus, Campylobacter, Escherichia coli were detected following four collections of fecal samples (J1, J35, J59 et J83). No Salmonella was found in the feces of pigs during this project. Antimicrobial resistance profiles were determined for each bacterial species for a total of 32 different antibiotics. Thirty percent of isolate/antibiotic combinations proved to be resistant (311/1046 combinations). The experimental structure of this project did not show if there was a resistance selection process during the growing-finishing stage.

The number of resistant isolates tended to increase in lots where animals were given tylosin, while this number tended to decrease in lots where animals were given salinomycin and tended to remain stable in control lots. The main resistance cases observed from the studied bacteria were: ampicillin, lincomycin, streptomycin, sulfisoxazole, tetracycline and tylosin. These antibiotic resistance profiles are similar to observations made in the farm component of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). The characterization of antibiotic resistance within this project suggests that antibiotics used as growth promotants on a single lot are insufficient to exert a measurable selection pressure.

This research report suggests that the use of antibiotics (tylosin phosphate, salinomycin) as growth promotant does not improve growth performance sufficiently to justify their use in pigs with a conventional health status kept in conditions similar to those found in the field. In addition, the report shows that pigs are carriers of certain bacteria that are resistant to antibiotics, but it suggests that the use of antibiotics as growth promotants in a single lot will not create enough pressure for the selection of resistant strains.

Link to access the complete report: Centre de développement du porc du Québec inc.

The authors of the report are: Christian Klopfenstein1, Janie Lévesque2, Joël Rivest1.

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Short list of published articles (2010-2011)

**Virulence factors**

The Pho regulon and the pathogenesis of *Escherichia coli*
Crépin S, Chekabob SM, Le Bihan G, Bertrand N, Dozois CM, Harel J.
During the course of infection, bacteria must coordinately regulate gene expression in response to environmental stimuli. The phosphate (Pho) regulon is controlled by the two component-regulatory system PhoBR. PhoBR is activated during starvation and regulates genes involved in phosphate homeostasis. Several studies have highlighted the importance of the Pho regulon in bacterial pathogenesis, showing how induction of PhoBR, in addition to regulating genes participating in phosphate metabolism, leads to modulation of many cellular processes. The pleiotropic effects of Pho regulon activation include attenuated virulence and alteration of many virulence traits, including adhesion to host cells and resistance to cationic antimicrobial peptides, acidity and oxidative stresses. This review provides an overview of the relationship between the Pho regulon and virulence in *Escherichia coli* and illustrates that, in addition to regulating phosphate homeostasis, the Pho regulon plays a key role in regulating stress responses and virulence.


Structure-function analysis of the TibA self-associating autotransporter reveals a modular organization
Côté JP, Mourez M.
Some enterotoxigenic *Escherichia coli* strains express the TibA adhesin/invasin, a multifunctional autotransporter that mediates the autoaggregation of bacteria, biofilm formation, adhesion to cultured epithelial cells, and invasion of these cells. To elucidate the structure-function relationship in TibA, we generated mutants by transposon-based linker scanning mutagenesis and by site-directed mutagenesis. Several insertion mutants had a defect in either adhesion or autoaggregation. Mutants with a defect in autoaggregation were found in the N-terminal half of the extracellular domain, while mutants with a defect in adhesion were found in the C-terminal half. The deletion of the putative N-terminal autoaggregation domain abolished the autoaggregation of the bacteria but did not affect adhesion. The deletion of a proline-rich region located at the C terminus of the extracellular domain abolished the adhesion properties of TibA but did not affect invasion. This finding suggests that adhesion and invasion may rely on distinct mechanisms. Thus, our results reveal that TibA possesses a modular organization, with the extracellular domain being separated into an autoaggregation module and an adhesion module.


Cell type-dependent internalization of the *Escherichia coli* STb enterotoxin
Albert MA, Kojic LD, Nabi IR, Dubreuil JD.
Previous studies have suggested that internalization of the *Escherichia coli* STb enterotoxin in human and rat intestinal epithelial cells is involved in STb pathogenesis, but toxin uptake in porcine jejunum epithelium, the *in vivo* target tissue, still remains elusive. Using flow cytometry, we studied the internalization of fluorescein isothiocyanate-labelled STb in porcine intestinal epithelial IPEC-J2 and murine fibroblast NIH-3T3 cell lines. In contrast to the selective pronase resistance of STb in NIH-3T3 cells at 37 °C, but not at 4 °C, indicative of toxin internalization, most of the toxin was pronase-sensitive at both temperatures in IPEC-J2 cells, indicating reduced uptake, but significant cell surface binding. Actin reorganization is required for STb internalization by NIH-3T3 cells, confirming STb endocytosis in these cells. The toxin receptor, sulfatide, could not explain these internalization differences because both cell lines possessed surface sulfatide and internalized antisulfatide antibodies over time at 37 °C. Inhibition of lipid rafts endocytosis, known to contain sulfatide, with methyl-β-cyclodextrin or genistein, did not influence toxin uptake by either cell line. STb internalization is therefore differentially regulated depending on the cell type, possibly by factors other than sulfatide. Although a small STb fraction could be internalized by porcine intestinal epithelial cells, our findings suggest the ability of STb to induce, from the cell surface, intracellular signalling leading to fluid secretion in porcine intestinal epithelium.

