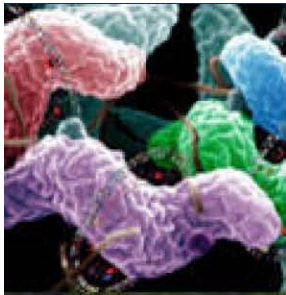


Conférence canadienne sur le *Campylobacter* 2008

2008 Canadian *Campylobacter* Conference



Program and abstracts  
of the

2008 *Canadian Campylobacter*  
Conference

Intercontinental Hotel  
Montréal, Québec  
September 25-26, 2008



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# Letter of Welcome

Dear Colleagues and friends,

The executive Committee of the 2008 Canadian *Campylobacter* Conference is pleased to welcome all delegates and invited guests to Montreal, one of Canada's most vibrant, creative and colourful cities. We hope that your visit to Montreal will be exciting and that the Conference will provide you with a unique opportunity to meet, listen to and interact with world-class experts in the field of *Campylobacter*.

The thematic direction of the 2008 Canadian *Campylobacter* Conference focuses on the "lab-epi-public health practice" interface. The programme of the Conference is designed to state the current situation of *Campylobacter* in Canada from isolation to interventions, and to discuss solutions to enhance our knowledge and capacity in order to reduce the burden of *Campylobacter* in our country. It is hoped that the Conference will foster partnerships among researchers and public health practitioners across the country and will lead to a Canadian position on research and intervention priorities. We will also take advantage of this venue to honour two Exceptional Canadian "*Campylobacter* Scientists".

The Executive Committee would like to thank all our National and International keynote speakers, presenters and registrants for their collaboration and contribution to this exciting event. Sincere thanks are also given to the members of the Scientific Committee for their work and involvement, and to our IT professional and administrative assistant for their tremendous work.

The Executive Committee takes this opportunity to also thank all sponsors for their support: Public Health Agency of Canada, Université de Sherbrooke, Université de Montréal, Canadian Institutes of Health Research, National Research Council, Fermentas, Oxoid, Roche, DiaMed Lab Supplies inc. and Sarstedt.

Your feedback on this venue will be greatly appreciated. We would therefore ask you to provide any general or specific comments by completing the evaluation form included in the participant's folder.

Warm welcome to all of you!

*The Executive Committee*

# Executive Committee

**Eric Frost**

*Faculté de médecine et des Sciences de la santé  
Université de Sherbrooke*

**Kathleen Laberge**

*Public Health Agency of Canada*

**Simon Lévesque**

*Faculté de médecine et des Sciences de la santé  
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**Sophie Michaud**

*Faculté de médecine et des Sciences de la santé  
Université de Sherbrooke*

**Pascal Michel**

*Public Health Agency of Canada and  
Université de Montréal*

## Scientific Program Steering Committee

**Réjean Dion**

*Institut national de santé publique du Québec*

**Ruff Lowman**

*Canadian Food Inspection Agency*

**Rita Finley**

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Université de Sherbrooke*

**Christine Szymanski**

*National Research Council –  
Institute for Biological Sciences  
University of Alberta,  
Department of Biological Sciences*

# Conference Objectives

The 2008 Canadian *Campylobacter* Conference focuses on the "lab-epi-public health practice" interface.

The objectives of the Conference are to:

- Provide a communication and exchange platform to enhance national partnerships and collaborations in the areas of *Campylobacter* fundamental and applied research; diagnostic and microbial characterisation methods; surveillance, prevention and control strategies
- Translate knowledge gained into interventions and policies specific to the Canadian context in order to reduce human campylobacteriosis
- Prepare the Canadian scientific community to the forthcoming International *Campylobacter*, *Helicobacter* and Related Organisms workshop which will take place in Japan (2009) and Canada (2011)
- Honour two Exceptional Canadian *Campylobacter* Scientists

## Scope of Concurrent Sessions

The Conference highlights four main areas of the *Campylobacter* field:

### **DIAGNOSIS, ANTIMICROBIAL RESISTANCE AND MOLECULAR TYPING**

Laboratory tools used to conduct optimal epidemiological surveillance and source attribution

### **PATHOGENESIS AND PHYSIOLOGY**

*Campylobacter*-host interactions; environmental persistence; pathogenesis; host colonization and transmission; animal models; stress responses; and general physiology and metabolism

### **SURVEILLANCE, PREVENTION AND CONTROL**

Outbreak investigations and identification of associated risk factors; prevention and control activities applicable from farm to fork; policies influencing the frequency of infections; epidemiological research and ecology

### **GENOMICS AND MOLECULAR APPROACHES**

*Campylobacter* and related organisms genomes, novel technique development and genome-wide approaches to investigate pathogenesis and physiology

## **Continuing Education Credits**

This is a collective training activity approved under the terms of Section I of the Maintenance of Certification Program of the Royal College of Physicians and Surgeons of Canada (RCPSC) by the Centre de formation continue de la Faculté de Médecine et des Sciences de la santé de l'Université de Sherbrooke. Each hour of participation in the training program will generate one training credit for a maximum of 12 hours and 15 minutes (12:15 hours), (that is to say 6 hours on September 25, 2008 and 6:15 hours on September 26, 2008).

The Ordre des médecins vétérinaires du Québec has assigned 12 hours and 30 minutes of continuing education credits for veterinarians attending the conference.

# Conference Schedule Summary

Time	Wednesday, September 24	Thursday, September 25	Friday, September 26
7 : 00		<b>Registration and Set up of Poster Presenters</b> <i>(Maisonneuve Room)</i>	
8 : 00			
8 : 15		<b>Welcoming Remarks</b> <i>(Maisonneuve Room)</i>	<b>Welcoming Remarks</b> <i>(Maisonneuve Room)</i>
		<b>PLENARY SESSIONS</b>	
8 : 30		<i>Campylobacter</i> : From Isolation to Intervention and the Role of Pulsnet Canada <i>(Maisonneuve Room)</i>	Effects of the Danish Intervention Strategies Aimed at Reducing <i>Campylobacter</i> in Broiler Meat : 2002-2007 <i>(Maisonneuve Room)</i>
9 : 10		Surveillance Gaps : Human Perspective <i>(Maisonneuve Room)</i>	Antimicrobial Resistance in <i>Campylobacter</i> : Global Trend, Mechanisms of Emergence and Ecological Fitness <i>(Maisonneuve Room)</i>
9 : 40		<i>Campylobacter</i> in Environmental Water : Where does it come from and Where does it go? <i>(Maisonneuve Room)</i>	
10 : 00			<b>Break and Poster Viewing</b>
10 : 10		<b>Break and Poster Viewing</b>	
10 : 30			CIPARS: an Integrated Approach to Surveillance and Control <i>(Maisonneuve Room)</i>
10 : 40		The Farm to Plate Approach in the Control of <i>Campylobacter</i> in Food Products <i>(Maisonneuve Room)</i>	
11 : 10			<b>Closing Remarks</b>
11 : 15			
11 : 20		<i>Campylobacter jejuni</i> and the Pathogenesis Cycle <i>(Maisonneuve Room)</i>	
11 : 30			<b>Lunch</b>
12 : 00		<b>Lunch and Poster Viewing</b>	
12 : 30			<b>ROUND TABLE DISCUSSION</b> <i>(Maisonneuve Room)</i>
		<b>CONCURRENT SESSIONS : ORAL PRESENTATIONS</b>	
13 : 00		<b>A</b> : Diagnosis, Antimicrobial Resistance and Molecular Typing <i>(Maisonneuve Room)</i>	<b>B</b> : Pathogenesis and Physiology <i>(Saint-Laurent Room)</i>
14 : 30		<b>Break and Poster Viewing</b>	
15 : 00		<b>C</b> : Surveillance, Prevention and Control <i>(Maisonneuve Room)</i>	<b>D</b> : Genomics and Molecular Approaches <i>(Saint-Laurent Room)</i>
15 : 30			
16 : 30		<b>Poster Viewing</b>	
17 : 00			
17 : 30		<b>Reception in Honour of Exceptional Canadian <i>Campylobacter</i> Scientists</b> <i>(Les Voûtes Room)</i>	
18 : 00			
19 : 30	Registration <i>(Main entrance)</i>		
21 : 00			

# Conference Program

**WEDNESDAY, SEPTEMBER 24, 2008**

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19:30 – 21:00 Registration

*Main Entrance*

**THURSDAY, SEPTEMBER 25, 2008**

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7:00 – 8:00 Registration and Set Up of Poster Presenters

*Maisonneuve Room*

8:15 – 8:30 Welcoming Remarks

*Maisonneuve Room*

**PLENARY SESSIONS :**

*Maisonneuve Room*

***Campylobacter in Canada: Current Situation***

*\*Please note there will be a 10 minute question period following each presentation*

**Chairs: Réjean Dion and Pascal Michel**

8:30 – 9:10 *Campylobacter* : From Isolation to Intervention and the Role of Pulsnet Canada  
**Guest speaker: Celine Nadon**, National Microbiology Laboratory

9:10 – 9:40 Surveillance Gaps : Human Perspective  
**Guest speaker: Eleni Galanis**, British Columbia Centre for Disease Control

9:40 – 10:10 *Campylobacter* in Environmental Water : Where does it come from and where does it go?  
**Guest speaker: Sophie Michaud**, Université de Sherbrooke

10:10 – 10:40 Break and Poster Viewing

10:40 – 11:20 The Farm to Plate Approach in the Control of *Campylobacter* in Food Products  
**Guest speaker: Sylvain Quessy**, Université de Montréal

11:20 – 12:00 *Campylobacter jejuni* and the Pathogenesis Cycle  
**Guest speaker: Erin Gaynor**, University of British Columbia

12:00 – 13:00 Lunch and Poster Viewing

## **THURSDAY, SEPTEMBER 25, 2008**

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### **13:00 – 14:30 CONCURRENT SESSIONS**

*\*Please note there will be a 5 minute question period following each presentation*

### **SESSION A : DIAGNOSIS, ANTIMICROBIAL RESISTANCE AND MOLECULAR TYPING**

*Maisonneuve Room*

**Chair: Sophie Michaud**

- 13:00 – 13:15 Comparison of nine isolation methods for the recovery of *Campylobacter*  
***Danielle Daignault***
- 13:15 – 13:30 Comparison of Pulsed Field Gel Electrophoresis and Fla typing for predicting Multilocus Sequence Typing clonal complexes of *Campylobacter jejuni*  
***Simon Lévesque***
- 13:30 – 13:45 Application of comparative genomics-based genotyping to molecular epidemiological studies of *Campylobacter jejuni*  
***Eduardo Taboada***
- 13:45 – 14:00 Antimicrobial resistance in *Campylobacter* isolated from sheep in Ontario -preliminary results  
***Richard Reid-Smith / Lisa Scott***
- 14:00 – 14:15 Evaluation of Pathatrix and Modified FDA/CFSAN-BAM On line Method for the Isolation of *Campylobacter* spp. From Chicken Parts  
***Alfonso Valdivieso-García***
- 14:15 – 14:30 Validation of a Simplified Enrichment for *Campylobacter* Detection in Food Samples  
***Bérengère Genest***
- 14:30 – 15:00 Break and poster viewing

### **SESSION B : PATHOGENESIS AND PHYSIOLOGY**

*Saint-Laurent Room*

**Chairs: Alain Stintzi and Christine Szymanski**

- 13:00 – 13:30 The Signaling Cascade that controls flagellar gene expression in *Campylobacter jejuni*  
***David Hendrixson***
- 13:30 – 13:45 The interaction of *Campylobacter jejuni* with a mucus secreting cell line HT29MTXE12  
***Abofu Alemka***
- 13:45 – 14:00 Post-transcriptional regulation of virulence-associated phenotypes in *Campylobacter jejuni*  
***Stuart Thompson***
- 14:00 – 14:15 Metabolomic analysis of *C. jejuni* 11168H reveals presence of novel flagellar glycans  
***Susan Logan***
- 14:15 – 14:30 *Campylobacter jejuni* induces rapid colitis and NF- $\kappa$ B signaling in IL-10<sup>-/-</sup>;NF- $\kappa$ BEGFP mice  
***Christian Jobin***
- 14:30 – 15:00 Break and poster viewing

## **THURSDAY, SEPTEMBER 25, 2008**

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### **SESSION C : SURVEILLANCE, PREVENTION AND CONTROL**

*Maisonneuve Room*

**Chair: François Milord**

- 15:00 – 15:15 Findings of Two years of Integrated *Campylobacter* Surveillance within a Community (C-EnterNet)  
*André Ravel*
- 15:15 – 15:30 Rare *Campylobacter jejuni* strain points to manure-contaminated mud as cause of bike race outbreak, British Columbia, 2007  
*Tammy Stuart*
- 15:30 – 15:45 The seasonality of campylobacteriosis: are we missing something?  
*Julie Arsenault*
- 15:45 – 16:00 Burden of illness associated with antimicrobial susceptible and resistant *Campylobacter* infections in the Perth and Wellington-Dufferin-Guelph Health Units  
*Anne Deckert*
- 16:00 – 16:15 Spatial analysis and modeling of interactions between environment and *Campylobacter jejuni* in Eastern Township surface waters  
*Djoan Bonfils*
- 16:15 – 16:30 Molecular characterization of *Campylobacter jejuni* strains linked to recent milk-related outbreaks and surveillance of California Central Valley dairy environments  
*William Miller*

### **SESSION D : GENOMICS AND MOLECULAR APPROACHES**

*Saint-Laurent Room*

**Chair: Eric Frost**

- 15:00 – 15:15 The identification of *Campylobacter jejuni* essential genes  
*Martin Stahl*
- 15:15 – 15:30 Characterization of the regulatory networks controlling iron metabolism in *Campylobacter jejuni*  
*James Butcher*
- 15:30 – 15:45 Development and application of multiplex PCR assays of cytolethal-distending toxin (cdt) genes for the characterization and differentiation of *C. jejuni* and *C. coli* species  
*Izhar Khan*
- 15:45 – 16:00 Expression, purification and structural characterization of CfrA, an iron transporter from *Campylobacter jejuni*  
*John Baenziger*
- 16:00 – 16:15 Identification of novel genes in the oral pathogen *Campylobacter rectus*  
*Deborah Threadgill*
- 16:15 – 16:30 Molecular typing of *Campylobacter* in the genomic era  
*Trudy Wassenaar*
- 16:30 – 17:30 Poster viewing

### **RECEPTION :**

*Les Voûtes Room*

- 17:30 – 19:00 **RECEPTION IN HONOUR OF EXCEPTIONAL CANADIAN CAMPYLOBACTER SCIENTISTS**  
**CAMPYLOBACTER HISTORY: A CANADIAN PERSPECTIVE**  
Honors presented by: *Mohamed Karmali*, Public Health Agency of Canada

## **FRIDAY, SEPTEMBER 26, 2008**

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8:15 – 8:30 Welcoming Remarks

*Maisonneuve Room*

### **PLENARY SESSIONS :**

*Maisonneuve Room*

#### **Strategies for *Campylobacter* Surveillance and Control**

*\*Please note there will be a 10 minute question period following each presentation*

**Chairs: Alain Stintzi and Kathleen Laberge**

8:30 – 9:15 Effects of the Danish Intervention Strategies Aimed at Reducing *Campylobacter* in Broiler Meat : 2002-2007

**Guest speaker: Hanne Rosenquist**, The National Food Institute, Denmark

9:15 – 10:00 Antimicrobial Resistance in *Campylobacter* : Global Trend, Mechanisms of Emergence and Ecological Fitness

**Guest speaker: Qijing Zhang**, Iowa State University, USA

10:00 – 10:30 Break and Poster Viewing

10:30 – 11:15 CIPARS: An Integrated Approach to Surveillance and Control

**Guest speaker: Rebecca Irwin**, Public Health Agency of Canada

11:15 – 11:30 Closing Remarks

11:30 – 12:30 Lunch

### **ROUND TABLE DISCUSSION :**

*Maisonneuve Room*

12:30 – 16:30 National *Campylobacter* Coordination and Action Plan for Prevention and Control, Characterization, Surveillance and Research

## Plenary Speakers

### NADON CELINE

National Microbiology Laboratory

**Title : *Campylobacter: From Isolation to Intervention and the Role of PulseNet Canada***

Dr. Celine Nadon is a research microbiologist and the head of PulseNet Canada at the Public Health Agency of Canada's National Microbiology Laboratory. Dr. Nadon received her Bachelor of Science (1996) and Master of Science (1998) degrees from the University of Manitoba, and her Ph.D. from Cornell University (2003). Prior to joining the Public Health Agency of Canada in 2006, Dr. Nadon trained at the United States Department of Agriculture's Food Safety and Inspection Service Office of Public Health Science in Washington, D.C. Dr. Nadon's interests include molecular subtyping of bacterial pathogens, cluster and outbreak detection for diarrheal diseases, the distribution and genetic diversity of *Salmonella*, *Shigella*, *E. coli* and *Campylobacter*, and the epidemiology of foodborne disease. As the Head of PulseNet Canada, she oversees national molecular surveillance for bacterial foodborne diseases. Dr. Nadon is also an Adjunct Professor in the Department of Medical Microbiology and Infectious Diseases at the University of Manitoba, Faculty of Medicine.

