Swine and Poultry Infectious Diseases Research Center

- Dr. Josée Harel, Director

May 27, 2013 - CAHLN
CRIPA Organization

CRIP 2006-2013 strategic group grant from FRQ-NT (government of Quebec)
CRIPA 2013-2019

40 Researchers:
- 6 Universities
- 1 CEGEP
- 2 Private corporations
- 5 Governmental agencies

Interdisciplinary:
- Bacteriology and virology
- Immunology
- Chemistry
- Pharmacology
- Epidemiology

Expertise:
- 2 Canada research chairs of excellence
- 1 NSERC Industrial research chair in Food Safety
- 1 Industrial research chair in poultry
- OIE Reference laboratory (EcL)
- Bioaerosol laboratory
Swine and avian industry are important for the development of the Quebec farming.

<table>
<thead>
<tr>
<th>Annual Data</th>
<th>Swine</th>
<th>Poultry</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production QC (2011) Canadian %</td>
<td>7,5 M</td>
<td>420 M kg</td>
<td>113 M douz.</td>
</tr>
<tr>
<td></td>
<td>25 %</td>
<td>28 %</td>
<td>14 %</td>
</tr>
<tr>
<td>Value at the farm QC, M $</td>
<td>933</td>
<td>678</td>
<td>124</td>
</tr>
<tr>
<td>Exportation, %</td>
<td>60</td>
<td>12</td>
<td>- - -</td>
</tr>
<tr>
<td>Jobs</td>
<td>19 800</td>
<td>13 000</td>
<td></td>
</tr>
<tr>
<td>Repercussions QC, billion $</td>
<td>1,25</td>
<td></td>
<td>2,061</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(transformation included)</td>
</tr>
<tr>
<td>Economic loss related to infectious diseases</td>
<td>SRRP 30 M $</td>
<td>LTI* 1,97 M $</td>
<td>S. enteritidis 1,25 M $</td>
</tr>
</tbody>
</table>

* 2010
CRIPA ❖ Mission

Fight against infectious diseases, costly for *swine and avian industries*, and that might have an impact on public health
CRIPA Research program

Multidisciplinary approach integrated in order to improve the swine and poultry health

Increase scientific knowledge about pathogenic agent

Line 1 Infectious agent
- Food safety
- Zoonosis

Line 2 Epidemiology & diagnostic
- Models for infectious diseases

Line 3 Vaccines & other alternatives
- Prevention
- Biosecurity

Goals

Approaches

Niches

Effects

Improve veterinary public health

Improve animal health

Use that knowledge to make new diagnostic tools, preventive or therapeutic strategies

Increase scientific knowledge about pathogenic agent

Use that knowledge to make new diagnostic tools, preventive or therapeutic strategies

Improve veterinary public health

Improve animal health
CRIPA  ❖  Added value

• Integrated research program
• Relationship with partners
  – Swine industries
  – Poultry and eggs industries
  – Pharmaceuticals
  – MAPAQ
• Collaborations & student co-directions
• Equipment parks (technology platforms)
• New initiatives grants
• Swine and Avian Immunology Tool Bank
• Knowledge transfer & translation
CRIPA 🟢 Swine Infectious Diseases Center Activities

• Student Training
  ✓ Technologic workshops
  ✓ Studies & Congress grants – Oral and Poster presentation
  ✓ International training grants
  ✓ Over 200 students trained since 2006

• Annual Symposium
  ✓ Gathered swine industry stakeholders, researchers, students and pharmaceutical companies

• Communication
  ✓ CRIPA Bulletin – Monthly newsletter
  ✓ Info-CRIPA – Annual research report
  ✓ Café-CRIPA – Annual knowledge transfer meeting with industry
  ✓ Website – www.crip.umontreal.ca/en/home
  ✓ SITB website
CRIPA  Moving forward

• Enhance national & international networking
• Increase our networking towards a Canadian cluster
CRIPA ᵉ Leadership

- Basic research
- Technology Transfer
- Applied research
- Diagnostic
- Vaccine Therapeutic alternative
- Antimicrobial alternatives
  - Judicious use of ATBs
- Leadership
  - Swine/Poultry health
  - Food safety
  - Public health
Characterization of a virulence factor (bacteria or viral)