**Novel genes associated with biofilm formation of *Actinobacillus pleuropneumoniae***

Grasteau A, Tremblay YD, Labrie J, Jacques M.

*Actinobacillus pleuropneumoniae* is a Gram-negative bacterium and is the causative agent of swine pleuropneumonia, a highly contagious respiratory disease. Biofilm formation is an important ability possessed by numerous bacterial pathogens. The purpose of this study was to identify and characterize biofilm mutants of *A. pleuropneumoniae* serotype 1 strain S4074 created using a mini Tn-10 transposon. The transposon library was screened to identify mutants with a modified ability to form biofilms in polystyrene microtiter plates. A total of 1200 mutants were screened and the analysis identified 24 mutants that exhibited abnormal biofilm formation, at least 16 unique genes were identified. Most genes identified in the enhanced-biofilm mutants encoded proteins with unknown functions, whereas most genes identified in the biofilm-reduced mutants encoded proteins related to transport, protein synthesis and nucleic acid synthesis. Approximately 50% of genes, including hns, *potD2*, *ptsI*, tig and rpmF, identified in our screen have been previously associated with biofilm formation in *A. pleuropneumoniae* and other bacterial species, and thus validated the screening method. The rest of genes identified, such as APL_0049, APL_0637 and APL_1572, have not been previously associated with biofilm formation. Interestingly, gene APL_0049 was previously seen among the genes differentially expressed during a natural infection of pig lungs. Preliminary characterization of the mutants was also initiated by assessing their hydrophobicity, their biofilm matrix composition and their ability to adhere to a polystyrene surface or NPTr cells. Based on the preliminary characterization, some of the mutants identified appear to have deficiencies during the initial attachment or growth of the biofilm. In conclusion, transposon mutagenesis analysis allowed the identification of new genes associated with biofilm formation in *A. pleuropneumoniae*.


**Structure determination of *Streptococcus suis* serotype 2 capsular polysaccharide***

Van Calsteren MR, Gagnon F, Lacouture S, Fittipaldi N, Gottschalk M.

The capsular polysaccharide (CPS) of *Streptococcus suis* serotype 2 was isolated, purified, chemically modified, and characterized. Sugar and absolute configuration analyses of the CPS gave the following composition: D-Gal, 3; D-Glc, 1; D-GlcNAc, 1; D-Neu5Ac, 1; L-Rha, 1. Sialic acid was found to be terminal, and the CPS was quantitatively desialylated by mild acid hydrolysis. The CPS was also submitted to periodate oxidation followed by borohydride reduction and Smith degradation. Sugar and methylation analysis, 1H and 13C nuclear magnetic resonance, and mass spectrometry of the native CPS or of its specifically modified products allowed to determine the repeating unit sequence: [4][Neu5Ac(alpha2-6)Gal(beta1-4)GlcNAc(beta1-3)]Gal(beta1-4)[Gal(alpha1-3)]Rha(beta1-4)Glc(beta1-)]. The backbone sequence was found to be identical to that of *Streptococcus agalactiae* or group B *Streptococcus* (GBS) type VIII and *Streptococcus pneumoniae* type 23F. The *S. suis* CPS shares the sequence Neu5Ac-Gal-GlcNAc-Gal in common with GBS types Ia, Ib, II, III, and IV CPSs but differs from them by the presence of rhamnose and the fact that sialic acid is 2,6- rather than 2,3-linked to the following Gal. A correlation between the *S. suis* CPS sequence and genes of the serotype 2 cps locus encoding putative enzymes responsible for the biosynthesis of the repeating unit was tentatively established.

Critical role for *Streptococcus suis* cell wall modifications and suilysin in resistance to complement-dependent killing by dendritic cells

Lecours MP, Gottschalk M, Houde M, Lemire P, Fittipaldi N, Segura M.

*Streptococcus suis* is an emerging zoonotic agent of septicemia and meningitis. Knowledge on host immune responses toward *S. suis* and strategies used by this pathogen for subversion of these responses is scarce. Here, *S. suis* modulation of dendritic cell (DC) functions were assessed for the first time. Using *S. suis* knockout mutants in capsular polysaccharide (CPS) expression, it was shown that CPS blocks DC phagocytosis and impairs cytokine release by hindering cell wall components. Mutants impaired in D-α-aminolactic acid (LTA) or N-deacetylation of peptidoglycan (PG) further demonstrated the importance of cell wall in modulation of DC activation. Notably, LTA/PG modifications were identified as major players in resistance to complement-dependent killing by DCs. Finally, *S. suis* hemolysin was partially involved in cytokine release and also contributed to bacterial escape of opsonophagocytosis. Overall, *S. suis* uses its arsenal of virulence factors to modulate DC functions and escape immune surveillance.