### GALANIS ELENI

British Columbia Centre for Disease Control



**Title : *Surveillance Gaps: Human Perspective***

Dr. Eleni Galanis obtained her medical degree from the Université de Sherbrooke in 1995 and a Master of Public Health from Harvard University. She trained in Community Medicine at the University of Toronto as well as in the Health Canada Field Epidemiology Training Program. Dr. Galanis is currently working on enteric and zoonotic disease surveillance, control and prevention at the BC Centre for Disease Control. Her interests include communicable disease epidemiology, outbreak investigation, surveillance methods and international health issues. Recent relevant work includes the impact of the 2004 BC avian influenza outbreak on *Campylobacter* incidence rates, the investigation of a large *C. jejuni* outbreak associated with a bike race in BC in 2007 and the integrated surveillance of pathogens along the foodchain in BC.

## Plenary Speakers

### MICHAUD SOPHIE

Université de Sherbrooke



**Title : *Campylobacter* in Environmental Water : Where does it come from and where does it go? The CAMPYLOGIS project**

Dr Michaud obtained her Doctorate in Medicine (M.D.) in 1991, and her Royal College of Physicians and Surgeons of Canada Specialist Certificates in Internal Medicine (1995), in Medical Microbiology (1997) and in Infectious Diseases (1997) at the Université de Sherbrooke, Québec. She practiced for one year and then completed a Masters of Public Health at Harvard University in Boston (2000) and a fellowship in Molecular Epidemiology at Boston University (2000). She is presently Associate Professor at the Department of Microbiology and Infectious Diseases at the Faculté de Médecine de l'Université de Sherbrooke and works as a Medical Microbiologist and an Infectious Diseases consultant at the Centre Hospitalier Universitaire de Sherbrooke (CHUS). She is a member of the Infectious Diseases Axis of the Centre de Recherche Clinique Étienne LeBel of the CHUS. Her current research seeks to understand the clinical and molecular epidemiology of *Campylobacter* for developing effective infection control measures.

### QUESSY SYLVAIN

Université de Montréal,  
Faculté de médecine vétérinaire



**Title : The Farm to plate approach in the control of *Campylobacter* in food products**

Dr Sylvain Quessy is a graduate of the Université de Montréal (DVM, 1984). He worked as a private practitioner and as a meat hygienist for the Canadian Food Inspection Agency (CFIA) before the completion of his PhD in microbiology and immunology (Montreal, 1994). He then worked for Health Canada as a scientific researcher and head of the environmental microbiology section of the Health of Animals and Food Laboratory at St-Hyacinthe where he studied the molecular epidemiology and the control of food-borne and water-borne pathogens. In 1999, he accepted a position as a professor of veterinary hygiene and food safety at the Faculty of Veterinary Medicine of the Université de Montréal. Between 1999 and 2005 he was a research chair in food safety where he supervised the work of many graduate students, working on the genetic characterization, control and epidemiology of food-borne and environmental pathogens. He is currently the Head of the pathology and microbiology Department at the Faculty of Veterinary Medicine. He acted as a scientific counsellor for many governmental, professional or producer organizations in the development of food safety policies, on-farm HACCP-based models, and risk analysis. He has been recognized as an expert in microbial risk assessment by the World Health Organization. He published and presented numerous scientific papers on the molecular epidemiology, pathogenesis and control of pathogens such as *Salmonella*, *Yersinia*, *Cryptosporidium* and *Campylobacter*.

## Plenary Speakers

### GAYNOR ERIN

University of British Columbia



**Title : *Campylobacter jejuni* and the Pathogenesis Cycle**

Dr. Erin Gaynor received her Ph.D. from the University of California, San Diego, in 1997. During her graduate work with Dr. Scott Emr, Dr. Gaynor characterized molecular mechanisms underlying retrograde Golgi-ER transport and vesicle-mediated secretory trafficking in the yeast *Saccharomyces cerevisiae*. During her postdoctoral fellowship with Dr. Stanley Falkow at Stanford University, Dr. Gaynor initiated several projects exploring *Campylobacter jejuni* pathogenesis and developed new genetic and whole-genome tools to identify genes important for the pathogen-host cell interaction. This led to the characterization of a *C. jejuni* signal transduction pathway and a global stress response in *C. jejuni* and *Helicobacter pylori* that is important for virulence and transmission. Since 2003, Dr. Gaynor has been an Assistant Professor and Canada Research Chair in the Department of Microbiology and Immunology at the University of British Columbia. Her laboratory continues to investigate regulatory mechanisms, stress responses, and other factors important for *C. jejuni* pathogenesis.

### ROSENQUIST HANNE

The National Food Institute, Denmark



**Title : Effects of the Danish Intervention Strategies aimed at reducing *Campylobacter* in broiler meat, 2002-2007.**

Dr. Hanne Rosenquist is Ph.D. in Food Microbiology. Since 1998 she has been employed at the National Food Institute, Technical University of Denmark, working on topics related to microbial food safety; e.g. monitoring projects, intervention studies and quantitative risk assessments. Her main expertise is within broiler processing and *Campylobacter*. In 2004 she became senior scientist and research coordinator of activities within production hygiene and food control. She is the project leader of a national research project on control of *Campylobacter* in the broiler production and task leader of a project on *Campylobacter* source attribution. Further, she participates in international projects and working groups on *Campylobacter*; e.g. MedVetNet Work Package 24 on comparison of *Campylobacter* risk assessments, and the Codex working group on guidelines for control of *Campylobacter* and *Salmonella* spp. in broiler chicken meat. Since April 2008 she has been the head of the Danish Zoonosis Centre.

## Plenary Speakers

**ZHANG QIJING**  
Iowa State University



**Title : Antimicrobial Resistance in *Campylobacter*: Global Trend, Mechanisms of Emergence, and Ecological Fitness**

Dr. Zhang is a Professor in Veterinary Microbiology and the Frank Ramsey Endowed Chair in Veterinary Medicine at Iowa State University. Dr. Zhang received his PhD in immunobiology from Iowa State University and postdoctoral training in molecular microbiology from University of Missouri-Columbia. He was an Assistant Professor at the Ohio State University prior to his current appointment at ISU. Dr. Zhang's research program focuses on antimicrobial resistance in *Campylobacter*. His work covers the ecology, molecular basis of emergence and transmission, and fitness mechanisms of antibiotic resistant *Campylobacter*. He was the Panel Manager for the USDA NRI grant program: Epidemiological Approaches for Food Safety during 2005-2007, and recently became the Chair-Elect of Division Z (Animal Health) of the American Society for Microbiology.

**IRWIN REBECCA**  
Public Health Agency of Canada



**Title : CIPARS : An Approach to Surveillance and Control**

Dr. Rebecca Irwin is a veterinary epidemiologist and Director of the Antimicrobial Resistance Program, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada. She is responsible for the management and coordination of surveillance, research and risk assessment initiatives in support of the Public Health Agency of Canada and the Veterinary Drugs Programme of Health Canada. She leads a workforce of 25 professional and support personnel located across Canada to integrate antimicrobial resistance surveillance and research activities amongst government, academic and industry sectors. Her main focus of activity is with the coordination and operation of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). This program has been running since 2002 with the reporting of integrated data on antimicrobial use and antimicrobial resistance among enteric organisms from humans and major food animal production species.

## Plenary Speakers

### **HENDRIXSON DAVID R.**

The University of Texas Southwestern Medical Center

**Title : The Signaling Cascade that Controls Flagellar Gene Expression in *Campylobacter jejuni***



Dr. David Hendrixson received his Ph.D. from Washington University in St. Louis, MO in 1999 under the mentorship of Dr. Joseph St. Geme. For his graduate thesis, Dr. Hendrixson characterized Hap-mediated adherence of *Haemophilus influenzae* to respiratory epithelial cells. During his post-doctoral fellowship with Dr. Victor DiRita at the University of Michigan Medical School, Dr. Hendrixson developed and used new genetic technologies for *Campylobacter jejuni* to explore how flagellar genes are regulated and identify colonization factors of *C. jejuni* required for infection of the natural avian host. Dr. Hendrixson has been an Assistant Professor in the Department of Microbiology at the University of Texas Southwestern Medical School since 2004. His laboratory continues to understand the functional role of virulence and colonization factors of *C. jejuni* and how the bacterium controls the proper expression of genes encoding these factors.

# Plenary Speaker Abstracts

**THURSDAY, SEPTEMBER 25, 2008**

**NADON CELINE**

***Campylobacter: From isolation to intervention and the role of PulseNet Canada***

PulseNet Canada is the National Molecular Subtyping Network for Foodborne Disease Surveillance. Through the application of pulsed-field gel electrophoresis subtyping, PulseNet facilitates the identification of foodborne disease outbreaks at the earliest possible stage, links geographically dispersed cases, and provides a communication platform that connects the provincial public health laboratories across Canada. Presently, PulseNet is active for foodborne pathogens *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes*. As for other enteric bacterial pathogens, the laboratory methods for the detection and isolation of *Campylobacter* from stool specimens involve the use of selective media and recovery conditions that are optimized for survival and growth. However, slight method differences between laboratories may have the potential to affect the recovery of *Campylobacter* from clinical specimens. Additionally, the forwarding of *Campylobacter* isolates from clinical laboratories to provincial laboratories for further characterization may vary across Canada. These issues, basic components of laboratory surveillance, are among the factors that will be compared and contrasted for *Campylobacter* and other enteric bacterial pathogens. The challenges and potential for capturing *Campylobacter* in the PulseNet Canada program will be presented and discussed.

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**GALANIS ELENI**

***Surveillance gaps: Human perspective***

*Campylobacter* is the most common cause of human bacterial enteritis in Canada. Close to 10,000 cases are reported every year in this country. Apart from the handling and consumption of chicken, sources of infection are unclear. This review was undertaken to describe the gaps in surveillance of *Campylobacter* infection in humans in Canada and to suggest some potential solutions.

Campylobacteriosis is a reportable condition in Canada. Surveillance among humans is based on laboratory reporting of cases of *Campylobacter* infection to public health authorities. Few laboratories conduct further subtyping or share these data on a routine basis. Clusters of cases are investigated but interviewing of sporadic cases to identify risk factors varies across jurisdictions. The source of infection in outbreaks and sporadic cases is rarely identified. Risk factor data are not routinely centralised. Subtyping of isolates and collection of case data have not, for the most part, improved our ability to control *Campylobacter* infection in humans. This is in stark contrast with other bacterial enteric diseases where cases and outbreaks can be attributed to specific sources and these can often be mitigated.

The lack of epidemiologically-relevant subtyping tools and the lack of understanding of sources of infection other than poultry are major limitations in surveillance and control. Potential solutions include 1) the greater integration of epidemiological and microbiological tools in the investigation of cases and outbreaks and during research to identify useful subtyping techniques and 2) research focused on identifying the reservoir and sources of *Campylobacter* infection in humans.

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# Plenary Speaker Abstracts

**THURSDAY, SEPTEMBER 25, 2008**

**MICHAUD SOPHIE**

***Campylobacter in environmental water : Where does it come from and where does it go? The CAMPYLOGIS project***

Identification of the sources and routes of transmission of *Campylobacter* is essential for the development of targeted and efficient prevention measures. Recent studies suggest that the importance of drinking water as a source of sporadic *Campylobacter* infections in humans may have been markedly underestimated. An area of current interest is the possible contribution of drinking water and exposure to animals (e.g., cattle) to the observed differences in the epidemiology of *Campylobacter* infections in rural vs. urban areas. Since just a few hundred viable organisms represent an infectious dose, even apparently low levels of contamination could result in infection. Determination of fecal indicator organisms is used as a routine indicator of the microbiological safety of drinking water, but *Campylobacter* levels in water supplies are not correlated with the levels of marker organisms. To assess the risk from *Campylobacter* accurately requires new, independent methods and criteria for evaluating and monitoring the quality of water used for recreational and drinking purposes.

The CAMPYLOGIS project consists in (i) developing a Geographical Information System (GIS)-based human campylobacteriosis monitoring system integrating environmental and health data in the Eastern Townships, to evaluate the infectious risk related to water quality and land use; and (ii) validating the causal relationships suggested by the GIS by comparing by MLST *Campylobacter jejuni* isolates collected in the Eastern Townships, Québec, Canada, during a 2-year period from cases of human diarrhoea, environmental water (rivers, tributaries and wells), and whole fresh market chickens together with bovine, wild animal and bird feces. The prevalence and the quantity of *Campylobacter*, fecal coliforms and *E. coli* in environmental water

were evaluated weekly in 32 river and stream sample sites in the Eastern Townships, to determine the real importance of establishing a protocol for the specific detection and quantification of *Campylobacter* in environmental and untreated drinking water. MLST will validate the conclusions generated by the GIS monitoring system regarding the causal relationship between the incidence of campylobacteriosis in humans and water quality, and test the hypothesis that sources of campylobacteriosis acquired in rural or semi-rural regions differ from those acquired in urban regions.

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**QUESSY SYLVAIN**

***The Farm to plate approach in the control of Campylobacter in food products***

It is now widely accepted that control of food-borne pathogens starts at the farm level by decreasing the number of animals or birds entering in the food processing chain. In order to do so for *Campylobacter*, there is a need for an appropriate knowledge of the epidemiology of this pathogenic micro-organism at the animal population level and in the various steps of food production. In this presentation, we will review the current knowledge and some of our recent findings on the (molecular) epidemiology of *Campylobacter* in order to appreciate the feasibility of the farm to plate approach in the control of this pathogen.

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# Plenary Speaker Abstracts

THURSDAY, SEPTEMBER 25, 2008

GAYNOR ERIN

## *Campylobacter jejuni and the pathogenesis cycle*

For many disease-causing organisms, “pathogenesis” is a term that is often used as a synonym for “virulence.” For *Campylobacter jejuni*, however, it is becoming increasingly clear that not only are all aspects of the pathogenesis cycle – colonization, transmission, and virulence – critical for the organism’s ability to cause human disease, but also that factors controlling one aspect of this cycle often modulate one or both of the others as well. Furthermore, although our understanding of *C. jejuni*’s pathogenesis mechanisms has increased significantly over the past several decades, at least two big-picture questions remain: (1) how, specifically, does it make us sick, and (2) how does it navigate so many different environmental and *in vivo* niches despite fastidious laboratory growth characteristics?

In this talk, I will first briefly review several well-characterized *C. jejuni* pathogenesis determinants and discuss their roles in and implications for *C. jejuni* disease, transmission, and host colonization. I will then focus on several projects from our laboratory that were initiated to address the questions above, and resultant findings that have revealed intriguing links between *C. jejuni* pathogenesis and more “basic” processes such as stress survival, biofilm dynamics, metabolism, and cell envelope maintenance. These findings also continue to support the hypothesis that the genetics underlying the ability of a given *C. jejuni* strain to survive or thrive throughout the pathogenesis cycle may correlate with its success in disease etiology.

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HENDRIXSON DAVID R.

## *The Signaling Cascade that Controls Flagellar Gene Expression in Campylobacter jejuni*

Flagellar motility of *Campylobacter jejuni* is an important virulence and colonization factor involved in infection of human and avian hosts. Development of the flagellar organelle requires the correct temporal expression of over 40 genes and the proper ordered secretion and interaction of flagellar proteins. Genes encoding the flagellar basal body, rod, and hook components are dependent upon  $\sigma^{54}$  for expression. In previous studies, we determined that expression of  $\sigma^{54}$ -dependent flagellar genes requires the flagellar export apparatus (FEA), the FlgSR two-component system, and the putative GTP-binding protein FlhF. Recent work has provided evidence for how the functions of these systems converge to culminate in expression of  $\sigma^{54}$ -dependent flagellar genes. By using a constitutively active form of the FlgR response regulator, we determined that the FlgSR system functions downstream of the FEA to activate flagellar gene expression. This finding suggests that the FlgS sensor kinase may detect formation of the FEA or secretion of flagellar proteins by the apparatus to begin a phosphorelay event culminating in activation of FlgR. By analyzing mutants with functional defects in the FEA and *in vitro* biochemical assays with, we determined that FlgS likely interacts with the apparatus to detect when a proper FEA is formed. Additional analyses suggest that FlhF most likely functions downstream of the FlgSR system to activate expression of  $\sigma^{54}$ -dependent flagellar genes. This protein may influence production of  $\sigma^{54}$  or translation of proteins encoded by  $\sigma^{54}$ -dependent flagellar genes. Our work has provided insight into a complex regulatory network consisting of different protein systems that function together to ensure the proper expression of flagellar genes, production of flagellar proteins and synthesis of the flagellum.