• Toxin, adhesin, capsule, surface protein....
• Gene identification
• Expression regulation
• Inactivation of gene and mutant characterization
• Virulence in animal (natural host)
• Host response
<table>
<thead>
<tr>
<th>Name</th>
<th>Specialization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marie Archambault</td>
<td>Bacteriology, Antimicrobial resistance</td>
</tr>
<tr>
<td>Younes Chorfi</td>
<td>Mycotoxins</td>
</tr>
<tr>
<td>Sylvie d’Allaire</td>
<td>PRRSV epidemiology</td>
</tr>
<tr>
<td>John M. Fairbrother</td>
<td><em>E. coli</em> reference laboratory, OIE</td>
</tr>
<tr>
<td>Philippe Fravalo</td>
<td>Food safety</td>
</tr>
<tr>
<td>Carl A. Gagnon</td>
<td>Virology</td>
</tr>
<tr>
<td>Marcelo Gottschalk</td>
<td>Serology, <em>S. Suis</em>, App</td>
</tr>
<tr>
<td>Josée Harel</td>
<td>Molecular microbiology</td>
</tr>
<tr>
<td>Ann Letellier</td>
<td>NSERC industrial chair in food safety</td>
</tr>
</tbody>
</table>
Diagnostic services (PCR, serology, phylogenetic analysis)
Epidemiosurveillance
DNA Microarrays
R & D
CRIPA  ❖  Outcomes: biosecurity

Letellier, D’Allaire, Klopfenstein, Vaillancourt

- *Salmonella*, HACCP from farm to fork: **CRSV**
- *Campylobacter*
- *Staphylococcus aureus* MRSA
- Virus SRRP:
  - Gilt acclimatization
  - Air filtration
  - Project key
Examples of some works from members of the CRIPA

- Laboratory of Diagnostic in Veterinary Virology (LDVV)
- Molecular Diagnostic Laboratory (LDM)
- Serology, *S. suis*, App
- Bacteriology
- Food Safety (CRSV)
- EcL laboratory
- Mycotoxins
Laboratory of Diagnostic in Veterinary Virology

Carl Gagnon

- Development of a viral microarray to identify viral agents (C. Belhumeur)
- SRRP genome sequencing (Carl Gagnon)
- Effects of mycotoxins in food on viral infections (PRRSV, PCV2) (Chorfi, Gagnon, Segura and Lessard)
- APHIS/USDA: Seroprevalence of swine pathogens (VSRRP, PCV2, App, S suis) in population of feral swine.
CRIPA ❖ Activities in 2012-2013

Laboratory of Diagnostic in Veterinary Virology (LDVV) - FMV

PRRSV diversity (S. D’Allaire)

Evaluation of disinfectants efficiency against different viruses

Identification of new microorganisms never reported by scientific community:

• Spp. Mycoplasma in porks (Donald Tremblay)
• Herpesvirus in a Beluga whale
• New porcine parvovirus genotype (PARV4-like virus)
• Bovine herpesvirus type 4
Phylogenetic tree of the HA gene nucleotide sequences of recent H3N2 triple reassortant Canadian influenza virus isolates.

Molecular Diagnostic Laboratory (LDM)  
Faculty of medicine veterinary

Josée Harel, Director  
Carl A. Gagnon, Molecular Virology Director  
Marie Archambault, Molecular Bacteriology Director

Donald Tremblay, Coordinator  
Véronique Allard, Research agent  
Andrée Déry, Technician

- Infectious agents, zoonosis (bacteria, viruses, parasites)  
- Different animal species  
- Real-time PCR, sequencing, microarrays  
- R&D
Detection of several thousands of genes in a single experiment
CRIPPA  ❖  International collaborations

Virulence and antimicrobial resistance microarrays

• Dog *E. coli* and *E. coli* UTI P. Boerlin U of Guelph and PICRA
• Dog and human colitis and Crohn’s E. coli K Simpsons Cornell U
• E. coli mastitis Schukken Cornell U
• EPEC; Afset Norway; Gruenheid U McGill
• Atypical E.coli LSPQ
• Environmental and UTI *E. coli* F. Ramirez, A. Guerrero Barrera U.Aguascalientes Mexico ; Brousseau and Masson Biotechnology Research institute NRC
• *S. aureus* ATBR Messier, Labrecque and Archambault MAPAQ
• *Brachyspira hyodysenteriae* ATBR JH Fairbrother MAPAQ
CRIPA  ❖  Molecular epidemiology
Statens Serum Institute (Denmark)


Table 5. Pathotypes of *E. coli* B2 isolates from UTI patients, community-dwelling humans, meat and food animals.