*J Infect Dis. 2011 Sep;204(6):919-29.*

Characterization of porcine dendritic cell response to *Streptococcus suis*

Lecours MP, Segura M, Lachance C, Mussa T, Surprenant C, Montoya M, Gottschalk M.

*Streptococcus suis* is a major swine pathogen and important zoonotic agent causing mainly septicemia and meningitis. However, the mechanisms involved in host innate and adaptive immune responses toward *S. suis* as well as the mechanisms used by *S. suis* to subvert these responses are unknown. Here, and for the first time, the ability of *S. suis* to interact with bone marrow-derived swine dendritic cells (DCs) was evaluated. In addition, the role of *S. suis* capsular polysaccharide in modulation of DC functions was also assessed. Well-encapsulated *S. suis* was relatively resistant to phagocytosis, but it increased the relative expression of Toll-like receptors 2 and 6 and triggered the release of several cytokines by DCs, including IL-1β, IL-6, IL-8, IL-12p40 and TNF-α. The capsular polysaccharide was shown to interfere with DC phagocytosis; however, once internalized, *S. suis* was readily destroyed by DCs independently of the presence of the capsular polysaccharide. Cell wall components were mainly responsible for DC activation, since the capsular polysaccharide-negative mutant induced higher cytokine levels than the wild-type strain. The capsular polysaccharide also interfered with the expression of the co-stimulatory molecules CD80/86 and MHC-II on DCs. To conclude, our results show for the first time that *S. suis* interacts with swine origin DCs and suggest that these cells might play a role in the development of host innate and adaptive immunity during an infection with *S. suis* serotype 2.


Administration of probiotics influences F4 (K88)-positive enterotoxigenic *Escherichia coli* attachment and intestinal cytokine expression in weaned pigs


This study evaluated the effect of the probiotics *Pediococcus acidilactici* and *Saccharomyces cerevisiae boulardii* on the intestinal colonization of O149 enterotoxigenic *Escherichia coli* harbouring the F4 (K88) fimbriae (ETEC F4) and on the expression of ileal cytokines in weaned pigs. At birth, different litters of pigs were randomly assigned to one of the following treatments: 1) control without antibiotics or probiotics (CTRL); 2) reference group in which chlortetracycline and tiamulin were added to weaning feed (ATB); 3) *P. acidilactici*; 4) *S. cerevisiae boulardii*; or 5) *P. acidilactici* + *S. cerevisiae boulardii*. Probiotics were administered daily (1 × 109 CFU per pig) during the lactation period and after weaning (day 21). At 28 days of age, all pigs were orally challenged with an ETEC F4 strain, and a necropsy was performed 24 h later. Intestinal segments were collected to evaluate bacterial colonization in the small intestine and ileal cytokine expressions. Attachment of ETEC F4 to the intestinal mucosa was significantly reduced in pigs treated with *P. acidilactici* or *S. cerevisiae boulardii* in comparison with the ATB group (P = 0.01 and P = 0.03, respectively). In addition, proinflammatory cytokines, such as IL-6, were upregulated in ETEC F4 challenged pigs treated with *P. acidilactici* alone or in combination with *S. cerevisiae boulardii* compared with the CTRL group. In conclusion, the administration of *P. acidilactici* or *S. cerevisiae boulardii* was effective in reducing ETEC F4 attachment to the ileal mucosa, whereas the presence of *P. acidilactici* was required to modulate the expression of intestinal inflammatory cytokines in pigs challenged with ETEC F4.

Potential use of a recombinant replication-defective adenovirus vector carrying the C-terminal portion of the P97 adhesin protein as a vaccine against Mycoplasma hyopneumoniae in swine

Okamba FR, Arella M, Music N, Jia JJ, Gottschalk M, Gagnon CA.

Mycoplasma hyopneumoniae causes severe economic losses to the swine industry worldwide and the prevention of its related disease, enzootic porcine pneumonia, remains a challenge. The P97 adhesin protein of M. hyopneumoniae should be a good candidate for the development of a subunit vaccine because antibodies produced against P97 could prevent the adhesion of the pathogen to the respiratory epithelial cells in vitro. In the present study, a P97 recombinant replication-defective adenovirus (rAdP97c) subunit vaccine efficiency was evaluated in pigs. The rAdP97c vaccine was found to induce both strong P97 specific humoral and cellular immune responses. The rAdP97c vaccinated pigs developed a lower amount of macroscopic lung lesions (18.5 + or - 9.6%) compared to the unvaccinated and challenged animals (45.8 + or - 11.5%). rAdP97c vaccine reduced significantly the severity of inflammatory response and the amount of M. hyopneumoniae in the respiratory tract. Furthermore, the average daily weight gain was slightly improved in the rAdP97c vaccinated pigs (0.672 + or - 0.068 kg/day) compared to the unvaccinated and challenged animals (0.568 + or - 0.104 kg/day). A bacterin-based commercial vaccine (Suvaxyn MH-one) was more efficient to induce a protective immune response than rAdP97c even if it did not evoke a P97 specific immune response. These results suggest that immunodominant antigens other than P97 adhesin are also important in the induction of a protective immune response and should be taken into account in the future development of M. hyopneumoniae subunit vaccines