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# Plenary Speaker Abstracts

FRIDAY, SEPTEMBER 26, 2008

## ROSENQUIST HANNE

### *Effects of the Danish intervention strategies aimed at reducing Campylobacter in broiler meat, 2002-2007*

B. Borck, H. Rosenquist, L. Boysen, S. Nordentoft, B. Helwigh, S. Ethelberg, C. Galliano

Thermotolerant *Campylobacter* spp. are the most common bacterial cause of human gastrointestinal disease in Denmark. In 1997, the Danish authorities initiated a strategy to control *Campylobacter*. A Danish quantitative risk assessment of *C. jejuni* in chicken meat, indicated that a key risk reducing control option is to reduce the concentration of *Campylobacter* in the chicken meat. In 2002, the industry began allocating meat from *Campylobacter* negative broilers to the production of fresh chilled products and meat from *Campylobacter* positive broilers to the production of frozen products. The intervention strategy was developed further in 2003 comprising: 1) Reduction of infection at the farm level, 2) Reduction of contamination at slaughter by preventing cross-contamination, and 3) Education of consumers. In this study, we have compiled and analysed monitoring data to evaluate possible effects of the strategy. Since 2002, the percentage of *Campylobacter* positive broiler flocks has decreased from 43% to 27% in 2007. At processing, the percentage of *Campylobacter* positive samples of fresh chilled chicken meat has dropped from 18% in 2004 to 8% in 2007. Furthermore, the number of reported human campylobacteriosis cases decreased from 4,379 cases in 2002 to 3,868 cases in 2007. Even though the number of human cases may be influenced by factors such as changes in consumption patterns and increased import of fresh chicken meat, we believe that the observed decrease in the number of positive samples from farm to processing and the overall reduced number of human cases is a result of the implemented control strategy.

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## ZHANG QIJING

### *Antimicrobial resistance in Campylobacter: global trend, mechanisms of emergence, and ecological fitness*

Antimicrobial resistance in *Campylobacter* presents a significant threat to public health. During the past decade, significant advances have been made in understanding the epidemiology and development mechanisms of antibiotic resistant *Campylobacter*. This presentation will discuss the global prevalence of antibiotic resistance in *Campylobacter*, the mechanisms involved in the emergence and transmission of antibiotic resistant *Campylobacter*, and the potential impact of acquiring antibiotic resistance on the ecological fitness of *Campylobacter* in animal reservoirs. Special emphasis will be given to recent finding on how *Campylobacter* develop resistance to various antibiotics and how antimicrobial usage in animal production influences the emergence of antimicrobial resistant *Campylobacter*. Particularly, the resistance to fluoroquinolones and macrolides, which are important for clinical treatment of campylobacteriosis, will be examined using specific examples. The role of natural transformation in horizontal spread of antimicrobial resistance and the impact of antibiotic withdrawal on the prevalence of antibiotic resistant *Campylobacter* will also be discussed.

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# Plenary Speaker Abstracts

FRIDAY, SEPTEMBER 26, 2008

**IRWIN REBECCA**

***CIPARS : An Integrated Approach to Surveillance and Control***

*Campylobacter* remains an important cause of bacterial enteritis in Canada. Campylobacteriosis is a notifiable disease with cases being tracked through convoluted trail of paperwork from personal physicians, to municipal, provincial and federal public health authorities. Ultimately cases are captured through the National Notifiable Diseases Program in PHAC. Studies conducted by PHAC have estimated that for every case of Campylobacteriosis reported to NND, between 23 and 49 cases may actually be occurring in the Canadian population. Significant underestimation of the true incidence coupled with a lack of information on exposure sources limits focussed public health interventions.

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was developed as a concerted suite of surveillance pilot projects that span the animal, food and human sectors to capture data to assess the human health impact of antimicrobial use in agri-food and aquaculture on human health. This model of an integrated surveillance system can be used to develop broader strategies for national enteric organism surveillance. In CIPARS, *Campylobacter* data is currently limited to isolates captured from the abattoir and retail surveillance components. Recently, a pilot project examining human surveillance isolates of *Campylobacter* from Saskatchewan was conducted to assess capacity for better integration within CIPARS.

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## Concurrent Sessions : Oral Abstracts

### **SESSION A: DIAGNOSIS, ANTIMICROBIAL RESISTANCE AND MOLECULAR TYPING**

#### **A01 Comparison of nine isolation methods for the recovery of *Campylobacter***

Danielle Daignault<sup>1</sup> Marie-Josée Champagne<sup>1</sup>  
and Lucie Dutil<sup>1</sup>

<sup>1</sup>Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Saint-Hyacinthe Unit, Québec, Canada.

**Background :** *Campylobacter* is a leading cause of enteric illness in many developed countries. The Laboratory for Foodborne Zoonoses (LFZ) of the Public Health Agency of Canada (PHAC) performs *Campylobacter* isolation for two surveillance programs, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) and C-EnterNet. There are numerous *Campylobacter* isolation methods although not one is designated as a standard method. Ten *Campylobacter* isolation methods were compared in the LFZ laboratory.

**Methods :** Samples from CIPARS Abattoir (n=94) and C-EnterNet On-Farm component (n=52) were analysed using ten isolation methods. Abattoir samples were taken from bovine caeca and On-Farm samples were faeces (porcine and bovine) taken from pen floors. The first 9 methods consisted of streaking 3 different types of agar (mCCDA, Karmali and Campy-Cefex) with a) the original sample, 2) a 1:10 dilution in buffered peptone water (BPW) and 3) the sample after enrichment in Bolton Broth. The tenth method used Hunt Enrichment Broth (HEB) and mCCDA.

**Results :** Direct plating on mCCDA, Karmali and Campy-Cefex agar of the Abattoir samples gave 40%, 41% and 29% recovery rate respectively whereas On-Farm samples showed recovery rate of 23%, 17% and 15%. Enrichment in Bolton Broth followed by plating on the 3 types of agar gave 32%, 32% and 26% recovery rate for Abattoir samples and 8%, 17% and 17% for the On-Farm ones. Dilution of the samples in BPW plated on the e types of agar gave 23%, 21% and 23% for Abattoir and 23%, 21% and 23% for On-Farm. Finally, the method using HEB and mCCDA showed recovery rate of 72% and 69% for Abattoir and On-Farm samples respectively.

**Conclusion :** The HEB-mCCDA method yielded superior results for caecal and faecal samples. Bovine caecal samples showed better *Campylobacter* recovery rate than the bovine faecal samples taken from pen floors.

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#### **A09 Comparison of Pulsed Field Gel Electrophoresis and Fla typing for predicting Multilocus Sequence Typing clonal complexes of *Campylobacter jejuni***

S. Lévesque<sup>1</sup>, E. Frost<sup>1</sup>, R.D. Arbeit<sup>2</sup> and S. Michaud<sup>1</sup>

<sup>1</sup>Département de microbiologie et infectiologie, Faculté de Médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada <sup>2</sup>Infectious Diseases Section, Tufts University School of Medicine, Boston, MA.

**Background :** Several molecular strain typing systems have been described for *C. jejuni*; however, there have been relatively few comparative studies and the most effective typing method for particular questions remains unresolved. This study describes the congruence among the genotypic relationships defined by three strain typing methods and considers their

relative utility for epidemiologic and surveillance studies of *C. jejuni*.

**Methods :** We applied two different methods, MLST and *flaA* SVR, to 289 isolates (163 human, 56 chicken, 34 raw milk, 36 environmental water) collected in Québec over three years. In addition, the analysis included the PFGE typing results of a subset of 131 isolates previously studied.

**Results :** All three typing systems were discriminatory (index >0.9). Among 131 isolates analyzed by PFGE, each of the 20 types represented by  $\geq 2$  isolates corresponded to a single clonal complex (CC) by MLST. In contrast, among the 14 most prevalent *flaA* SVR types (5 to 27 isolates each), eight (57%) included isolates that represented multiple different CCs. The basis for these discordant results was uncertain. There were no simple linear relationships among the typing systems. Individual sequence type (ST) could be associated with multiple *flaA* SVR types; conversely, a single *flaA* SVR type could comprise isolates with different ST.

**Conclusion :** Each of these molecular typing systems has particular strengths and limitations as well as significant operational differences. The method of choice may vary depending on the specific question at hand. At this time, MLST appears to be the single most effective tool for molecular strain typing of *C. jejuni* and uniquely suitable for extended, collaborative investigations.

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## **A12 Application of comparative genomics based genotyping to molecular epidemiological studies of *Campylobacter jejuni***

Susan L. Ross<sup>1</sup>, Joanne Mackinnon<sup>2</sup>, Julie Johnson<sup>4</sup>, Michael Roberts<sup>5</sup>, William Mauro<sup>3</sup>, James E. Thomas<sup>5</sup>, Kris Rahn<sup>2</sup>, Victor J. P. Gannon<sup>5</sup>, Eduardo N. Taboada<sup>5</sup>.

<sup>1</sup>University of Lethbridge, Lethbridge, AB;

<sup>2</sup>Public Health Agency of Canada, Guelph, ON;

<sup>3</sup>Canadian Food Inspection Agency, Lethbridge, AB;

<sup>4</sup>Health Canada, Lethbridge, AB; <sup>5</sup>Public Health Agency of Canada, Lethbridge AB.

**Background :** Comparative genomics can be used to assess the genetic similarity of organisms at the whole-genome level and represents an alternate approach to conventional genotyping methods for comparing bacterial isolates. We have developed Comparative Genomic Fingerprinting (CGF), a rapid method for *Campylobacter jejuni* genotyping that assesses the conservation status of 20 genes previously described as having high intraspecies variability. CGF was tested in a molecular epidemiological study of *C. jejuni* isolated in southern Alberta.

**Methods :** 590 *C. jejuni* isolates were obtained from over 2000 environmental, animal and human, clinical samples collected in the region of southern Alberta during a 3-year study (2004-2006). Isolates were genotyped using both CGF and *flaA*-RFLP analysis. BioNumerics v5.1 was used to assign clonal clusters based on fingerprint data and to examine the concordance between methods.

**Results :** While similar typing results were obtained with both genotyping methods, CGF was able to confirm possible epidemiological relations for strains bearing different *flaA* alleles. CGF clusters composed of water/animal isolates with matching profiles to contemporary clinical isolates were obtained, suggesting potential transmission

routes. Of the 108 CGF profiles found among 260 clinical isolates analyzed, 6 profiles accounted for 31% of all clinical isolates in our dataset. These profiles are not linked with outbreaks, have persisted over multiple sampling years and may represent endemic clones with enhanced virulence to humans.

**Conclusion :** CGF analysis, which is based on the comparative genomic analysis of a subset of highly variable genes in *C. jejuni*, presents a rapid and cost-effective genotyping method for molecular epidemiological studies. As this approach can be adapted to platforms with increased throughput, the CGF concept promises to deliver even greater resolution in a fraction of the time and cost required for leading conventional genotyping methods.

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#### **A08 Antimicrobial resistance in *Campylobacter* isolated from sheep in Ontario - preliminary results**

Scott, L.<sup>1</sup>; Moon, C.<sup>1</sup>; Menzies, P.<sup>1</sup>; Reid-Smith, R. J.<sup>1,2</sup>; Berke, O.<sup>1</sup>; Avery, B. P.<sup>1,2</sup>; Janecko, N.<sup>1</sup> McEwen, S. A.<sup>1</sup>

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The human health impact of antimicrobial resistant *Campylobacter* associated with antimicrobial use in food animals is a major concern. *Campylobacter* has been isolated from sheep feces and lamb meat. *Campylobacter*, primarily *C. fetus* and *C. jejuni*, is significant cause of abortion in sheep. Sheep are a minor food animal commodity in Canada and antimicrobial resistance in sheep bacteria has been little studied; the one previous study did not investigate *Campylobacter*. Although antimicrobial use is not common in the Canadian sheep industry, it does occur, and

much, due to the paucity of approved products, is extra-label. Fifty sheep flocks and one feedlot in Ontario were recruited into an ongoing one year study of antimicrobial use and resistance. Fecal samples are collected from pens and pastures at an initial visit and after 12 months; samples from 5 animals are pooled for each of 4 groups: nursing, market and replacement lambs, and adult ewes. *Campylobacter* isolates are tested for susceptibility to 8 antimicrobials (azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin) using Sensititre® broth microdilution. Preliminary results are available from initial sampling at 48 farms; 60.1% (83/138) of the pooled samples were positive for *Campylobacter*. Of 81 isolates speciated, 86.4% were *C. jejuni*, 12.3% were *C. coli*, and 1.2%, *C. lari*. Of 75 isolates with susceptibility results, 53.3% were resistant to one or more antimicrobials, 2.7% to 3 antimicrobials, and none to 4 or more. Resistance was observed to 4 of the 8 antimicrobials: tetracycline, 49%; ciprofloxacin and nalidixic acid, 10%; and telithromycin, 1%. Overall, the proportional occurrence of *C. jejuni* in the feces of sheep is higher than has been observed in cattle (<45% of *Campylobacter* isolates) and pig (<5%) feces in Canada. Resistance in *Campylobacter* from Ontario sheep is infrequent; however, resistance to drugs important in human medicine was observed.

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#### **A17 Evaluation of Pathatrix and Modified FDA/CFSAN-BAM Online Method for the Isolation of *Campylobacter* spp. From Chicken Parts**

A. Valdiviesco-Garcia<sup>1</sup>, E. Gonsalves<sup>1</sup>, K. Harris<sup>1</sup>, M. Varughese<sup>1</sup> and R.J. Reid-Smith<sup>1</sup>  
<sup>1</sup>Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada.

**Background :** *Campylobacter* is the most common cause of bacterial gastroenteritis in humans worldwide. Transmission to people can

be through consumption of contaminated foods, particularly chicken. Determining the prevalence of *Campylobacter* in chicken products is critical in epidemiological studies of *Campylobacter*. Implementation of more sensitive techniques in surveillance programs and epidemiological research provides more accurate prevalence data.

**Objective :** To compare the Pathatrix system with a standard method for isolation of *Campylobacter* from chicken meat samples.

**Methods :** One hundred and four fresh retail chicken samples were enriched as described by the FDA/CFR-BAM *Online* (BAM) method. After 24 h of shaking incubation at 42° C, aliquots were taken and plated onto Campy-Line agar (CLA), charcoal cefoperazone deoxycholate agar (CCDA), and hydrophobic grid membrane filter-semisolid medium (HGMF-SSM) plates. The rest of the enrichment broth was run in the Pathatrix unit, and aliquots plated onto CLA, CCDA and HGMF-SSM.

**Results :** The highest isolation rate was obtained with the BAM method and plated onto HGMF-SSM (48.08%), followed by CCDA (42.31%) and CLA (39.42%). The Pathatrix results were as follows: HGMF-SSM (37.50%), CCDA (28.85%) and CLA (23.08%). The McNemar's test indicated significant disagreement between Pathatrix and the BAM method using HGMF-SSM ( $p=0.009$ ), CCDA ( $p=0.0002$ ), and CLA ( $p<0.0001$ ) media when detecting chicken samples as positive for *Campylobacter*. There were no samples that the Pathatrix identified as positive and the BAM method negative; but the reverse did occur, Pathatrix negative, BAM positive (HGMF-SSM=10.58%, CCDA=13.46%, CLA=16.35%). Among the post-enrichment media, HGMF-SSM yielded the highest number of isolations of *Campylobacter* in both the BAM and Pathatrix methods.

**Conclusions :** The BAM and the Pathatrix methods disagreed, with the BAM method detecting more naturally *Campylobacter* contaminated chicken samples than the Pathatrix system. For these samples, the Pathatrix was less sensitive than, and did not offer an improvement over, the BAM method.

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### A19 Validation of a Simplified Enrichment for *Campylobacter* Detection in Food Samples

Florence Gorse<sup>1</sup>, Jean-Louis Pittet<sup>1</sup>, Bérenghère Genest<sup>1</sup>  
<sup>1</sup>bioMérieux R&D Microbiologie Industrielle

**Introduction :** Resuscitation and growth of thermophilic *Campylobacter* from food require specific cultural conditions like microaerobic atmosphere and/or blood or other components in complex media. Setting of these enrichments is cumbersome and expensive and there is a need for simplified and optimized methods for *Campylobacter* detection.

**Purpose:** The aim of this study was to evaluate a new method using a modified Stomacher bag, named combibag, to promote the microaerophily directly inside, associated with the use of the Bolton broth without blood.

**Methods :** Food samples, 1/10 diluted in Bolton broth without blood were cultured in Combibag for 48 hours at 41.5°C. Microaerobic atmosphere was generated by addition of a gas generator in a small pocket inside the Combibag. After incubation, *Campylobacter* were detected after streaking on selective agar plates or by the use of an immunoassay test. The complete alternative method was compared to the ISO 10272-1 reference method for the recovery of *Campylobacter* from 180 naturally contaminated poultry samples.

**Results :** The new method, associated to the immunoassay (VIDAS CAM) as the detection

system, detected 104 positive samples versus 96 for the traditional method. Statistical analysis (binomial law) showed no difference between the new method and the reference method. Aspect of selective agar plates obtained after streaking of enrichment broths of the new method or the reference method were also registered. Enumeration of *Campylobacter* and the ratio *Campylobacter*/contaminant flora were always higher with the new enrichment protocol.