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>UTI patients (n=52)</th>
<th>Community-dwelling humans (n=36)</th>
<th>Broiler chicken meat (n=5)</th>
<th>Danish chickens (n=17)</th>
<th>Broiler Pork (n=3)</th>
<th>Danish Pork (n=27)</th>
<th>Pigs (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>ExPEC</td>
<td>92</td>
<td>75</td>
<td>20</td>
<td>77</td>
<td>29</td>
<td>33</td>
<td>89</td>
</tr>
<tr>
<td>UPEC</td>
<td>46</td>
<td>36</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>MNEC</td>
<td>12</td>
<td>31</td>
<td>0</td>
<td>15</td>
<td>6</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>APEC</td>
<td>65</td>
<td>53</td>
<td>20</td>
<td>54</td>
<td>29</td>
<td>33</td>
<td>89</td>
</tr>
<tr>
<td>Atypical EPEC</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Potentially non-pathogenic</td>
<td>6</td>
<td>0</td>
<td>40</td>
<td>8</td>
<td>47</td>
<td>33</td>
<td>4</td>
</tr>
</tbody>
</table>

Microarray-based detection of extended virulence and antimicrobial resistance gene profiles in phylogroup B2 *Escherichia coli* of human, meat and animal origin

CRIPA ✤ From research to diagnostic
Marcelo Gottschalk

*Actinobacillus pleuropneumoniae*

- Characterization of atypical strains
  - Cross-reactions between North American serotype 13 strains with serotype 10 strains: data used in serology

- Standardization of a new serotype-specific PCR
  - New real-time PCR for direct detection of App serotype 1 from tonsils

- Comparison of different serological tests for App
  - CFT, LC-LPS ELISA, ApxI-Tnb ELISA (commercial kit) and ApxIV (commercial kit)
  - In collaboration with ISU: it was shown that the LC-LPS is the most specific and sensitive test to detect App antibodies
Prevalence of pathogens in wild boars

• Serological diagnosis of
  - App (all serotypes)
  - *Mycoplasma hyopneumoniae*
  - *Lawsonia intracellularis*
  - Salmonella
  - PRRS*
  - PCV-2*

• PCR detection in tonsils
  - *S. suis* (all serotypes)
  - *S. suis* serotype 2 and 14 (zoonotic serotypes)

*Collaboration Dr. Carl Gagnon
CRIPA    From diagnostic to research
Marcelo Gottschalk

Use of strains recovered from the diagnostic lab

• Untypable strains of *S. suis*
  -Characterization of virulence potential

• *S. suis* serotype 2 strains
  -Characterization of two major sub-populations with different virulence potential: identification of virulence factors

• General bank of *S. suis* strains
  -Validation of a multiple-PCR to individually identify all serotypes of *S. suis* (test developed in collaboration with Japanese researchers)
CRIPA Food Safety

Ann Letellier
(NSERC Industrial Research Chair in Meat Safety)
Sylvain Quessy
Philippe Fravalo
Marie Archambault

• Characterization and epidemiology of zoonotic bacteria
  *Campylobacter, Salmonella, Listeria, MRSA*

• Antibioresistance and Integrated Canadian Program on Antimicrobial Resistance (PICRA)
From the farm to the table approach

**HACCP:** Hazard analysis and critical control points

- **FeedAssure**
  - milling industry

- **PASA**
  - ex:CQA

- **BPTP**
  - transportation

- **PASA**
  - abattoir

- **Good hygiene practices**

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**Research:**
- Vaccines development
- Efficacy of antibiotic alternatives
- Antibioreistance
- Molecular characterization of foodborne pathogens
- Animal digestive ecosystem analysis

**Support to industries and gouvermentual authorities:**
- Hazard analysis (HACCP)
- Food safety (CTIA)
- Support to industry
- *Salmonella* surveillance plan (pork)
- Specific projects

**Diagnostic services:**
- Detection of antibiotic residues in eggs, feed and meats
- Detection of foodborne pathogens
- Evaluation of the meat’s microbiological quality

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*FeedAssure™ = HACCP milling industry, PASAF: Food safety Enhancement Program on farm, CQA™=Canadian Quality assurance; BPTP=Bonnes pratiques de transport des porcs; PASA=Food Safety Enhancement Program*
The OIE Reference Laboratory for *Escherichia coli* (EcL)

John Morris Fairbrother, BVSc, PhD

“Integrated and ecosystemic approach to the study and surveillance of *E. coli* in the animal-human-environment interface”
CRIPA  ✦ Development of mycotoxins measuring methods in domestic animal’s serum and urine

• Developing these measuring methods allowed to:
  – Offer this service to the veterinary practitioners in the field of animal production
  – Have an expertise in the biomarker field
  – Obtain 2 grants (swine cluster and MAPAQ) to conduct research relating to the impact of mycotoxins on animal health and production (pork et dairy cattle)
# REAGENTS

## ELISA

Commercially available ELISA kits are not included. Only match pairs or homologues ELISAs are included. The objectives reducing high costs normally related to the use of kits.