*Vaccine. 2010 Jul 5;28(30):4802-9.*

Flagellin produced in plants is a potent adjuvant for oral immunization

Girard A, Saran W, Bergeron-Sandoval LP, Sarhan F, Archambault D.

The aim of this study was to produce adjuvant with high biosafety, efficacy and low cost. Towards this goal, the plant *Nicotiana benthamiana* transient expression system was successfully used to express *Salmonella* typhimurium's flagellin (FljB). The yield of the expressed FljB was 280 mg per kg of fresh weight (FW) leaves. The lyophilized plant powder containing plant expressing FljB was mixed with ovalbumin (OVA) and used for oral immunization of BALB/c mice. The ELISA analysis showed higher and accelerated OVA-specific serum antibody responses in mice given the mixture when compared to animals receiving OVA alone. Furthermore, FljB elicited a mixed Th1/Th2 response as shown by the presence of specific anti-OVA IgG1, IgG2a and IgG2b isotypes. OVA-specific IgAs were also detected in mice given the mixture. Cell-mediated immune response to OVA was induced by FljB as determined by a spleen lymphocyte specific proliferation test. No immune response was generated against FljB. In conclusion, our results showed for the first time the production of FljB in plants and the efficient use of the crude lyophilized extract as an adjuvant for oral immunization

*Vaccine. 2011 Sep 2;29(38):6695-703.*

Polyelectrolyte Complex of Carboxymethyl Starch and Chitosan as Protein Carrier: Oral Administration of ovalbumin

Assaad E, Blemur L, Lessard M, Mateescu MA.

A novel carboxymethyl starch (CMS)/chitosan polyelectrolyte complex (PEC) was proposed as an excipient for oral administration of ovalbumin. The dissolution of ovalbumin from monolithic tablets (200 mg, 2.1×9.6 mm, 50% loading) obtained by direct compression was studied. When CMS was used as an excipient, more than 70% of the loaded ovalbumin remained undigested after 1 h of incubation in simulated gastric fluid (SGF) with pepsin. The complete dissolution, after transfer of tablets into simulated intestinal fluid (SIF) with pancreatin, occurred within a total time of about 6 h. Higher protection (more than 90% stability in SGF) and longer dissolution (more than 13 h) were obtained with 50% CMS/50% chitosan physical mixture or with PEC excipients. A lower proportion of chitosan was needed for PEC than for the CMS/chitosan mixture to obtain a similar dissolution profile. The high protection against digestion by pepsin, the various release times and the mucoadhesion properties of these excipients based on CMS favor the development of suitable carriers for oral vaccinations

Chicken infectious anaemia vaccinal strain persists in the spleen and thymus of young chicks and induces thymic lymphoid cell disorders
Vaziry A, Silim A, Bleau C, Frenette D, Lamontagne L.

The chicken infectious anaemia virus (CIAV) infection may induce immunosuppression and persistent infection. The use of vaccination in young chicks is still controversial due to its low immune efficiency. In order to verify the viral persistency of a vaccinal strain of CIAV and its associated-lymphoid cell disorders, 54 1-day-old specific pathogen free chicks were vaccinated (CIAV-VAC®; Intervet, Millsboro, Delaware, USA) and haematologic examination, expression of viral VP3 gene, humoral response and phenotyping of lymphoid cells were studied in lymphoid organs at various times post vaccination (p.v.). No clinical signs were observed but light heteropaena was detected in CIAV-vaccinated chicks. The VP3 gene of CIAV was detected by polymerase chain reaction in the thymus and spleen from day 7 until 28 days p.v. Thymic larger CD4(+)CD8(+) cells increased only at 7 days p.v. while smaller CD4(+)CD8 (+) cells decreased after 14 and 28 days in CIAV-vaccinated birds. The CD4 expression, in contrast to that seen for CD8, decreased in thymocytes from the CIAV-vaccinated group. In the spleen and bursa, the percentage of CD8(+) cells increased at 7 and 28 days p.v. only, while CD4(+) cells decreased simultaneously. The vaccinated chicks also exhibited a higher number of splenic CD3(-)CD8(+) cells (natural killer cells). The anti-CIAV antibody responses, however, remained low in most vaccinated chicks and did not persist up to 18 days p.v. These results suggest that the vaccinal virus strain is clinically attenuated but persists in the thymus and spleen in some birds, inducing a low humoral immune response and altering thymopoiesis.