**Significance :** This study showed that the new method is comparable to the ISO 10272-1 method for the recovery of *Campylobacter* from poultry samples and that the confirmation of positive samples was facilitated. The new system, to promote microaerobic incubation, greatly simplifies and standardizes the enrichment step for an optimized detection of *Campylobacter* from food samples and allows the use of enrichment broth not containing blood.

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## **SESSION B:** **PATHOGENESIS AND PHYSIOLOGY**

### **B01 The interaction of *Campylobacter jejuni* with a mucus secreting cell line HT29MTXE12**

Abofu Alemka<sup>1,2</sup>, Marguerite Clyne<sup>1,2</sup> and Billy Bourke<sup>1,2</sup>

<sup>1</sup>UCD School of Medicine and Medical Science

<sup>2</sup>The Childrens' Research Centre, Our Ladys' Hospital for Sick Children, Crumlin, Dublin 12

**Introduction:** *Campylobacter jejuni* is the commonest cause of bacterial gastroenteritis worldwide. It is also responsible for severe postinfectious sequelae such as reactive arthritis, irritable bowel syndrome and the Guillain-Barre syndrome.

**Aims and Background :** Current understanding of the pathogenesis of *Campylobacter* disease lags behind that of other enteric pathogens, partly due to the lack of a reliable animal model of the disease. *In vitro* models of *Campylobacter* infection lack a mucus layer and hence may not accurately reflect the situation *in vivo*. The aim of this study was to characterise the interaction of *C. jejuni* with a cell line, HT29MTXE12 (E12), that produces an adherent mucus gel when cultured on transwell inserts.

**Methods :** Cell culture, Confocal Microscopy, Immunofluorescence Microscopy, Gentamycin Protection Assay.

**Results :** *C. jejuni* readily colonised and reproduced in the overlying mucus layer, adhered to underlying epithelial cells, invaded and translocated to the basolateral chamber of the transwell in this model. Compared to the parent cell line HT29, which lacks an adherent mucus layer, the presence of mucus markedly increased the internalisation of *C. jejuni*. The integrity of the epithelial barrier was compromised 48 hours post infection as shown

by a drop in transepithelial resistance values. Also, *C. jejuni* aligned with tight junctions, and colocalised with the tight junction protein occludin, supporting the paracellular route of translocation.

**Conclusion :** These results demonstrate the exciting potential of the E12 model for studying the virulence of *C. jejuni in vitro*, in a manner that more accurately reflects conditions *in vivo*.

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### **B06 Post-transcriptional regulation of virulence-associated phenotypes in *Campylobacter jejuni***

Joshua A. Fields<sup>1</sup> and Stuart A. Thompson<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology Medical College of Georgia

**Background :** *Campylobacter jejuni* is a leading cause of gastroenteritis, and must survive multiple conditions during its lifestyle in animals, in the environment, and in humans. It is therefore critical for *C. jejuni* to regulate its protein expression, yet *C. jejuni* has relatively few transcriptional regulators. In other bacteria, post-transcriptional regulation can occur via the CsrA system, which is involved in the expression of numerous phenotypes including those related to pathogenesis. Typically, the CsrA protein binds to target mRNAs, affecting both translation and mRNA stability. Regulation of CsrA activity involves small RNAs, which bind to CsrA, modulating CsrA mRNA binding activity.

**Methods :** The *C. jejuni* 81-176 *csrA* gene was mutated by insertion of a non-polar chloramphenicol-resistance cassette, and then complemented by expressing a wildtype copy of *csrA* in the *csrA* mutant. These strains (wildtype, *csrA* mutant, and complement) were tested for virulence-associated traits, including motility (soft agar test), biofilm formation (on polystyrene plates), oxidative stress

(atmospheric oxygen and hydrogen peroxide), and host cell adherence and invasion (INT407 human epithelial cells).

**Results :** While the *csrA* mutant did not have apparent *in vitro* growth defects, it exhibited changes in all of the virulence-associated phenotypes tested. The mutant was characterized by decreases in motility, in biofilm formation, in resistance to oxidative stress and in adherence to human epithelial cells. Conversely, the *csrA* mutant showed a dramatic increase in the ability to invade human epithelial cells. All phenotypes were complemented by a wildtype copy of *csrA*.

**Conclusion :** *C. jejuni* CsrA plays a role in the expression of several virulence-associated characteristics, presumably by post-transcriptional regulation of proteins required for these traits. This suggests that understanding the *C. jejuni csrA* system will enhance our knowledge of the pathogenic mechanisms of this important pathogen. Experiments to define the *C. jejuni* CsrA regulon are ongoing.

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### **B07 Metabolomic analysis of *C. jejuni* 11168H reveals presence of novel flagellar glycans**

Susan M. Logan<sup>1</sup>, E. Soo<sup>2</sup>, E. Vinogradov<sup>1</sup>, J. Kelly<sup>1</sup>, A. Aubry<sup>1</sup>, J. Hui Sarah Howard<sup>3</sup> and B. Wren<sup>3</sup>

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**Background :** Glycosylation of flagellin structural proteins is essential for production of flagellar filaments in *Campylobacter* and the

presence of unique glycan moieties has been shown to be critical to virulence. Genomic comparisons have revealed that the flagellar glycosylation island (FGI) is one of the most variable loci amongst isolates. Initial studies of flagellar glycosylation in *C. jejuni* 81-176 and *C. coli* VC167 have demonstrated that these strains are able to synthesise novel legionaminic and pseudaminic acid derivatives which are present on flagella.

**Methods :** We now examine the metabolome and flagellin proteins of *C. jejuni* 11168H to determine the flagellar glycan biosynthetic capacity of this strain. We have used HILIC-MS to purify sugar nucleotide activated sugars and NMR to determine structure. Top down mass spectrometry was employed to characterise the flagellin protein.

**Results and Conclusion :** In addition to CMP-Pse, CMP-PseAm, CMP-LegAm, and CMP-Neu5Ac, the metabolome of this strain contained two novel related CMP-linked sugars of mass 390 Da and 389 Da. We have purified the CMP-389 nucleotide activated sugar and determined the structure by NMR to be a novel pseudaminic acid derivative. Analysis of purified flagellin protein by MS confirmed the presence of the novel glycan on the surface of the *Campylobacter jejuni* 11168 cells attached in *O*-linkage to the flagellin protein. Comparative phylogenomics of *Campylobacter* isolates revealed a cluster of six genes (Cj1321-26) within the flagellar locus which are specific markers for a “livestock clade” and these genes are present in the FGI of 11168H. We examined the metabolomes of select isogenic mutants in 11168H to determine the identity of the corresponding “livestock specific” glycan synthesized by these genes. In addition, we compared the metabolic profiles of livestock and non-livestock isolates to confirm the presence of this specific flagellar glycan as a biomarker for livestock colonisation.

### **B08 *Campylobacter jejuni* induces rapid colitis and NF- $\kappa$ B signaling in IL-10<sup>-/-</sup>;NF- $\kappa$ B<sup>EGFP</sup> mice**

Elisabeth Lippert<sup>1</sup>, Thomas Karrasch<sup>1</sup>, Young-Eun Joo<sup>1</sup>, Brigitte Allard<sup>1</sup>, Hans H. Herfarth<sup>1</sup>, Deborah Threadgill<sup>1</sup>, Christian Jobin<sup>1</sup>  
<sup>1</sup>Dept. of Medicine and Center for GI Biology and Disease, University of North Carolina

**Background :** Limited information is available on the molecular mechanisms associated with *Campylobacter jejuni*-induced pathogenesis. This is likely due to the lack of reliable experimental murine models. This study aims at establishing a model of *C. jejuni*-induced intestinal inflammation and to define the molecular mechanisms associated with the pathogenesis.

**Methods :** Axenic IL-10<sup>wt/wt</sup>;NF- $\kappa$ B<sup>EGFP</sup> and IL-10<sup>-/-</sup>;NF- $\kappa$ B<sup>EGFP</sup> mice were mono-associated with different inoculum of *C. jejuni* (strain 81-176; 10<sup>2</sup>-10<sup>9</sup>cfu/mouse). Macroscopic and confocal microscopic EGFP imaging (NF- $\kappa$ B-activation), qPCR analysis and histological analysis were utilized to characterize the bacteria-induced host responses. Inflammation was visualized by colonoscopy using a STORZ coloview mini-endoscope. NF- $\kappa$ B signaling analysis (transcriptional activity, I $\kappa$ B degradation) and pro-inflammatory gene expression were performed in the mouse colonic CMT93 cells.

**Results :** *C. jejuni* associated IL-10<sup>-/-</sup>;NF- $\kappa$ B<sup>EGFP</sup> mice developed severe inflammation and bloody diarrhea 14 days post-infection as depicted by colonoscopy. Macroscopic analysis showed elevated EGFP expression throughout the colon of *C. jejuni*-associated IL-10<sup>-/-</sup>;NF- $\kappa$ B<sup>EGFP</sup> mice. Confocal microscopy analysis revealed EGFP positive enterocytes and lamina propria immune cells after bacterial colonization. Histological analysis showed

severe crypt hyperplasia, goblet cell depletion, ulcers and immune cell infiltration. TNF and IL-12p40 mRNA were significantly induced in the colon of these mice compared to WT mice. In vitro analysis showed that *C.jejuni*-infected CMT93 cells induced I $\kappa$ B degradation (2 hours post-infection) followed by enhanced NF- $\kappa$ B transcriptional activity (18h; >5 fold) and increased IL-6, MIP-2 and Nod2 mRNA accumulation. Importantly, *C.jejuni*-induced NF- $\kappa$ B transcriptional activity as well as IL-6, MIP-2 and Nod2 mRNA accumulation were blocked by molecular delivery of I $\kappa$ B (Ad5I $\kappa$ BAA). Pharmacological NF- $\kappa$ B blockade with BAY 11-7085 failed to prevent inflammation but rather increased intestinal bacterial load.

**Conclusion :** Our findings indicate that *C. jejuni* induces rapid and severe intestinal inflammation in a susceptible host, which correlates with enhanced NF- $\kappa$ B activity. Our data suggest that NF- $\kappa$ B signalling may be necessary to prevent *C. jejuni* spreading.

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## **SESSION C:** **SURVEILLANCE, PREVENTION AND CONTROL**

### **C02 Findings of Two years of Integrated *Campylobacter* Surveillance within a Community (C-EnterNet)**

André Ravel<sup>1</sup>, Barbara Marshall<sup>2</sup>, Katarina Pintar<sup>1</sup>, Angela Cook<sup>1</sup>, Andrea Nesbitt<sup>1</sup>, Nancy Sittler<sup>3</sup>, Michele Van Dyke<sup>4</sup>, Frances Jamieson<sup>5</sup>, Clifford Clark<sup>6</sup>, Frank Pollari<sup>2</sup>

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**Abstract :** Integrated surveillance and source attribution have been advocated in Canada and abroad to reduce the burden of enteric diseases in general, and of campylobacteriosis in particular. In Canada, C-EnterNet, a multi-partner integrated sentinel site surveillance program, was launched in 2005 in the first (pilot) sentinel site (Region of Waterloo, Ontario) for the surveillance of *Campylobacter* and other enteric pathogens in both the human population and various exposure sources. Active monitoring of those pathogens on farms (swine, dairy, beef, broiler chicken operations), retail raw meat (raw chicken breasts, pork chops, and ground beef) and surface untreated water was initiated within the sentinel site boundaries: culture-based detection (and molecular based methods for water only), speciation, antimicrobial resistance testing, and enumeration (in food only) were performed. In parallel, enhanced epidemiological and microbiological data were collected for the human cases in the community, based on a

strong collaboration with the local public health unit and both private and public diagnostic laboratories. Endemic human campylobacteriosis cases during the first two surveillance year will be described, including incidence rate, age and gender distribution, temporal distribution, and results from the risk factor analysis. *Campylobacter* contamination rates in the various sources over the same two year period will be described, including subtyping data.

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### **C07 Rare *Campylobacter jejuni* strain points to manure-contaminated mud as cause of bike race outbreak, British Columbia, 2007**

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<sup>1</sup>Canadian Field Epidemiology Program, Public Health Agency of Canada <sup>2</sup>Vancouver Coastal Health Authority <sup>3</sup>British Columbia Centre for Disease Control

**Background :** One of the largest reported *C. jejuni* outbreaks in Canada occurred in June 2007 in British Columbia, associated with a mountain bike race that took place in wet and muddy conditions on a trail frequented by people and domestic and wild animals.

**Methods :** A retrospective cohort study was conducted, with multivariate logistic regression analysis in STATA v.9. Samples of mud and water from the race course were collected and tested for *Campylobacter*, generic *Escherichia coli* and total coliform counts. Multi locus sequence typing (MLST) was performed on clinical *C. jejuni* isolates.

**Results :** 787 individuals participated in the race. Of those who responded, 25 (5%) met the laboratory-confirmed case definition and 200 (36%), the clinical case definition. Racers who inadvertently consumed mud had a relative risk

(RR) of illness of 2.1 (95% CI 1.5-3.0). Individuals who drank cups of water from official water stations also had an increased risk of illness (RR = 2.0, 95% CI 1.4-2.9). Multivariate adjustment showed that consumption of water was not associated with illness. Mud samples tested negative for *Campylobacter*, but positive for generic *E. coli*, with coliform counts exceeding 24,192/100mL. All 14 *C. jejuni* clinical isolates were identical by MLST, belonging to ST-538 which clusters within the ST-45 clonal complex. ST-538 has only been isolated from 2 humans and 1 horse worldwide.

**Conclusion :** Contaminated mud was the likely source of *Campylobacter*. A single *C. jejuni* strain was responsible for the outbreak. As horses were present on the trail prior to the race, it is possible that horse manure may have contributed to this outbreak. Recommendations for future races include removal of visible animal feces from the course and educating racers on risks of accidental mud ingestion. MLST may be helpful in characterising the source of *C. jejuni* outbreaks.

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### **C09 : The seasonality of campylobacteriosis: are we missing something?**

Julie Arsenault<sup>1</sup>, André Ravel<sup>2</sup>, Olaf Berke<sup>3</sup>, Pierre Gosselin<sup>4</sup>, Pascal Michel<sup>2</sup>

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**Abstract :** In developed countries, the incidence rate of campylobacteriosis is characterized by a strong and consistent seasonal pattern, for which there is no consensual explanation. In general, for the northern hemisphere, the incidence increases from the beginning of May up to a main peak occurring between mid-June and mid-July, with a secondary peak often observed in fall. There is an important knowledge gap

about the factors underlying seasonality of campylobacteriosis, although various hypotheses have been proposed. Understanding the reasons for seasonality is fundamental to clarify the epidemiology of the disease, with principal impacts on determining more targeted, timely intervention strategies for public health and veterinary medicine.

The objectives of this study are to present the current hypotheses on campylobacteriosis seasonality through a comprehensive review of the literature, and to identify scientific evidences supporting or refuting these hypotheses. Mechanisms related to human population behaviors, pathogen-to-pathogen interactions, and environmental effects on the bacteria will be considered. As an exploratory analysis to assess the importance of agro-environmental factors on seasonality, the seasonal patterns of campylobacteriosis incidence in Quebec will be described according to the characteristics of case residence area (urban, rural with few agricultural activities, rural with high agricultural activities). The analysis will be based on culture-confirmed cases of campylobacteriosis reported to regional health units between 1996 and 2006. Classification of regions will be done using census data from Statistics Canada (urban/rural) and Quebec's Ministry of Agriculture data on agricultural activities at the farm level. Smoothed time series of the weekly incidence of campylobacteriosis will be computed to present data.

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### **C11 Burden of illness associated with antimicrobial susceptible and resistant *Campylobacter* infections in the Perth and Wellington-Dufferin-Guelph Health Units**

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L<sup>7</sup>; Boerlin, P<sup>2</sup>; Dewey, C<sup>1</sup>; Irwin, R<sup>2</sup>; McEwen, S. A.<sup>1</sup>

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**Abstract :** A multidisciplinary, two year project (2002-2004) was conducted in two health units in Ontario, Canada to investigate: the burden of illness associated with antimicrobial susceptible and resistant *Campylobacter*; risk factors for human infection with susceptible and resistant *Campylobacter*; retail chicken *Campylobacter* prevalence; and the occurrence of susceptible and resistant *Campylobacter* isolates in retail chicken and human cases. Laboratory confirmed human cases, each with two age-matched controls from the same health unit area, were administered a phone questionnaire that incorporated: potential risk factors for antimicrobial susceptible and resistant *Campylobacter* infection; prior and concurrent antimicrobial usage; and the burden of illness. Burden of illness indicators included the reported type, duration, and severity of clinical signs, activity limitations, and health care utilization. Retail chicken was sampled from randomly selected stores in the Wellington-Dufferin-Guelph and Perth District Health Unit areas. *Campylobacter* isolates from chicken and laboratory confirmed human cases were tested for susceptibility to ampicillin (AMP), amoxicillin-clavulanic acid (AMC), chloramphenicol (CHL), clindamycin (CLI),

ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), tetracycline (TCY) and trimethoprim-sulfamethoxazole (SXT) using the E-test®. Questionnaire data were collected from 250 human cases and 512 controls. Cases had an age range of 0.3 to 85 years and were comprised of 140 males and 110 females. *Campylobacter* isolates were available from 131 human cases. Of these, 2.3% were *C. coli* and 97.7% were *C. jejuni*. Seven human isolates (5.3%) were resistant to CIP. No resistance to AMC, CHL, or GEN was found in the human isolates and 59.5% were susceptible to all antimicrobials tested. An overall assessment of the burden of illness of campylobacteriosis in this study, as well as the impact of antimicrobial resistance on the burden of illness indicators will be presented.