<table>
<thead>
<tr>
<th>Tool</th>
<th>Description</th>
<th>Protocol/Reagents</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>Matched-bonds: R&amp;D Systems MAB6111 &amp; 6A4</td>
<td>Available</td>
<td>M. Gottschalk &amp; M. Segura</td>
</tr>
<tr>
<td>IL-6</td>
<td>Matched-bonds: R&amp;D Systems: AP664 &amp; BAF654b</td>
<td>In development</td>
<td>M. Gottschalk &amp; M. Segura</td>
</tr>
<tr>
<td>IL-8</td>
<td>Matched-bonds: R&amp;D Systems: AP668 &amp; BAF689m</td>
<td>Available</td>
<td>M. Gottschalk &amp; M. Segura</td>
</tr>
<tr>
<td>IL-12/23 p40</td>
<td>Matched-bonds: R&amp;D Systems: MAB5421 &amp; BAF522a</td>
<td>Available</td>
<td>M. Gottschalk &amp; M. Segura</td>
</tr>
<tr>
<td>IL-10</td>
<td>Matched-bonds: R&amp;D Systems: MAB9991 &amp; 6A403e</td>
<td>Available</td>
<td>M. Gottschalk &amp; M. Segura</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Matched-bonds: R&amp;D Systems: Mab75-2-1 &amp; Mab75-3 (multiplex)</td>
<td>Available</td>
<td>M. Gottschalk &amp; M. Segura</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Matched-bonds: R&amp;D Systems: MAB990 &amp; 6A403e</td>
<td>Available</td>
<td>M. Gottschalk &amp; M. Segura</td>
</tr>
</tbody>
</table>

## RT-PCR (semi-quantitative)

<table>
<thead>
<tr>
<th>Tool</th>
<th>Description</th>
<th>Protocol/Reagents</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-18</td>
<td>Primers for target and housekeeping genes and protocol details under request</td>
<td>Available</td>
<td>M. Gottschalk</td>
</tr>
<tr>
<td>IL-6</td>
<td>Available</td>
<td>M. Gottschalk</td>
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</tr>
<tr>
<td>IL-8</td>
<td>Available</td>
<td>M. Gottschalk</td>
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<tr>
<td>IL-9</td>
<td>Available</td>
<td>M. Gottschalk</td>
<td></td>
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</table>

## Quantitative Real-Time PCR (qRT-PCR)

<table>
<thead>
<tr>
<th>Tool</th>
<th>Description</th>
<th>Amplification (bp)</th>
<th>Protocol/Reagents</th>
<th>Contact</th>
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</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>SYBER-Green</td>
<td>In development</td>
<td>M. Lessard</td>
<td></td>
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<tr>
<td>IL-12p35</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>Y. Boutin</td>
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<tr>
<td>IFN-γ</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>Y. Boutin</td>
<td></td>
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<tr>
<td>MCP-1</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>Y. Boutin</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>Y. Boutin</td>
<td></td>
</tr>
<tr>
<td>TGF-β1</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>Y. Boutin</td>
<td></td>
</tr>
<tr>
<td>INF-γ</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>M. Lessard</td>
<td></td>
</tr>
<tr>
<td>INOS</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>C. Dozois</td>
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<tr>
<td>β-defensin 2x</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>M. Lessard</td>
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<tr>
<td>Lipocalin</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>M. Lessard</td>
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<tr>
<td>COX-2</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>M. Lessard</td>
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<tr>
<td>FOXO3</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>M. Lessard</td>
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<tr>
<td>PPAR-γ</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>M. Lessard</td>
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<tr>
<td>TLR4</td>
<td>SYBER-Green</td>
<td>Available</td>
<td>Y. Boutin</td>
<td></td>
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</tbody>
</table>
CAFÉ CRIPA le 29 mai 2013

En PRIMEUR et avec SAVEUR

Carl Gagnon, UdeM,
Jane C. Hennings, U. of Minnesota,
John Harding, U. of Saskatchewan,
Marcelo Gottschalk, UdeM,
Maria Calvijo, U. of Minnesota,

Séquençage haut débit SRRP
Diagnostic du virus SRRP
Brachyspira update
Streptococcus suis mise à jour
Diagnostic de M. hyorhinis

Organisé conjointement avec le 12e congrès du CAHLN/RCTLSA : 26 -29 mai 2013
Canadian Animal Health Laboratorians Network (CAHLN)

Lieu: Faculté de médecine vétérinaire de Saint-Hyacinthe
Visitez le site Web : http://www.cahln-rctlsa.com
ACKNOWLEDGEMENTS

Regroupements stratégiques FRQ_NT

Université de Montréal

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