Host-pathogene interactions

Amoeba host model for evaluation of Streptococcus suis virulence
Boufait L, Charette SJ, Fillion G, Gottschalk M, Grenier D.

The Gram-positive bacterium Streptococcus suis is a major swine pathogen worldwide that causes meningitis, septicemia, and endocarditis. In this study, we demonstrate that the amoeba Dictyostelium discoideum can be a relevant alternative system to study the virulence of S. suis.


Increased persistence of Salmonella enterica serovar Typhi in presence of Acanthamoeba castellani
Douesnard-Malo F, Daigle F.

Salmonella enterica serovar Typhi (S. Typhi) is the etiological agent of the systemic disease typhoid fever. Transmission occurs via ingestion of contaminated food or water. S. Typhi is specific to humans and no animal or environmental reservoirs are known. As the free-living amoeba Acanthamoeba castellani is an environmental host for many pathogenic bacteria, this study investigates interactions between S. Typhi and A. castellani by using co-cultures. Growth of both organisms was estimated by cell count, viable count, flow cytometry and fluorescence microscopy. Results indicate that S. Typhi can survive at least three weeks when grown with A. castellani, as opposed to less than 10 days when grown as singly cultured bacteria under the same conditions. Interestingly, amoebae growth after 14 days was similar in co-cultures or when singly cultured, suggesting that S. Typhi is not cytotoxic to A. castellani. Bacteria surviving in co-culture were not intracellular and did not require a physical contact with amoebae for their survival. These results suggest the possibility of a selective advantage for S. Typhi to be associated with A. castellani and that amoebae may contribute to S. Typhi persistence in the environment

Environmental characteristics associated with campylobacteriosis: accounting for the effect of age and season
Arsenault J, Michel P, Berke O, Ravel A, Gosselin P.
Campylobacteriosis is a leading cause of acute bacterial gastroenteritis. An ecological study was undertaken to explore the association between environmental characteristics and incidence of campylobacteriosis in relation to four age groups and two seasonal periods. A multi-level Poisson regression model was used for modelling at the municipal level. High ruminant density was positively associated with incidence of campylobacteriosis, with a reduced effect as people become older. High poultry density and presence of a large poultry slaughterhouse were also associated with higher incidence, but only for people aged 16-34 years. The effect of ruminant density, poultry density, and slaughterhouses were constant across seasonal periods. Other associations were detected with population density and average daily precipitation. Close contacts with farm animals are probably involved in the associations observed. The specificity of age and season on this important disease must be considered in further studies and in the design of preventive measures.
Epidemiol Infect. 2011 Apr 14:1-12. [Epub ahead of print]

Evaluation of the relationship between personality traits, experience, education and biosecurity compliance on poultry farms in Québec, Canada
Racicot M, Venne D, Durivage A, Vaillancourt JP.
Biosecurity compliance is an issue in all types of animal production. Poor compliance is frequently related to lack of knowledge or comprehension. Human dimensions, such as personality and attitudes were also suggested as being related to compliance. As part of a larger study, personality traits, experience, education and training of employees, visitors and growers were evaluated to assess their relationship with their compliance with biosecurity measures when entering and exiting poultry barns. Biosecurity compliance was evaluated using hidden cameras. One hundred fourteen individuals involved in a total of 2379 visits on 23 poultry farms responded to a personality test. Results demonstrated that several determinants of compliance exist, and some are related to personality, experience and education. Three personality traits were significantly associated with compliance: responsibility, complexity and action-oriented. Such information has important implications for the selection of job applicants or task attribution and to enhance effectiveness of training programs.

Risk signals of an influenza pandemic caused by highly pathogenic avian influenza subtype H5N1: Spatio-temporal perspectives
Zhang Z, Chen D, Chen Y, Davies TM, Vaillancourt JP, Liu W.
Highly pathogenic avian influenza (HPAI) subtype H5N1 is a trans-boundary animal disease that has crossed the animal-human species barrier and over the past decade has had a considerable impact on the poultry industry, wild bird populations and on human health. Understanding the spatio-temporal patterns of H5N1 outbreaks can provide visual clues to the dynamics of disease spread and of areas at risk, and thus improve the cost-effectiveness of disease control and prevention. This study describes the characteristics and investigates the temporal, spatial and space-time dynamics of H5N1 outbreaks in domestic poultry between December 2003 and December 2009 using a global database. The study found that the start date of the epidemic wave was postponed, the duration of the epidemic was prolonged and its magnitude reduced over time, but the disease transmission cycle was not efficiently interrupted. Two 'hot-spot' regions of H5N1 outbreaks were identified: well-documented locations in East and Southeast Asia, as well as a novel location at the boundaries of Europe and Africa, where enhanced surveillance should be conducted. The risk of a pandemic due to H5N1 remains high.
Vet J. 2011 Sep 21. [Epub ahead of print]
Antibiotics impacts and resistance

In growing pigs, chlortetracycline induces a reversible green bone discoloration and a persistent increase of bone mineral density dependent of dosing regimen

Guillot M, Alexander K, Pomar C, Del Castillo JR.