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### **C16 Spatial analysis and modeling of interactions between environment and *Campylobacter jejuni* in Eastern Township surface waters**

Djoan Bonfils<sup>1</sup>, André Lavoie<sup>1</sup>, Simon Lévesque<sup>1</sup>, Eric Frost<sup>1</sup>, Karen St-Pierre<sup>2</sup>, Rémy Desbiens<sup>1</sup>, Nathalie Carrier<sup>2</sup>, Goze Bertin Béné<sup>1</sup>, Sophie Michaud<sup>2</sup>

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**Background :** The sources and the epidemiology of *Campylobacter* in environmental water are not well understood. We developed a spatial analysis methodology able to identify the main environmental factors predicting the presence and quantity of *Campylobacter* in river water.

**Methods :** For two years, water samples were cultured weekly for *Campylobacter* and fecal

coliforms at 32 sampling sites of the hydrographic network of the Eastern Townships, Québec. The quantity of *Campylobacter* in water was estimated using the Most Probable Number method. The 32 sampling sites were linked to their catchment area; 10 of them were excluded from the analysis, because their hydrographic basin was not independent from the other sites. For each site, the following environmental variables were included: Water flow, slope, land-cover, land-use including type of farming, animal density, total precipitation in the 3 days prior to water sampling. A stepwise multivariate regression was realized across the different analysis windows to define the size of the area upstream from the sampling sites (from 1.5 to 24 km) which was the most closely associated with the mean quantity of *Campylobacter*, and which environmental factors were associated with a higher mean quantity of *Campylobacter* in water.

**Results :** Preliminary results show that an area defined by a radius of 14 km upstream of the sample site was the most contributing zone for the bacteria ( $r^2=0.382$ ,  $p=0.002$ ). Within this 14 km area, the only significant variable associated with a higher mean quantity of *Campylobacter* was bovine density ( $p=0.002$ ). When analyzing the data within a 120 m buffer zone across 14 km upstream of the sampling sites ( $r^2=0.396$ ,  $p=0.001$ ), the only significant variable associated with a higher mean quantity of *Campylobacter* was the percentage of agricultural surface ( $p=0.001$ ).

**Conclusion :** These results suggest a strong implication of bovine density in conjunction with crops and associated manure spreading on the presence and quantity of *Campylobacter* in environmental water.

#### **C04 Molecular characterization of *Campylobacter jejuni* strains linked to recent milk-related outbreaks and surveillance of California Central Valley dairy environments**

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**Background :** Dairy products, primarily milk, have been implicated in multiple *Campylobacter* outbreaks: 49 milk-related outbreaks were reported in the U.S. alone from 1978 to 2003. Recently, two milk-related, *C. jejuni* outbreaks were reported in California. The first (1644 cases: May 2006) occurred at 11 California correctional/mental health facilities and represents the largest U.S. milk-related, *C. jejuni* outbreak to date. This outbreak was linked epidemiologically to consumption of pasteurized milk from a dairy (“Dairy 1”) that supplied milk to all 11 facilities. The second outbreak (8 confirmed cases: Nov-Dec 2007) was traced back to raw milk produced by another dairy (“Dairy 2”).

**Methods :** *C. jejuni* clinical outbreak strains were obtained from the state health departments. Farm investigations were conducted to collect environmental samples at the implicated California dairies. Additional *Campylobacter* strains were cultured from samples collected at other Central Valley dairies and creameries. All *Campylobacter* strains were characterized by a combination of multilocus sequence typing (MLST) and major outer membrane protein (Cmp) typing.

**Results :** Approximately 300 *Campylobacter* strains were typed: 23 MLST sequence types and 49 Cmp types were identified. In both

California investigations, strains with the same ST and Cmp type as the clinical outbreak strain were found in environmental samples on the dairies: in Dairy 1, the outbreak strain was isolated from dairy waste water and in Dairy 2, the outbreak strain was isolated from cattle feces. Surveillance sampling of other Central Valley dairy environments revealed the presence of multiple *C. jejuni* STs but only two *C. coli* STs (STs-894 and 1068).

**Conclusion :** MLST and Cmp typing were useful epidemiological tools for source-tracking *C. jejuni* strains during an outbreak investigation. Molecular typing also revealed high genetic diversity within *C. jejuni* samples isolated from dairy environments, but a highly clonal *C. coli* population isolated from the same environments.

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## **SESSION D:** **GENOMICS AND MOLECULAR** **APPROACHES**

### **D01 The identification of *Campylobacter jejuni* Essential Genes**

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The identification of *C. jejuni* genes required to sustain life is of considerable interest. The function of these essential genes could reveal novel and important metabolic and biochemical pathways in *C. jejuni*. In order to identify these genes, a genome-wide mutagenesis approach was employed. First, a transposon mutant library of *Campylobacter jejuni* NCTC11168 comprising 7201 individual mutants was constructed. Second, the transposon insertion sites of all the mutants were mapped using a microarray transposon tracking approach. The transposons were found to be randomly inserted throughout the genome. Among the 1654 predicted genes from this organism, 295 had no detectable transposon insertion. As a consequence, these genes were predicted to be essential. The absence of transposon within several of the identified genes was further confirmed by using a PCR approach. As expected, many of these indispensable genes encode proteins involved in the basic DNA replication and RNA transcription machineries, and other basic cellular processes such as RNA translation and modification, protein processing and cell division. Genes coding for proteins involved in energetic and intermediary metabolisms were also identified. However, the essentially of these genes might be dependent on the set of metabolites absent in the Mueller-Hinton growth medium used in this study. Interestingly, 135 essential genes (~46%) encode proteins of unknown function,

highlighting our lack of knowledge of *Campylobacter* physiology. Computational comparison of *C. jejuni* essential genes to the core set of genes indispensable for growth in eight other bacterial species, including *Helicobacter pylori*, *Escherichia coli*, and *Bacillus subtilis*, revealed a set of 48 essential genes (16%) common to more than three other bacteria, while 27% were essential in only one or two. In conclusion, we identified the core set of genes in *C. jejuni* indispensable to sustain its growth, opening new research avenues for drug development and physiological studies.

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### **D02 Characterization of the Regulatory Networks Controlling Iron Metabolism in *Campylobacter jejuni***

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Iron is known to catalyze a wide range of biochemical reactions essential for most living organisms, including *C. jejuni*. Paradoxically, this iron reactivity is also responsible for the generation of the biotoxic hydroxyl radical ( $\cdot\text{OH}$ ). Thus microorganisms must achieve an effective iron homeostasis by tightly regulating the expression of genes involved in iron acquisition, metabolism and oxidative stress defense. Interestingly, in addition to the classical ferric uptake regulator Fur, *C. jejuni* carries another member of the Fur family of metalloregulators, PerR. PerR is a peroxide-sensing regulator and typically regulates peroxide stress response in Gram-positive bacteria.

To define the Fur and PerR regulons, we constructed their isogenic mutants and complemented strains in *C. jejuni* NCTC 11168. First, the contribution of Fur and PerR to oxidative stress resistance was assessed by disk inhibition assays. The  $\Delta$ *perR* mutant was more resistant to H<sub>2</sub>O<sub>2</sub> and cumene hydroperoxide, and more sensitive to menadione. The  $\Delta$ *fur* mutant was more resistant to H<sub>2</sub>O<sub>2</sub> and more sensitive to cumene hydroperoxide and menadione. Second, the Fur and PerR regulons were characterized by genome-wide transcriptome profiling. A total of 53 and 81 genes were found to be Fur and PerR regulated respectively. PerR and Fur appear to regulate gene expression both dependently and independently of the presence of iron and/or H<sub>2</sub>O<sub>2</sub>, suggesting the presence of complex regulatory mechanisms. Many genes were found to be both Fur and PerR regulated, indicating that the PerR and Fur regulons overlap. Third, the genes under the direct control of Fur and PerR are currently being identified and characterized by electrophoretic mobility shift assays, ChIP-on-chip experiments, and surface plasmon resonance analysis. Overall, these data reveal the interplay between the PerR and Fur regulons.

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### **D03 Development and application of multiplex PCR assays of cytolethal-distending toxin (*cdt*) genes for the characterization and differentiation of *C. jejuni* and *C. coli* species**

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Thermophilic *Campylobacter* species such as *C. jejuni* and *C. coli* have been significantly implicated in human gastrointestinal infections. These species commonly occur in poultry, bovines, pigs and humans. In order to better understand the occurrence and epidemiology of *Campylobacter* species prevalent in the

environment, rapid methods are warranted to determine the prevalence of virulence and toxin genes among *Campylobacter* species. The study was designed to develop novel triplex PCR assays to identify and discriminate between species of *C. jejuni* and *C. coli* based on the cytolethal-distending toxin (*cdt*) genes. CDT affects the epithelial cell layer and causes progressive cellular distension and death in cell lines. The CDT activity is encoded by the *cdt* genes, which consist of three adjacent or slightly overlapping genes named *cdtA*, *cdtB* and *cdtC*. Species-specific primers for each gene were derived from highly variable sequences in the target gene. Specificity of the primers and PCR conditions were verified using other species of *Campylobacter* as well as different negative control species. To evaluate specificity and novelty, the newly developed method is being applied to characterize *C. jejuni* and *C. coli* strains isolated from various aquatic ecosystems across Canada.

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### **D05 Expression, Purification and Structural Characterization of CfrA, An Iron Transporter from *Campylobacter jejuni***

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*Campylobacter* ferric receptor (CfrA) is a putative siderophore-iron transporter localized in the outer membrane of the enteric food-borne pathogen *C. jejuni*. CfrA is essential for the growth of *C. jejuni*, and is thus a potential therapeutic target. We cloned the *cfrA* gene from chromosomal DNA and examined the expression of 6-, 8-, and 10-His-tagged constructs under different temperatures and concentrations of the inducer IPTG. Several detergents, including dodecylmaltoside, were effective at solubilizing CfrA from the pelleted membrane fraction, although a substantial

portion of the expressed protein was resistant to solubilization and likely targeted to inclusion bodies. FTIR spectra recorded from purified and membrane-reconstituted CfrA are highly characteristic of a  $\beta$ -sheet protein ( $\sim 50\%$   $\beta$ -sheet and  $\sim 10\%$   $\alpha$ -helix) and are similar to spectra that have been recorded from other siderophore-mediated iron transporters. Membrane incorporated CfrA undergoes relatively extensive peptide hydrogen-deuterium exchange upon exposure to  $^2\text{H}_2\text{O}$  and is resistant to thermal denaturation at temperatures up to  $95^\circ\text{C}$  - denaturation was only achieved after boiling CfrA for 1 hour. The secondary structure, relatively high aqueous solvent exposure, and high thermal stability are all consistent with a transmembrane  $\beta$ -barrel structure containing a plug domain. Sequence alignments suggest that CfrA contains many of the structures conserved in other transporters, including the Ton box, PGV, IRG, RP, and LIDG motifs of the plug domain. A homology model constructed for CfrA suggests that regions expected to play a role in substrate binding exhibit unexpected sequence variations relative to other siderophore-mediated iron transporters, including the ferric-enterobactin transporter, FepA. CfrA may have a broader substrate specificity than other members of this protein family. The unique structural features of the putative ferric-siderophore binding site suggest that CfrA may be a therapeutic target for the treatment of *C. jejuni* infections.

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#### **D10 Identification of novel genes in the oral pathogen *Campylobacter rectus***

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<sup>1</sup>Department of Genetics, CB, University of North Carolina <sup>2</sup>SPIRE Postdoctoral Fellowship Program, University of North Carolina

A poorly described bacteria, *Campylobacter rectus*, has been implicated as an etiological agent of periodontal disease. The aim of this

study was to use a comparative genomics approach to identify genes that contribute to the lifestyle of *C. rectus* as an oral pathogen.

Suppressive subtractive hybridization (SSH) was used to identify genes encoded by *C. rectus* ATCC 33238, but not present in the genome of a related *Campylobacter* species, *Campylobacter jejuni* ATCC 11168. SSH identified 154 unique DNA sequences from the *C. rectus* genome. Ninety-two of the 154 clones were classified as *C. rectus* specific, as they did not show significant sequence homology to genes identified in any strain of *C. jejuni* (BLAST E-value  $>1\text{E-}3$ ). BLAST analysis predicted that the 92 *C. rectus* specific gene fragments play a role in a variety of biological processes including signal transduction mechanisms (histidine kinase, response regulators, diguanylate cyclases, chemotaxis receptor) and potentially virulence (S-layer RTX and cysteine desulhydrase). Further analysis of the *C. rectus* specific clones identified a group of 20 genes sharing homology with genes only identified in members of the genus *Campylobacter* that commonly reside within the oral cavity of humans; and fragments sharing homology with genes previously not identified in any member of the genus *Campylobacter*. These data provide the first substantial insights of the genomic content of *C. rectus*, a significant oral pathogen. The genes identified in this study are a valuable resource for initiating new research on the virulence of *C. rectus* during periodontitis.

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## **D08 Molecular typing of *Campylobacter* in the genomic era**

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The molecular epidemiology of *Campylobacter jejuni* and *C. coli* is still an enigma that nearly two decades of typing investigations have not been able to solve. Technical improvements and standardization of methodology have resulted in reliable and portable methods, but the data have not brought the expected insights in source attribution, relative risk, or recognition of higher or lower virulent subpopulations, despite numerable efforts. The latest addition to the firmament of typing methods has been complete genome hybridization by micro-array analysis. This may be the way forward, but technical improvements are needed to improve the output, increase the throughput and reduce the costs. In addition, it is necessary to complete our understanding of the true genetic potential of the organism, by generating and interpreting additional complete genome sequences. From analysis of 15 genome sequences currently available it is shown what insights can be gathered on core genes (present in all genomes of a given species) and dispensable genes of *C. jejuni* and related species. The total gene pool of a species, the 'pan-genome' should be the basis of micro-arrays that sufficiently cover the genetic repertoire potentially present in individual isolates. Implications of such insights on the current practice of molecular epidemiology will be discussed. It can be envisaged that the generation of complete genome sequences may be the ultimate fine-tuned typing method of the future, provided technical improvements and reduced costs continue their current trends.

## Concurrent Sessions : Poster Abstracts

### **SESSION A: DIAGNOSIS, ANTIMICROBIAL RESISTANCE AND MOLECULAR TYPING**

#### **A02 Epidemiology and antimicrobial susceptibility of 71 *Campylobacter fetus* subsp. *fetus* strains isolated from 67 patients in Québec, Canada, from 2001 to 2006**

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**Objectives :** Review of 71 *Campylobacter fetus* subsp. *fetus* (*C. fetus*) strains isolated from 67 patients in Québec from 2001 to 2006.

**Methods :** Epidemiological data, antimicrobial susceptibility by agar dilution (CLSI),  $\beta$ -lactamase and pulsed-field gel electrophoresis (PFGE) with the enzyme *Sma*I were determined.

**Results :** Nine to 15 patients were infected yearly with *C. fetus*. The male-to-female ratio was 2.2 to 1.0; 48% of the patients were  $\geq$  60 years old. The isolation site, age, sex and Québec socioeconomic region (QSR) of the patients were:

Isolation site*	Stools	Blood	P
# patients	25	41	
Age of patients	8-84 (median 51 years)	32-94 (median 67 years)	0.0003
# men	20 (80%)	25 (61%)	0.18
QRS # 06	17 (68%)	14 (34%)	0.016

\*The isolation site was unknown for one man (1.5%). Five patients suffered a relapse (7.5%), the same strain (as confirmed by PFGE) having been isolated 25 days to five months apart. In

2001- 2002, four men, 24 to 44 years old, living in QRS # 6, suffered an enterocolitis caused by a *C. fetus* pulsovar 1. All *C. fetus* strains were susceptible to ampicillin, erythromycin, imipenem and meropenem with MIC<sub>90</sub>s of 1, 1, 0.125, 0.125 mg/L respectively. One (1.5%), four (6%), 14 (21%) and 17 (25%) patients were respectively infected with strains resistant to gentamicin, ciprofloxacin, tetracycline and cefotaxime. All strains were  $\beta$ -lactamase negative. From 2001 to 2004, 18 *C. fetus* strains isolated from stools showed 12 different PFGE profiles.

**Conclusion :** In Québec, there was an increase in the male-to-female ratio of the 67 patients from 2001 to 2006 in comparison to 92 patients from 1989 to 2000 (ratio of 1.1 to 1.0) with a *C. fetus* infection. The patients with an enterocolitis were younger and more often from the QSR 06 than patients with septicemia and were men for 80%.