We studied in growing pigs the effects of exposure to dietary chlortetracycline on bone mineral density (BMD) and bone color. Pigs were randomly allocated to a drug-free diet (n=48) or a diet fortified with 800 ppm of chlortetracycline, starting either at 28- or 84-d of age, and for either a 28- or 56-d duration (n=16 pigs/group). The lumbar vertebral discoloration and BMD of randomly chosen pigs were evaluated at 28-d intervals up to 168-d of age. The odds of bone discoloration increased with dosing duration and age at treatment onset, and decreased with the withdrawal time and age at treatment onset interaction (p < or = 0.001). The measured trabecular BMD linearly increased with age and squared treatment duration (p < or = 0.005). Therefore, TC-induced bone discoloration is reversible, and may be prevented with proper dosing regimen design. Moreover, TC induces a persistent increase on BMD that could be detected with quantitative computed tomography.


Continuous feeding of antimicrobial growth promoters to commercial swine during the growing/finishing phase does not modify faecal community erythromycin resistance or community structure


To investigate the effect of continuous feeding of antimicrobial growth promoters (tylosin or virginiamycin) on the swine faecal community. The study consisted of two separate on-farm feeding trials. Swine were fed rations containing tylosin (44 or 88 mg kg(-1) of feed) or virginiamycin (11 or 22 mg kg(-1) of feed) continuously over the growing/finishing phases. The temporal impact of continuous antimicrobial feeding on the faecal community was assessed and compared to nondosed control animals through anaerobic cultivation, the analysis of community 16S rRNA gene libraries and faecal volatile fatty acid content. Feeding either antimicrobial had no detectable effect on the faecal community. Erythromycin methylase genes encoding resistance to the macrolide-lincosamide-streptogramin B (MLS(B) ) antimicrobials are present at a high level within the faecal community of intensively raised swine. Continuous antimicrobial feeding over the entire growing/finishing phase had no effect on community erm-methylase gene copy numbers or faecal community structure. Antimicrobial growth promoters are believed to function by altering gut bacterial communities. However, widespread MLS(B) resistance within the faecal community of intensively raised swine likely negates any potential effects that these antimicrobials might have on altering the faecal community. These findings suggest that if AGP-mediated alterations to gut communities are an important mechanism for growth promotion, it is unlikely that these would be associated with the colonic community.


Transcriptional analysis of antibiotic resistance and virulence genes in multiresistant hospital-acquired MRSA

Pruneau M, Mitchell G, Moisan H, Dumont-Blanchette E, Jacob CL, Malouin F.

The staphylococcal chromosome cassette mec cannot solely explain the multiresistance phenotype or the relatively mild virulence profile of hospital-acquired methicillin-resistant Staphylococcus aureus (HA-MRSA). This study reports that several multiresistant HA-MRSA strains differently expressed genes that may support antibiotic resistance, modify the bacterial surface and influence the pathogenic process. Genes encoding efflux pumps (norA, arsB, emrB) and the macrolide resistance gene ermA were found to be commonly expressed by HA-MRSA strains, but not in the archetypal MRSA strain COL. At equivalent cell density, the agr system was considerably less activated in all MRSA strains (including COL) in comparison with a prototypic antibiotic-susceptible strain. These results are in contrast to those observed in recent community-acquired MRSA isolates and may partly explain how multiresistant HA-MRSA persist in the hospital setting.

Use of a bacterial antimicrobial resistance gene microarray for the identification of resistant *Staphylococcus aureus*

As diagnostic and surveillance activities are vital to determine measures needed to control antimicrobial resistance (AMR), new and rapid laboratory methods are necessary to facilitate this important effort. DNA microarray technology allows the detection of a large number of genes in a single reaction. This technology is simple, specific and high-throughput. We have developed a bacterial antimicrobial resistance gene DNA microarray that will allow rapid antimicrobial resistance gene screening for all Gram-positive and Gram-negative bacteria. A prototype microarray was designed using a 70-mer based oligonucleotide set targeting AMR genes of Gram-negative and Gram-positive bacteria. In the present version, the microarray consists of 182 oligonucleotides corresponding to 166 different acquired AMR gene targets, covering most of the resistance genes found in both Gram-negative and positive bacteria. A test study was performed on a collection of *Staphylococcus aureus* isolates from milk samples from dairy farms in Québec, Canada. The reproducibility of the hybridizations was determined, and the microarray results were compared with those obtained by phenotypic resistance tests (either MIC or Kirby-Bauer). The microarray genotyping demonstrated a correlation between penicillin, tetracycline and erythromycin resistance phenotypes with the corresponding acquired resistance genes. The hybridizations showed that the 38 antimicrobial resistant *S. aureus* isolates possessed at least one AMR gene.