#### **A03 Phenotypic and genotypic characterization of *Campylobacter*, *Helicobacter* and *Arcobacter* isolated in a Montreal hospital from 1999 to 2007**

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**Objectives :** To determine the characteristics of *Campylobacter* (*C.*), *Helicobacter* (*H.*) and *Arcobacter* (*A.*) isolated at HSL from 1999 to 2007.

**Methods :** Epidemiological data was reviewed. Susceptibility to erythromycin (E), tetracycline (T) and ciprofloxacin (Ci) was done using agar dilution method (CLSI). Pulsed-field gel electrophoresis (PFGE) was determined with the enzymes *SmaI* and/or *KpnI*. The *fla* gene was analysed by PCR-sequencing. Stools were cultured on CCDA at 37°C in 5% O<sub>2</sub> for 72 h.

**Results :** From 1999 to 2007, 58% of 909 patients with an enteropathogenic bacteria, were infected with a *C.*, *H.* or *A.* Three *H. cinaedi* and 1.6% of the 516 *C.* were isolated from blood. Compared to *C. jejuni* acquired locally (n=250), *C. jejuni* acquired abroad (n=174) were more resistant (R) to Ci (66% vs 12%, P < 0.000001) but not to E (2% vs 5%, P = 0.16) nor to T (57% vs 58%, P = 0.99). *C. coli* acquired abroad (n=19) were more R to Ci (74% vs 14%, P = 0.003) but not to E (11% vs 7%, P = 1) nor to T (42% vs 50%, P = 0.9) than those acquired locally (n=14). Eleven *C. fetus fetus*, seven *C. upsaliensis*, three *C. lari*, three *C. hyointestinalis* and nine *A. butzleri* were also isolated. Three clusters were documented: 27 men who have sex with men (MSM) had a *C. jejuni* R to E and Ci, S to T, untypeable by PFGE and *fla* type I or II, 16 MSM had a *C. jejuni* R to Ci, S to E and T, PFGE pulsovar 1 and four MSM had a *C. fetus* pulsovar 1.

**Conclusion :** From 1999 to 2007, six *C.* species and one *H.* and *A.* species were identified. Three clusters of *C.* were documented in MSM.

#### A04 Comparison of disk diffusion and agar dilution methods for antimicrobial susceptibility testing of *Campylobacter coli* and *Campylobacter jejuni*

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**Objectives :** to compare the results obtained by disk diffusion (DD) and by agar dilution methods for erythromycin (E), tetracycline (T) and ciprofloxacin (Ci) of 100 *C. coli* and for T of 289 *C. jejuni*.

**Methods :** agar dilution method (CLSI) was done at HSL. Disk diffusion testing was done at HSL, HSC and LSPQ.

**Results :** the MICs (HSL) and zone diameters (HSL, HSC) for the susceptible (S) and resistant (R) *C. coli* and *C. jejuni* are :

	Erythro <i>C. coli</i>	Cipro <i>C. coli</i>	Tetra <i>C. coli</i>	Tetra <i>C. jejuni</i>
S* MIC (µg/ml)	≤0.06-4 (# 88)	≤0.06-1 (# 69)	≤0.06-2 (# 59)	≤0.06-4 (# 133)
S* DD (mm)	17-42	28-47	24-49	26-56
R* MIC (µg/ml)	64->128 (# 12)	4-64 (# 31)	16->128 (# 41)	16->128 (# 154)
R* DD (mm)	6	6-18	6-17	6-19

The 69 *C. coli* S to Ci and 31 R to Ci had respectively presence and absence of a zone around the nalidixic acid disk (NA). The 2 *C. jejuni* intermediate to T had a MIC of 8 µg/ml and zones of 22 and 25 mm. At LSPQ, the 25 *C. coli* S to Ci and 30 of the 31 R to Ci had respectively presence and absence of a zone for NA.

**Conclusion :** the DD interpretative criteria suggested for *C. coli* and *C. jejuni* from this study and from a precedent one are :

	Susceptible	Resistant
Erythromycin	> = 20 mm	= 6 mm
Tetracycline	> = 21 mm	< = 17 mm
Ciprofloxacin and NA	> = 26 to Ci and > 6 mm to NA	< = 19 to Ci and 6 mm to NA

If an intermediate zone diameter is obtained by DD, a MIC by DA or Etest is recommended.

## **A06 Incidence and molecular characterization of *Campylobacter* in surface water from the Pike River watershed**

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Agricultural practices are suspected to play a role in the dissemination of pathogens in the environment, especially in watercourses. It was reported that surface water might be a source of contamination for human because it may contain pathogens like *Campylobacter*. The current study was conducted to determine the incidence of *Campylobacter* spp. in water and to evaluate the genetic diversity of recovered bacteria. Surface water samples were taken from four sites located in a rural area of the Pike River watershed (Montérégie, QC) during Summer 2006. Water was filtered to retain bacteria and the filter was incubated in an enrichment broth. *Campylobacter* was detected from the enrichment broth both by PCR and by plating on selective media. By plating, *Campylobacter* spp. was isolated from 37% (14/38) of water samples; of which 93% were identified as *C. jejuni*. The incidence of *Campylobacter* spp. was found to be similar by PCR but some samples were positive for more than one species. Among the PCR-positive samples, 58% contained only *C. jejuni*, 25% contained both *C. jejuni* and *C. coli* and 17% contained all at once *C. jejuni*, *C. coli*, and *C. lanienae*. Genetic characterization of *C. jejuni* was performed by *flaA*-typing. Among the 11 typeable *C. jejuni* isolates, six distinct *flaA* patterns were observed. One pattern was shared by five *C.*

*jejuni* isolated from different sampling sites and at different dates. It was not possible to associate a *flaA* pattern with a specific site. Identifying the sources of contamination of *Campylobacter* is a complex task considering the genetic diversity of the bacteria. Matching genotypes from farm animals and water should help to better understand the *Campylobacter* distribution in the environment as the bacteria can survive in surface water.

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## **A07 Specific detection and identification of *Campylobacter jejuni* and *Campylobacter coli* by Culture and Polymerase Chain Reaction (PCR) in Human Faeces in a Tertiary Care Hospital of North India**

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**Background :** *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are thermophilic, Gram-negative, curved bacilli that causes gastro-enteritis worldwide. Recent studies suggest *C. jejuni* as an antecedent infection in Guillain-Barre syndrome (GBS – a type of peripheral neuropathy). Scarcity in reports from India in this subject has prompted us to undertake this study. Further, fastidious growth requirements and largely non-specific biochemical properties of these organisms' makes isolation and identification difficult and time-consuming. Hence, implementation of a molecular technique, such as PCR, along-with culture has been suggested for its reliable detection and identification.

**Methods :** A prospective study is being done at All India Institute of Medical Sciences, New Delhi since September 2003. A total number of 376 stool samples (147 samples from adult diarrhea patients, 170 samples from pediatric

diarrhea patients and 59 samples from pediatric GBS patients) has been screened by culture and PCR [(i) PCR for *Fla-A* gene for co-identification of *C.jejuni* and *C.coli*, (ii) PCR for *Hip-O* gene for specific identification of *C.jejuni*] was done for all the samples. mCCDA (modified Charcoal Cefoperazone Deoxycholate Agar, Oxoid, U. K.) with selective supplements ( 32mg/L Cefoperazone, 8 mg/L Amphotericin B and 4mg/L vancomycin) is used for isolation of these organisms from fecal samples. Plates are incubated at 37°C for 48-72 hrs in microaerophilic atmosphere ( a gas mixture of 5% O<sub>2</sub>, 10% CO<sub>2</sub> and 85% N<sub>2</sub> was filled in an aerobic jar by aid of an automated equipment, Mart, Switzerland))

**Results : In adult group of diarrhea patients :** One out of 147 patients were positive by culture, (0.68%), 04 samples were positive by PCR (2.72%)

**In pediatric group of diarrhea patients :** seventeen out of 170 patients were positive by culture (10%), 20 out of 170 samples were positive by PCR (11.76%)

**Among GBS Patients:** Three out of 59 patients (5.08%) were positive by culture, 04 out of 59 samples (6.77%) were positive by PCR .

**Conclusion :** Use of molecular technique such as; PCR, in conjunction with culture increases the sensitivity and helps in early detection of these organisms. Screening of stool samples from GBS patients for *Campylobacter Spp.* to know the etiology may be beneficial to plan possible treatment for better outcome This study shows that pediatric age group is at more risk to infection by this group of organisms.

## **A10 PCR Identification of Thermotolerant *Campylobacters* from Produce and Abattoir Samples: a Comparison of PCR Gene Targets**

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A number of PCR assays have been described for identification of *Campylobacter jejuni* and *C. coli*. Comparisons of PCR assays for *Campylobacter* identification of reference strains and clinical isolates have revealed discrepancies in identification when different gene targets are compared. Our goal was to compare several published PCR assays with biochemical analysis for identification of isolates of *Campylobacter* recovered from food and animal samples that were obtained as part of the Canadian Integrated Program for Antibiotic Resistance Surveillance (CIPARS). PCR assays were performed on isolates of *Campylobacter* recovered from 94 produce samples and 21 abattoir samples. Four PCR assays were compared: two multiplex assays for *C. jejuni* and *C. coli* identification, targeting a UDP-N-acetylglucosamine acyltransferase (*lpxA*) gene and random DNA; a commercial multiplex assay for *C. spp.*, *C. jejuni* and *C. coli* (GenPoint); and a simpleplex assay for detection of the hippuricase gene (*hip*) of *C. jejuni*. Examination of *C. jejuni* identification revealed that 88.8% of the produce samples (71/80) showed complete concordance between biochemical analysis and the 4 PCR assays and 70% concordance in abattoir samples (7/10). In addition, 7.8% (7/90) of the isolates identified as *C. jejuni* by biochemical analysis and 3 of the PCR assays were negative using the *hip* PCR. *C. coli* comparisons revealed only 25% concordance in produce (2/8), wherein 6/8 samples that were identified as *C. coli* using biochemical analysis were identified as *C. jejuni* by all 4 PCR assays including *hip*. One hundred percent concordance was observed between

analyses in abattoir samples (8/8). In conclusion, discordant results were obtained in identification of *C. jejuni* and *C. coli* between biochemical and PCR methods. *C. jejuni* samples will be sub-typed using High Resolution Melt analysis of the Clustered Regularly Interspersed Short Palindromic Repeat locus to determine whether non concordant samples can be predicted using this genotyping method.

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### **A11 Application of analytic Models to Ciprofloxacin Minimum Inhibitory Concentrations of Enteric *Campylobacter jejuni* Isolates from Human Patients in Saskatchewan 1999-2005**

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Antimicrobial resistance in *Campylobacter jejuni* from animals, food and humans is a global public health concern. Ciprofloxacin resistance in particular can increase the burden of illness of campylobacteriosis. Resistance surveillance data are typically dichotomized into sensitive and resistant, potentially obscuring subtle temporal shifts in minimum inhibitory concentration (MIC). MIC shift detection is important for early identification of antimicrobial selection pressure. Representative resistance data for human *Campylobacter* is scant in Canada; however, the Saskatchewan Disease Control Laboratory (SDCL) tests a large proportion of provincially-reported *Campylobacter* cases. The objective of this

study was to compare the ability of statistical analytic models for dichotomized and MIC data to detect temporal changes in ciprofloxacin resistance. Ciprofloxacin MICs were determined by Etest<sup>®</sup> for 1014 *C. jejuni* isolated from human feces submitted to the SDCL from 1999 to 2005. A resistance breakpoint of  $\geq 4.0$ ug/mL was used for dichotomization. A logistic model was applied to the dichotomized data to determine the effect of time on the predicted probability of a non-susceptible isolate. A discrete-time survival model, using concentration-to-inhibition of growth as the “time-to-event”, was utilized to compare the predicted hazards for the range of MIC dilutions over the study period. Overall, the prevalence of ciprofloxacin resistance was 8.8% (89/1014). The logistic model showed a decrease in the annual log-odds of resistance from 1999 to 2004, with a subsequent increase in 2005. The discrete-time survival model showed an annual increase in the hazard probabilities for low MIC dilutions (0.064-0.25 ug/mL) from 1999 through 2005. This indicates a similar trend for ciprofloxacin resistance to the logistic model. The MIC survival model was not demonstrably more sensitive than the logistic model, due in part to the relatively low number of moderate to high-MIC isolates. Further comparison of the two models using a larger dataset is warranted.

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### **A13 Evaluating The Potential of Comparative Genomic Fingerprinting for High-Resolution Genotyping of *Campylobacter jejuni***

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**Background :** Comparative genomics, the study of similarities and differences between two or more genomes, can be used to assess the genetic similarity of strains, thus representing an alternate approach to conventional genotyping. In previous work, we developed a comparative genomics-based genotyping assay (Comparative Genomic Fingerprinting or CGF) that uses four multiplex PCRs to assess the conservation status of 20 genes known to be variably absent among *Campylobacter jejuni* strains.

**Methods :** A panel of strains with varying levels of genetic similarity was used to compare the results obtained by CGF to those obtained by conventional molecular typing methods (*flaA*-RFLP, *flaA*-SVR sequencing and MLST). We also used computer simulations based on comparative genomic data to examine the effect of gene selection and the number of genes included in a CGF assay on typing results.

**Results :** Significant concordance exists between CGF and MLST, and to a lesser extent with *flaA*-SVR and *flaA*-RFLP, with CGF providing additional sub-typing resolution. Robust clusters obtained with one method were generally recovered with the other methods, although discrepancies were observed for isolates that did not form robust clusters using either method. We also found that gene

selection can have a significant effect on CGF results if the number of genes used to generate a profile is small (n=15), whereas an increasing number of genes (n>30-35) can approximate the results obtained from whole genome comparative genomic analysis.

**Conclusion :** Our results suggest that variability in the gene content can be used as a quick and reliable method for genotyping, provided that sufficiently large number of genes is targeted. As many hyper-variable genes encode potential virulence determinants, gene content analysis may prove to be an invaluable approach for generating epidemiologically and clinically relevant genotyping data.

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### **A14 Molecular typing of *Campylobacter* isolates from environmental water using Amplified Fragment-Length Polymorphism (AFLP)**

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**Background :** Surface waters may be the major reservoir from which *Campylobacter* are distributed between mammalian and avian host species and between these species and their immediate environment. We studied the molecular diversity of *Campylobacter* isolated from environmental water to further evaluate possible sources of water contamination.

**Methods :** *Campylobacter* isolates obtained weekly from 32 river water sampling sites in the Eastern Townships over 2 years were analysed by AFLP. Isolates were considered to have closely related genotypes based on molecular typing if their AFLP profiles were related at  $\geq 0.85$ .

**Results :** When the 878 *C. jejuni* isolates were analysed globally, the similarity coefficient (SC) was 66%, and 99% of isolates were part of 18 clusters of 2-670 isolates each. When analysing the data by sampling site, the SCs varied between 64 and 85%. The number of isolates analysed by site varied between 8 and 55 isolates each, of which 63-100% were distributed among 1-4 clusters of 2-43 isolates each. When we analysed the data from geographically near sampling sites belonging to the same catchment area together, we observed that 89%-100% of isolates were part of 2-6 clusters, with SCs varying between 67% and 78%. For *C. coli*, 46 of the 51 isolates analysed were distributed among 5 clusters of 2-23 isolates each, with a SC of 74%. For *C. lari*, 78 of the 82 isolates were distributed among 10 clusters of 2-32 isolates each, with a SC of 70%.

**Conclusion :** Although *Campylobacter* is known to be very heterogeneous genetically, the low genetic diversity observed among water isolates from the same sampling site suggests that there are few different contamination sources at a local level. This suggests that the size of the catchment area and land use may influence the species and genetic diversity of *Campylobacter* isolates from environmental water and could also indicate that a limited intervention strategy could have a considerable impact on *Campylobacter* prevalence in environmental water.

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### **A15 Evaluation of a PCR method for species identification of *Campylobacter jejuni* and *C. coli* as an alternative to conventional biochemical tests**

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**Background :** The objective of this study was to incorporate a PCR-based method into Health Canada's official method MFLP-46 (Identification of thermophilic *Campylobacter* from food) for rapid species identification of *C. jejuni* and *C. coli* from presumptive positive *Campylobacter* colonies isolated on selective plates.

**Methods :** Several published primer sets were initially tested for specificity and ease of use with DNA derived from pure cultures. The multiplex PCR system described by Persson and Olsen (2005) was selected for further evaluation. It consisted of primer pairs for the *C. jejuni* hippuricase gene, the *C. coli* aspartokinase gene and a universal 16S rDNA sequence used as an internal positive control for the PCR. This primer set was tested with *Campylobacter* strains isolated from foods and identified using the traditional biochemical tests described in MFLP-46, and the results of both methods were compared.

**Results :** A total of 230 *Campylobacter* strains were analyzed. Of these 204 were identified as *C. jejuni* and 26 were identified as *C. coli* using biochemical tests. All strains that were identified as *C. jejuni* biochemically were also identified as *C. jejuni* using the multiplex PCR method. Seven of the strains identified as *C. coli* using biochemical tests were found to be *C. jejuni* using the PCR method. The PCR identification was confirmed using a second

primer set with different gene targets. Misidentification by biochemical tests may have been due to variability in expression of the hippuricase gene.