Characterization of *Salmonella* Typhimurium isolates associated with septicemia in swine
Bergeron N, Corriiveau J, Letellier A, Daigle F, Quessy S.

*Salmonella* Typhimurium is frequently isolated from pigs and may also cause enteric disease in humans. In this study, 33 isolates of *S. Typhimurium* associated with septicemia in swine (CS) were compared to 33 isolates recovered from healthy animals at slaughter (WCS). The isolates were characterized using phenotyping and genotyping methods. For each isolate, the phage type, antimicrobial resistance, and pulsed-field gel electrophoresis (PFGE) DNA profiles were determined. In addition, the protein profiles of each isolate grown in different conditions were studied by Coomassie Blue-stained sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot. Various phage types were identified. The phage type PT 104 represented 36.4% of all isolates from septicemic pigs. Resistance to as many as 12 antimicrobial agents, including some natural resistances, was found in isolates from CS and WCS. Many genetic profiles were identified among the PT 104 phage types. Although it was not possible to associate one particular protein with septicemic isolates, several highly immunogenic proteins, present in all virulent isolates and in most isolates from clinically healthy animals, were identified. These results indicated that strains associated with septicemia belong to various genetic lineages that can also be recovered from asymptomatic animals at the time of slaughter.


Unusual central nervous system lesions in slaughter-weight pigs with porcine circovirus type 2 systemic infection
Drolet R, Cardinal F, Houde A, Gagnon CA.

Porcine circovirus type 2 systemic infection was diagnosed in 2 slaughter-weight pigs based on postmortem examination. The infection was associated with unusual central nervous system lesions characterized by a multifocal lymphohistiocytic to granulomatous meningoencephalomyelitis with giant cell formation. The role of these nervous system lesions in the development of the clinical signs in these pigs remains uncertain.

Porcine reproductive and respiratory syndrome virus (PRRSV)-specific mAbs: supporting diagnostics and providing new insights into the antigenic properties of the virus

Van Breedam W, Costers S, Vanhee M, Gagnon CA, Rodríguez-Gómez IM, Geldhof M, Verbeeck M, Van Doorselaere J, Karniychuk U, Nauwynck HJ.

The porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most important viral pathogens in the swine industry. Despite great efforts of pig holders, veterinarians, researchers and vaccine developers, the virus still causes major production losses. It is clear that efficient and correct monitoring and rational development of vaccines are crucial in the combat against this pathogen. PRRS-specific monoclonal antibodies (mAbs) are essential tools for both diagnostic and research purposes. This study describes the production of PRRSV GP3-, GP5- and N-specific hybridomas and an extensive characterization of the mAbs. The N-specific mAbs generated in this study appear to be useful tools for diagnostics, as they were found to react with genetically very different PRRSV isolates and may serve to discriminate between European and American type PRRSV isolates. These mAbs also allowed detection of the PRRSV N protein in both formalin-fixed, paraffin-embedded tissue sections and frozen tissue sections of PRRSV-infected lungs, further illustrating their diagnostic value. Different neutralization assays pointed out that none of the GP3- and GP5-specific mAbs tested shows virus-neutralizing capacity. This is noteworthy, as these mAbs recognize epitopes in the predicted ectodomains of their target protein and since the GP5-specific antibodies specifically react with the antigenic region that corresponds to the "major neutralizing epitope" suggested for American type PRRSV. The current findings argue against an important role of the identified antigenic regions in direct antibody-mediated neutralization of European type PRRSV in vivo. However, it is also clear that findings concerning a specific PRRSV epitope cannot always be generalized, as the antigenic determinants and their biological properties may differ radically between different virus isolates


The role of porcine reproductive and respiratory syndrome (PRRS) virus structural and non-structural proteins in virus pathogenesis

Music N, Gagnon CA.

Porcine reproductive and respiratory syndrome (PRRS) is an economically devastating viral disease affecting the swine industry worldwide. The etiological agent, PRRS virus (PRRSV), possesses a RNA viral genome with nine open reading frames (ORFs). The ORF1a and ORF1b replicase-associated genes encode the polyproteins pp1a and pp1ab, respectively. The pp1a is processed in nine non-structural proteins (nsps): nsp1α, nsp1β, and nsp2 to nsp8. Proteolytic cleavage of pp1ab generates products nsp9 to nsp12. The proteolytic pp1a cleavage products process and cleave pp1a and pp1ab into nsp products. The nsp9 to nsp12 are involved in virus genome transcription and replication. The 3’ end of the viral genome encodes four minor and three major structural proteins. The GP(2a), GP3 and GP4 (encoded by ORF2a, 3 and 4), are glycosylated membrane associated minor structural proteins. The fourth minor structural protein, the E protein (encoded by ORF2b), is an unglycosylated membrane associated protein. The viral envelope contains two major structural proteins: a glycosylated major envelope protein GP5 (encoded by ORF5) and an unglycosylated membrane M protein (encoded by ORF6). The third major structural protein is the nucleocapsid N protein (encoded by ORF7). All PRRSV non-structural and structural proteins are essential for virus replication, and PRRSV infectivity is relatively intolerant to subtle changes within the structural proteins. PRRSV virulence is multigenic and resides in both the non-structural and structural viral proteins. This review discusses the molecular characteristics, biological and immunological functions of the PRRSV structural and nsps and their involvement in the virus pathogenesis