**Conclusion :** The PCR identification of *C. jejuni* and *C. coli* was generally in concordance with biochemical tests and in some cases appeared to give more reliable results. Addition of this PCR method to MFLP-46 will decrease the time and cost of *Campylobacter* species identification in positive samples, enabling rapid data accumulation for use in risk assessment in cases of food contamination with *Campylobacter*.

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#### **A16 Comparison of sample weighing vs sample rinsing for the detection of *Campylobacter* spp from poultry**

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**Background :** A study was undertaken to determine if sampling methods affected recovery of *Campylobacter* spp. from poultry. Currently, Health Canada's MFLP-46 method (Identification of thermophilic *Campylobacter* from food) requires that raw meats be sampled by analysis of 25 g of meat cut into 0.3-0.5 cm<sup>3</sup> pieces. Carcass rinsing is a more efficient method of sampling that may result in improved recovery of contaminating bacteria on the exterior and interior surfaces of the poultry samples.

**Methods :** The study included 29 retail poultry samples (whole birds, parts and offals). A 25 g portion was taken from each sample and the remaining sample was rinsed with 200 ml of 0.1% peptone water. The 25 g sample or 25 ml

of the rinse was enriched in Park and Sanders broth. After 24 and 48 hours incubation, the broth was plated on mCCDA, Preston Agar with blood, and mCEFEX agar. Identification of *Campylobacter* species was based on colony morphology, microscopic morphology and motility, biochemical tests, growth at different temperatures, rapid identification kit (API Campy or Vitek) and PCR.

**Results :** Thirteen of the 29 weighed samples (44.8%) and 18 of the rinse samples (62.1%) were positive for the presence of *Campylobacter*. The difference in recovery between the two methods was not statistically significant (P=0.1881). *Campylobacter* spp. were recovered by both methods in 23 out of 29 samples (79.3%). Of the 32 strains of *Campylobacter* isolated, 22 were identified by traditional biochemical or rapid kit methods as *C. jejuni ssp jejuni*, six as *C. coli* and four were only identified to the genus level. By PCR, 22 samples were identified as *C. jejuni*, seven as *C. coli* and one could not be identified.

**Conclusion :** The study results demonstrate that carcass rinsing is a viable alternative to sample weighing when using the Park and Sanders broth as directed by the MFLP-46 method.

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#### **A18 Parameters that influence the efficient use of filtration for the isolation of *Campylobacter* spp. from foods**

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**Background :** *Campylobacter* is a frequent cause of human bacterial gastroenteritis. Previous studies have indicated membrane filtration as an effective isolation technique for *Campylobacter* spp. from fecal samples. However, a large number of cells were required for detection. We determined the minimum

number of *Campylobacter* cells needed to pass the filter and the effect of the status of the cells. We also determined the minimum number of cells to pass the filter from enriched food samples.

**Methods :** To determine the minimum required cells that go through the filters, experiments were done with healthy (24-h under microaerobiosis), coccoid, centrifuged (20 min, 16,000 g) and non-flagellated mutant cells. We also determined the minimum number of cells needed to isolate *Campylobacter* spp. from naturally contaminated enriched retail broiler samples. Experiments included 0.65- $\mu$ m-pore membrane filters (Millipore Corp.) on modified Campy-Cefex agar plates. To determine the rate of passage of *Campylobacter* through the membrane filters, inoculated filters were harvested at different time intervals and analyzed with scanning electron microscopy (SEM).

**Results :** The mean values for the minimum number of cells required to go through the filters differed significantly and were contingent to cell status. The minimum number of healthy and centrifuged cells that went through the filters (2.2 and 2.1 log<sub>10</sub> CFU, respectively) was different ( $P < 0.05$ ) from the minimum number of coccoid and mutant cells (4.1 and 3.4 log<sub>10</sub> CFU, respectively). The use of SEM was not effective to observe the rate of passage of *Campylobacter* through the filters. Enriched samples showed  $\sim 2$  log<sub>10</sub> CFU of *Campylobacter* are needed to obtain pure colonies on agar plates.

**Conclusion :** These results demonstrate that cell status may determine the minimum number of cells that can go through the filter. The use of filter membranes is an effective method to obtain pure *Campylobacter* colonies from enriched food samples.

## **SESSION B:** **PATHOGENESIS AND PHYSIOLOGY**

### **B02 Hydrogen Peroxide Defenses in *Campylobacter jejuni***

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**Abstract :** *Campylobacter jejuni* infection results in gastroenteritis in humans. As a microaerophilic bacterium, *C. jejuni* has a growth requirement for reduced oxygen. Consequently, *C. jejuni* will be unavoidably exposed to reactive oxygen species (ROS) during the course of normal metabolism. During infection, *C. jejuni* is also exposed to ROS produced by the host immune system and by the host intestinal microbiota. Irrespective of their sources, ROS will damage DNA and proteins, and cause peroxidation of lipids. Identification of defenses against ROS is important for understanding how *Campylobacter* survives this environmental stress during infection. Construction of isogenic deletion mutants into genes encoding potential oxidative stress defense systems lead to the discovery of a novel oxidative stress defense gene, Cj1386. Growth inhibition assays showed that the  $\Delta$ Cj1386 mutant has an increased sensitivity to hydrogen peroxide but not to cumene hydroperoxide or menadione. In support of this data, chromosomal complementation of the  $\Delta$ Cj1386 mutant restored the wild-type sensitivity to hydrogen peroxide. Cj1386 is located directly downstream and in the same operon as *katA* (catalase). A double deletion mutant  $\Delta$ (*katA* + Cj1386) was constructed. This mutant was found to exhibit a similar sensitivity to hydrogen peroxide as is seen in the single mutants  $\Delta$ Cj1386 and  $\Delta$ *katA*. This observation suggests that Cj1386 may be involved in the

same detoxification pathway as catalase, KatA. Catalase activity assays are currently being performed to assess the function of Cj1386 in hydrogen peroxide detoxification. Interestingly, the  $\Delta$ Cj1386 mutant was out-competed by the wild-type strain for colonization of the gastrointestinal tract of neonate piglets. This result indicates an important role for Cj1386 in *Campylobacter* colonization and pathogenesis.

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### **B03 Characterization of an indispensable two-component regulator of *C. jejuni***

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**Abstract :** The food-borne pathogen *Campylobacter jejuni* experiences traumatic environmental stresses during its life cycle, and must adapt to different conditions in order to successfully survive, replicate and colonize its host. Until recently, knowledge of the process governing *Campylobacter* sensing of its environment has been extremely limited.

The bacterial wall is the first barrier from external threats, and thus the first communication line between the cell and its surroundings. External stimuli are typically sensed by two-component signal transduction systems composed of a sensor histidine kinase component and a response regulator. Examination of the *C. jejuni* genome indicates the presence of 11 potential two-component response regulators. Microarray and real-time RT-PCR analysis of the *C. jejuni* response to antimicrobial peptide exposure revealed the down-regulation of the two-component regulator Cj0355c. This observation suggests a role for this regulator in sensing membrane integrity. Screening of a library composed of

7201 individual transposon mutants indicated that the Cj0355c gene had no detectable transposon insertion, implying an indispensable role for Cj0355c. The function of this gene was further investigated by constructing a *C. jejuni* strain over-expressing Cj0355c. Phenotypic analysis of this over-expressing strain revealed a role for Cj0355c in biofilm and pellicle formation. Interestingly, the over-expressing strain was also found to be affected in its ability to colonize the gastrointestinal tract of piglets as compared to the wild-type strain. Finally, transcriptomic studies indicated a role for Cj0355c in maintaining membrane integrity. All together, our data show that Cj0355c is an essential two-component signal regulator likely sensing ruptures in the bacterial cell wall and counteracting membrane damages.

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### **B05 Determination of the age of initial colonization of *Campylobacter* in broiler flocks in two selected farms in Sri Lanka**

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Thermotolerant *Campylobacter* species are a well known cause of food borne infection worldwide although the available data about this pathogen in Sri Lanka is sparse.

However, a recent study revealed high prevalence of *Campylobacter* positive broiler flocks in selected parts of the country. More than 65% of broiler flocks in all four provinces were *Campylobacter* positive at the age of slaughter.

The present study was aimed at determining the age of initial colonization of broilers in two different rearing systems and the time of the year.

Data were collected during a period of 6 months (December 2007 –May 2008) from 20 broiler flocks belonging to two farms (Farm A and B) in the Central province of the country. Farm A where 500-1000 birds in a flock practiced “all in all out” system. In farm B, a flock of broilers consisted of 100-200 birds and birds of different age groups were reared in pens close to each other.

From each flock studied, cloacal swabs were collected from randomly selected 10 birds every other day from day one onwards until the flock became positive for *Campylobacter*. Of 7 visits out of 20, caeca and meat were collected from the same flocks at slaughter.

Isolation and identification of *Campylobacter* was performed according to the method described by ISO (10272).

All flocks tested in this study became positive for *Campylobacter*. Out of 20 flocks 2 flocks showed colonization below 14 days of age. In all other flocks colonization was first seen at the age of 14- 24 days. At slaughter, caeca and meat were positive for the organism. According to the findings of this study the rearing system has not shown a major impact at the age of colonization. Finding of the study, as to the colonization age is in agreement with the literature. As the prevalence over only half a year was studied the investigation should be extended to a one year period.

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### **B09 A 37° C Liquid Nematode Killing Assay for Assessment of *Campylobacter* Virulence**

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A significant obstacle to understanding *Campylobacter*'s pathogenicity to humans is the absence of a useful animal model. The soil

nematode *Caenorhabditis elegans* has been used successfully to study pathogenicity of several other human pathogens. Repeated attempts to reproduce the published killing assays with *Campylobacter* in *C. elegans* at 26° C were unsuccessful. If *Campylobacter* virulence related genes are not fully operational at 26° C, the assay may need to be performed at a temperature to which *Campylobacter* adapted *i.e.* 37° C. However, *C. elegans* mutants which are most commonly used in this kind of experiment are not viable at 37° C.

Nematodes were isolated from soil and compost samples and assessed for viability at different growth temperatures. *Campylobacter* strains, negative control *Escherichia coli* OP50 and positive killing control *Salmonella Typhimurium* ATCC 14028 were added to the nematodes in liquid culture and mortality was scored hourly. The assay was assessed at 26° C and 37° C.

Two wild nematode strains were isolated that can survive at 37° C for ≥1 day. The assay was successful using these isolates as the time for 50% worm death in the positive control was significantly less than the time when temperature related mortality commenced and differential killing was observed between the positive and negative controls, as well as among the *Campylobacter* strains, at 37° C..

Using *Campylobacter* isolates from clinical, food, animal, and environmental sources, experiments are now in progress to determine if clusters of epidemiologically similar isolates can be observed. Genetic content of *Campylobacter* isolates in clusters with high nematocidal activity will then be analyzed to determine if there are any commonalities in putative virulence gene content. This data would be useful in molecular risk assessment and in the development of strategies to mitigate the public health consequences of this important human pathogen.

## **B10 The glycome of *Campylobacter jejuni* – understanding bacterial N-linked protein glycosylation**

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**Background :** Since the demonstration that the *Campylobacter jejuni* N-linked protein glycosylation (*pgl*) locus can be functionally transferred into *Escherichia coli*, there has been a surge of activity to exploit this pathway for glycoprotein engineering. Yet, little is known about the regulation and function of protein glycosylation in the native host.

**Methods :** *Pgl* promoter activities were identified by Gfp fusions and analyzed by semi-quantitative fluorescence microscopy while transcriptional start sites were determined using 5'RACE. Transcriptional profiles were compared using whole genome DNA-microarrays and capsular polysaccharides (CPS) were analyzed by quantitative high resolution magic angle spinning NMR (HR-MAS-NMR). Free N-glycans (fOS) were characterized using a previously described universal glycomics technique and N-glycosylation profiles were compared by immuno-detection using N-glycan specific antisera.

**Results and Conclusions :** We identified *pgl* gene promoters for *gne*, *pglB*, *pglC*, *pglE* and *pglJ*. These activities were growth-phase dependent and differ when the locus is transferred into *E. coli*. The absence of known transcription factor binding sites in proximity to the transcriptional start sites indicates a novel

mode of gene regulation in *C. jejuni* for this pathway.

Transcriptome analyses of 11 *pgl* mutants identified over 250 genes to be differentially expressed when compared to the wild-type including genes for iron metabolism, protein glycosylation, and capsule biosynthesis. Detailed HR-MAS-NMR analysis of *pgl* mutant capsules verified a novel link between N-glycan and capsular heptose biosynthesis.

Using non-specific proteolytic digestion and mass spectrometry analyses of the total glycome, we identified a novel component derived from the N-glycan pathway: free oligosaccharide (fOS). Interestingly, PglB-dependent fOS release but not N-linked protein glycosylation was found to be growth phase dependent, decreases at high medium osmolarity, and is regulated on the level of PglB activity. As mutants completely lacking fOSs showed an impaired growth phenotype under conditions of high ionic strength we conclude that this novel pathway intermediate acts as an osmoprotectant in *C. jejuni*.

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**SESSION C:**  
**SURVEILLANCE, PREVENTION AND CONTROL**

**C03 Registry based epidemiological study of children *Campylobacter* infections in Quebec, 1999-2006 and association with environmental risk factors**

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**Background :** In Quebec, *Campylobacter* infections incidence rate rised from 30 cases/100 000 inhabitants in 1990 to more than 45 cases/100 000 inhabitants in 1997 and was particularly elevated in children aged between 0-4 years old. Studies worldwide seem to show the impact of livestock density and water quality on the rate of these increasing infections.

The objective of this study was to evaluate seasonal and annual rate trend of these infections from 1999-2006 as well as his association to livestock density, type of water consumed and socioeconomics situation of areas where infections took place.

**Methods :** Cases of *Campylobacter* infections in children aged 0-4 reported to the Quebec ministry of health were analyzed. We describe seasonal and annual pattern of this rate from 1999-2006 in children, excluding Quebec northern regions. Univariate and multivariate Poisson regression were used to estimate infections relative risk associated with

environmental risk factors investigated in municipalities less than 10 000 inhabitants. The number of cases in each municipality was used as outcomes in the regression models. The expected number of cases occurring was assumed to be proportional to the population size and the exponential of a linear combination of the environmental variables included in the analysis. SAS Statistical Software was used for the analysis.

**Results and conclusions :** There were 1564 infections notified to the national register from 1999-2006. The seasonal pattern shows a rate always higher in summer and the overall annual incidence rate was about 52 cases/100 000 children. In total, 535 of 1564 cases (34.2%) could be assigned to small sized municipalities. Analysis showed that animal densities categorized in quartiles: cattle (RR=2.38 p-trends<.0001), swine (RR=1.41 p-trends<.0001), small ruminants (RR=1.95 p-trends=0.06); type of water consumed (RR=1.008 p-trends=0.18) and socioeconomics situation (RR=1.01 p-trends=0.0004, material) (RR=1.01 p-trends=0.002, social) are significantly related to increased rate. This indicates contamination from environmental risk factors investigated may be important factors in explaining sporadic children campylobacteriosis in Quebec.

**C05 National Agri-Environmental Standards Initiative: Occurrence of *Campylobacter* species and other waterborne pathogens in agricultural watersheds across Canada (2005-07)**

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**Abstract:** As part of the National Agri-Environmental Standards Initiative (NAESI) under the Agricultural Policy Framework, 4557 water samples were collected for waterborne pathogen analyses from 27 sites in four agricultural watersheds from 2005-07. The watersheds were located in intensive farming areas for poultry (British Columbia), beef cattle (Alberta), dairy cattle (Ontario), and hogs (Quebec). Pathogens were more commonly detected at watershed sites near agricultural activities than at reference sites away from agriculture in each watershed. *Campylobacter* sp. was the most commonly detected pathogen in water samples (57%), followed by *Cryptosporidium* sp. (48%), *Giardia* sp. (36%), *Salmonella* sp. (3%), and *E. coli* O157:H7 (1%). A multiplex PCR assay was applied for detecting multiple *Campylobacter* species in water samples. *Campylobacter jejuni* was the most commonly detected species, followed by *C. coli* and *C. lari*. However, the occurrence of *Campylobacter* species varied significantly between watersheds, and *C. coli* was most prevalent in the watershed with intensive hog farming. Occurrence data for *Campylobacter* and other waterborne pathogens is being used for the purposes of developing an environmental

benchmark indicative of impairment of ambient water quality in agricultural watersheds with multiple water uses.

**C08 Lessons Learned from Unique Interventions Implemented in Iceland in Response to a National Epidemic of Campylobacteriosis**

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**Background :** In 1999, a national epidemic of campylobacteriosis was reported in Iceland. The epidemic was attributed to the recent availability of fresh retail poultry products. As a result, novel interventions were implemented within the poultry industry, and veterinary and public health sectors. The objectives of this study were to review the interventions to assess their impact on campylobacteriosis and contribute knowledge that could be applied in Canada to prioritize interventions.