Investigation of the species origin of the St. Jude Porcine Lung epithelial cell line (SJPL) made available to researchers

Silversides DW, Music N, Jacques M, Gagnon CA, Webby R.

CRIP Bulletin

The new CRIP Bulletin provides information on a regular basis about the organization’s ongoing activities, program deadlines and current events. To subscribe, simply drop a line to our coordinator, Dr. Cécile Crost (c.crost@umontreal.ca).

Grants awarded to CRIP students

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Since its inception, the CRIP has awarded 41 grants to students, including 17 M.Sc. students, 19 Ph.D. students and three postdoctoral fellows, in order to help them attend international calibre conferences and present their research. These grants represent a reasonable contribution to costs incurred.

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The CRIP has a “Short-Term Financial Support” program designed to assist the advancement of the research conducted by the students by bridging temporary funding gaps, whether they result from funding as yet to be received (at the beginning of a project) or the expiration of a funding agreement (at the end of a project). Since the inception of this program, financial support has been granted to 41 students (21 M.Sc. students and 20 Ph.D. students).

CRIP 2009-2011 Graduates

Doctorate (Ph.D.)


Carmen Calinescu, 11/2009. Matrices à base de carboxyméthyl amidon pour des formulations pharmaceutiques des agents bioactifs à administration orale; under the direction of Mircea-Alexandru Mateescu.

Nadia Bergeron, 11/2009. Étude de la pathogénie et mise au point d'un vaccin contre les infections à Salmonella Typhimurium chez le porc, under the direction of Sylvain Quessy and France Daigle.

Faust René Okamba Ondzia, 12/2009. Évaluation du potentiel vaccinal d’un adénovirus recombinant non réplicatif exprimant l’adhésine P97 de Mycoplasma hyopneumoniae contre la pneumonie enzootique porcine; under the direction of Maximilien Arella and Carl A. Gagnon.

Lan Tran Thi Quynh, 05/2010. Étude de l’efficacité de la vaccination à Salmonella enteritidis chez la poule pondeuse et de la protection contre l’infection; under the direction of Martine Boulianne, Sylvain Quessy and Ann Letellier.

Vincent Deslandes, 08/2010. Étude des gènes d’Actinobacillus pleuropneumoniae exprimés en condition d’infection; under the direction of Mario Jacques, Josée Harel and John Nash.

Marycruz Domínguez-Punaro 10/2010, Studies on the exaggerated inflammatory response caused by Streptococcus suis at both Systemic and Central Nervous System levels; under the direction of Marcelo Gottschalk, Serge Rivest and Mariela Segura. Honour List of the Dean of the Faculty of Graduate and Postdoctoral Studies.

Manon Racicot, 07/2011. Évaluation de stratégies pour améliorer l’observance de la biosécurité sur les fermes avicoles au Québec; under the direction of Jean-Pierre Vaillancourt and André Durivage.

Wilfried Saron, 09/2011. Expression chez les plantes de protéines recombinantes pour des procédures vaccinales cas de l’artériivirus porc et de la flagelline; under the direction of Denis Archambault.

Marie-Ève Lambert, 09/2011. Epidémiologie du syndrome reproducteur et respiratoire dans deux régions de densités porcines différentes au Québec; under the direction of Sylvie D’Allaire and Zvonimir Poljak.
**Master (M.Sc.)**

Jian Jun Jia, 12/2009. *Identification of a new cell line permissive to porcine reproductive and respiratory syndrome virus replication*; under the direction of Carl A. Gagnon.


Alexandra Grasteau, 02/2011. *Sélection de mutations affectant la formation de biofilm*; under the direction of Mario Jacques.

Cynthia Lévesque, 04/2011. *Modèles cellulaires pour étudier les interactions entre Actinobacillus pleuropneumoniae et le virus du syndrome reproducteur et respiratoire porcin*; under the direction of Mario Jacques and Carl A. Gagnon.

Michael Beaudry Ferland, 2011. *Methicillin – resistant Staphylococcus aureus (MRSA) infections in pigs : strain characterization and comparison with MRSA from humans*; under the direction of Marie Archambault and Ann Letellier.

**Direct transition from M.Sc. to Ph.D.**


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