**Methods :** From March 25 to 28, 2008, face-to-face interviews using open-ended questionnaires were conducted. Key informants from the poultry industry, veterinary and public health

were identified based on their expertise and involvement in the interventions implemented in response to the epidemic. Literature and document reviews were conducted.

**Results :** Ten key informants were interviewed. Veterinary, environment and health agencies reacted quickly and collaborated to implement the following interventions between 1999 and 2002: *Campylobacter* surveillance in poultry, enhanced farm biosecurity, mass consumer education, changes in poultry processing, and poultry packaging and freezing policies. Subsequently, domestic cases of campylobacteriosis decreased (157/100,000 in 1999 to 75/100,000 in 2002).

**Conclusions :** Iceland's swift and precautionary actions coupled with collaboration between agencies and with the poultry industry were integral to the mitigation of the epidemic. Initially, media interest and consumer awareness provided economic motivation for the poultry producers to enhance biosecurity and produce *Campylobacter*-negative flocks, however long-term sustainability was believed to be achieved by the freezing policy.

Iceland was the first country to implement a freezing policy for the sale of *Campylobacter*-positive poultry products. This policy is believed to have been the most effective intervention as market forces have driven the poultry industry to strive for the ability to sell fresh poultry products.

**Next Steps :** The impact of the interventions on the occurrence of campylobacteriosis in Iceland will be quantitatively assessed by temporal analysis to measure their effectiveness in controlling the epidemic.

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## **C10 A conceptual scheme of the environmental transmission pathways of *Campylobacter* spp**

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**Abstract :** In Canada, infection with *Campylobacter* spp. is the leading cause of bacterial gastroenteritis, with an estimated annual incidence rate of 0.8 to 1.7% in the general population. The natural site of *Campylobacter* amplification is the intestinal tract of warm-blooded animals. Wild birds, poultry, sheep and cattle seem to be of particular importance in the natural cycle of the bacteria, because they are easily infected and can excrete the bacteria in high numbers. The bacteria can be dispersed in the environment by direct fecal excretion or through manure spreading, and animal carcasses can be contaminated to various extent depending on the slaughtering processes. Humans are infected by ingestion following exposure to contaminated water, environment, or food. Mechanical vectors such as flies could also play a role in the transmission of the bacteria. However, the exact pathways by which humans are infected remains poorly understood as to their relative importance. The significance of each pathway is likely to differ according to population and environmental characteristics, since any factors influencing the ability of the bacteria to survive outside their host may influence the likelihood of human infection.

The objective of this study is to propose a detailed conceptual scheme of the environmental transmission pathways of *Campylobacter* spp. through a critical appraisal of the literature and expert consultation. Emphasis will be put on plausible environmental pathways from animal excretion

to human ingestion, without considering transmission among animal species or transmission through the food chain. This scheme will be used as a framework for orienting the modeling and interpretation of a statistical analysis of environmental and demographic factors associated with campylobacteriosis risk in Eastern Canada.

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### **C12 Role of rainfall and physicochemical parameters of water on the distribution of *Campylobacter* in the Eastern Townships**

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**Abstract :** *Campylobacter* is part of the intestinal flora of several animals and birds. When ingested by humans, it causes more or less severe diarrhea depending on the conditions. Given the large number of cases in the Eastern Townships, a research project was initiated in 2005 in order to identify possible environmental sources of contamination and spread using a geographic information system (GIS). One component of the project relates to surface runoff as a means of transporting the bacteria. The assumption of this part of the work is that heavy rains contribute to the transport and spread of the bacteria which in turn increases opportunities for human infection. We have therefore put in relation rain data from 19 weather stations in the Eastern Townships with the results of water analysis on 32 sampling sites on the same territory. Parameters like water temperature, conductivity, pH, dissolved oxygen, were also collected. Each site was sampled weekly between July 2005 and October 2007 for a total of 2880 water samples. The aim of the sampling was to have an estimation of the

presence of the dominant species of *Campylobacter* (*jejuni*, *lari*, *coli*) at each site along with fecal coliforms and *E. coli*. Due to the type and nature of the data, most of the statistical analysis performed on *Campylobacter* and the meteorological data were non parametric (for categorical data). Initial results show no immediate correlation between precipitation and daily fluctuations of *Campylobacter* ( $r$  Spearman = 0,264,  $p < 0.01$ ). On the other hand, an analysis based on monthly averages can be considered as an encouraging first relationship ( $r^2 = 0,45$ ). One possibility that remains to be examined is the cumulative effect of precipitation two or three days before the water sampling because every drainage basin does not have the same hydrologic response after a rain event.

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### **C13 Environmental Effects on the Incidence of *Campylobacter* Infection in Philadelphia**

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**Background :** *Campylobacter* infections are a common cause of acute gastroenteritis, and are often complicated by renal, neurological and rheumatologic sequelae. Although excess summertime campylobacteriosis has been observed, its seasonal occurrence has been described relatively recently and is poorly understood. Given concerns related to global climate change, we sought to investigate how environmental factors influence campylobacter occurrence.

**Methods :** The investigation included 1532 cases of *Campylobacter* infection reported in Philadelphia County between 1994-2007. Poisson regression was used to identify associations between weekly weather and river

patterns, and disease incidence. To examine the relationship between daily environmental fluctuations and case occurrence, a case-crossover approach was employed. Both methods controlled for seasonal factors that could confound relationships between weather effects and disease occurrence.

**Results :** Incidence was greatest in June and July, and spectral decomposition revealed a peak at 51.0 weeks, suggesting annual periodicity. Weekly incidence was associated with relative humidity, (IRR per %: 1.03 [95% CI 1.02-1.04]), and the Delaware river pH (IRR per unit: 0.66 [95% CI 0.57-0.96]). Case-crossover analysis suggested that occurrence was intransigent to daily fluctuations in humidity (OR: 1.01 [95% CI 0.96-1.06]) but was significantly affected by daily pH changes (OR: 0.48 [95% CI 0.24-0.97]).

**Conclusion :** We confirmed the summertime predominance of *Campylobacter* in a major urban center. Environmental predictors in Philadelphia are extended periods of humidity, and increased river acidity; these effects may help predict its behaviour and summertime seasonality. In particular, increased humidity may decrease host susceptibility to pathogens, while river basicity may decrease the fitness of the pathogens themselves.

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#### **C14 Characteristics of Recurrent *Campylobacter* and *Salmonella* Infections, Montérégie, 2001 to 2007**

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**Background :** Gastrointestinal infections constitute an important public health issue. Despite underreporting, they represent 20 to 30% of notifiable diseases. Ethelberg *et al.* (2004) in Denmark demonstrated that 4,8% of

*Campylobacter* cases shared an address with one or more other cases. Recurrence can be defined as several infections in the same person or several persons infected in the same household. Few authors have studied the characteristics of recurrent cases. This research shows that, for *Campylobacter* and *Salmonella* infections, differences exist between sporadic and recurrent cases.

**Methods :** In Montérégie, 4186 cases of *Campylobacter* (72,9%) or *Salmonella* (27,1%) infections were reported between 2001 and 2007. A total of 3858 cases (91,7%) corresponding to the same number of households were classified as sporadic and 328 cases (7,8%) corresponding to 157 households as recurrent. For both groups, characteristics of the cases (infectious agent, age, season) and of the households (county of residence, type and delay of recurrence) were described and tested for statistical significance.

**Results :** Descriptive analysis revealed that, like sporadic cases, recurrent cases were mainly due to *Campylobacter* infections and occurred during summer months. The proportion of children under 5 years was higher among recurrent cases (18,9% vs 9,3% among sporadic cases; OR=2,3; IC95%=1,7-3,1). Households with recurrent cases were located more frequently in rural counties (45,3% vs 25,8%; OR=2,4; IC95%=1,7-3,3). When several persons were infected in the same household, the recurrence occurred mostly within 3 months (87% of households). When the same person became infected twice, the recurrence occurred mostly over 3 months (65% of households).

**Conclusions :** Our results show that households with recurrent cases have specific characteristics and that the type of recurrence should be considered in the analysis. These households may be exposed to specific risk factors. A detailed study should be conducted to verify this hypothesis.

## **C18 WHO Salm-Surv: Global Interdisciplinary Collaboration in Lab-based Foodborne Disease Surveillance and Outbreak Detection and Response**

Jaap Wagenaar, Danilo Lo Fo Wong, Fred Angulo, Pat McDermott, Lai-King Ng, Henrik Wegener, Martyn Kirk, Marc Jouan, Carmen Varela, and WHO Global Salm-Surv

**Background :** Recognizing the global public health importance of foodborne diseases and the need to enhance capacity for laboratory-based surveillance, WHO Global Salm-Surv (WHO-GSS) was launched in 2000. The program is a collaborative effort among the World Health Organization, government, academic, and private institutions focusing on foodborne and other enteric infectious diseases.

**Methods :** The primary activity is conducting international training courses. Course participants are public health professionals working in food safety related areas of microbiology and epidemiology. Courses include theoretical and bench top training in laboratory techniques for *Salmonella*, *Campylobacter*, Shiga toxin-producing *E. coli*, *Vibrio cholerae*, *Brucella* or other organisms of regional concern. Epidemiologic training includes outbreak detection and investigation, determination of burden of illness, and improvement of surveillance systems. Epidemiologists and microbiologists collaborate on a country plan of action for enhancing foodborne disease surveillance and outbreak response. Courses were conducted in English, Spanish, Portuguese, Chinese, Russian, or Arabic. Other program activities are: an Electronic Discussion Group (EDG), an External Quality Assurance System (EQAS) for typing (*Salmonella*), species identification (*Campylobacter*) and determination of antimicrobial resistance, a Country Databank with the fifteen most frequently isolated *Salmonella* serotypes, free reference testing

services, and focused regional and national projects.

**Results :** A total of 51 courses were held in 14 WHO-GSS Training Sites from 2000 to 2008 with over 900 participants from over 100 countries. As a result of the training courses, two *Salmonella* regional projects in Asia and Africa have been developed, two national *Salmonella* surveillance projects in Fiji and the Philippines have been launched, and burden of illness studies in Jordan, Slovenia, and the Caribbean are completed. In most of the participating countries, the collaboration between epidemiologists and microbiologists has improved considerably.

**Conclusion :** WHO-GSS successfully trained participants around the world in foodborne laboratory-based surveillance and outbreak response. Through GSS, interaction between epidemiologists and microbiologists from public health, veterinary and food safety sectors is fostered. The program has markedly enhanced the global efforts to improve foodborne disease surveillance and develop response networks. Regional and national projects continue to develop from these efforts.

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## **SESSION D: GENOMICS AND MOLECULAR APPROACHES**

### **D04 Quantitative Real Time PCR for *Campylobacter jejuni*, *C. coli* and *C. lari* in Environmental and Untreated Drinking Water**

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**Background :** Untreated drinking water is an important risk factor for campylobacteriosis. The most commonly used method to quantify *Campylobacter* in water is the Most Probable Number method (MPN). This method is laborious, time-consuming and not really precise. This project aimed to develop a quantitative real time PCR (qRT-PCR) for *Campylobacter jejuni*, *C. coli* and *C. lari* in environmental and untreated drinking water. It is difficult to obtain a clean enough sample for PCR directly from environmental water samples by centrifugation or filtration, so we opted to grow *Campylobacter* for a limited time and then quantify this culture amplified sample.

**Methods :** The qRT-PCR was a FRET system (Lübeck and al. 2003, Perelle and al. 2004). To determine the *Campylobacter* growth curve, 100 ml of environmental water was spiked with bacteria, filtered and incubated in Preston broth. Aliquots were removed at different times. With the optimized method, 15 environmental water sites situated in the Eastern Townships, Québec were sampled weekly from 10-21-2007 to 11-25-2007. Water volumes of 1 x 2000, 1 x 500 and 3 x 10 ml for each site were analyzed according to the MPN method. Only the 2000 ml was analyzed by qRT-PCR, after 3 hr

incubation at 37°C and 13 hr at 42°C. DNA was extracted from an aliquot of 1.5 ml.

**Results :** *Campylobacter* growth increased linearly between 5 and 20 hrs when water was spiked with 100 or 1000 bacteria. The qRT-PCR was reproducible, with an efficiency of 95% and a slope of -3.46 and detected as little as 3 to 10 bacteria. A total of 88 site samples were analyzed by the MPN method and by qRT-PCR. Results of qRT-PCR correlated with the MPN method (rho coefficient = 0.8; p<000.1).

**Conclusion :** This quantitative qRT-PCR is promising, especially for prevention because it is sensitive and rapid.

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### **D06 Identification of clinically relevant *Campylobacter jejuni* genes by population-based screening using comparative-genomic hybridization**

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**Background :** In previous work, we developed a comparative genomics-based genotyping assay (Comparative Genomic Fingerprinting or CGF) and used it to analyze a large collection of animal, environmental, and human clinical *C. jejuni* isolates obtained from southern Alberta (2004-2006). This allowed us to identify a small number of CGF clusters (n=10) representing 40% of all clinical isolates in the dataset. We have subjected isolates from these clinically-associated CGF clusters to further genomic characterization in order to identify genetic determinants of possible clinical relevance.

**Methods :** We have designed two focused pan-genomic mini-microarrays targeting 238 accessory genes from the multiple publicly-available *C. jejuni* genomes using the ArrayTube (AT) platform (Clondiag GmbH). The presence/absence rate for each gene was determined by using the AT microarrays in comparative genomic hybridization (CGH) experiments against a panel of strains representing clinical CGF clusters and non-clinical controls. Statistical analysis of the CGH data using Westfall-Young corrected Fisher's Exact Test was used to identify genes strongly associated with clinical CGF isolates.

**Results :** Although most genes tested by population-based screening showed no significant association towards clinical isolates, a small number of genes showed significant differences in conservation rates among clinical isolates with respect to non-clinical controls. Both positive and with negative associations towards clinical isolates were uncovered.

**Conclusions :** Application of population-based comparative genomic screens represents a powerful approach for uncovering genes whose presence/absence may be of clinical relevance.

These represent strong leads as possible genetic determinants of virulence and whose function should be evaluated. Because these genes are strongly associated with clinical isolates, they can also provide a basis for next-generation diagnostic assays capable of discriminating between pathogenic and non-pathogenic strains.

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## **D07 X-ray Crystallographic Analysis of the Sugar N-Acetyltransferases PglD and PseH, Enzymes Involved in the Biosynthesis of 2,4-diacetamido-Bacillosamine and 5,7-diacetamido-Pseudaminic acid**

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**Abstract :** The biosynthesis of oligosaccharide-repeat units often involves sugar-nucleotide precursors or building blocks, with nucleotides such as UDP, GDP or TDP linked to the sugar moiety. Several bacterial cell-surface polysaccharides, including those associated with lipopolysaccharide, capsule, pilin and enterobacterial common antigen contain sugar moieties that have been *N*-acetylated at a sugar-nucleotide precursor stage. In addition, 5,7-diacetamido-pseudaminic acid (Pse), which decorates the flagella of *Helicobacter pylori* and *Campylobacter jejuni*, as well as 2,4-diacetamido-bacillosamine, the linkage sugar of the *C. jejuni* *N*-linked glycan (Pgl), are *N*-acetylated during their biosynthesis. In order to better understand the relationship between structure and molecular function of *N*-acetyltransferases that transfer an acetyl group from acetyl-CoA to the amino-sugar moiety of UDP-sugar intermediates, we determined the crystal structures of *C. jejuni* PglD and *H. pylori* PseH. PseH adopts the core GNAT *N*-acetyltransferase fold that has been identified in a variety of small-molecule acetyltransferases. Crystallographic trapping of PseH with various combinations of substrate and product

molecules reveals that the active site is located in a similar location as compared with other GNAT acetyltransferase structures, and shares an active site Tyr that can potentially act as an acid to protonate the CoA-leaving group. The structure of PglD reveals a completely different structure, a trimeric, left-handed  $\beta$ -helix. Both molecular modeling of the PglD tetrahedral intermediate and site-directed mutagenesis are consistent with His125 as the most likely residue to function as a general base in the catalytic mechanism. Supported by the NRC-GHI and CIHR (GSP-48370).

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### **D09 Comparison of *Campylobacter* flaSVR genotype isolated from humans and poultry in three European regions**

T.M. Wassenaar

**Abstract :** The genetic diversity of *Campylobacter* in human infection and in poultry was assessed in three different European regions by sequencing of the short variable region (SVR) of *flaA*. Randomly chosen isolates originated from Norway, Iceland, and Basque country in Spain. A total of 300 strains was investigated, 100 per country of which 50 originated from either host.

The results indicate extensive diversity in both hosts, and identified differences in the nature and distribution of genotypes between the countries. These differences could not be related to climate, so that *Campylobacter* from Iceland and Norway were not more similar to each other than either was to Basque country. Differences between the countries exceeded the observed differences between human and poultry isolates within a country. Thus, regional differences are extensive and should not be ignored when comparing genotyping data originating from different international studies. This study was performed with support from CampEC, a SafeFood Era network.

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## Potential Conflict of Interests Disclosure

**These resource persons did not declare any potential conflict of interests:**

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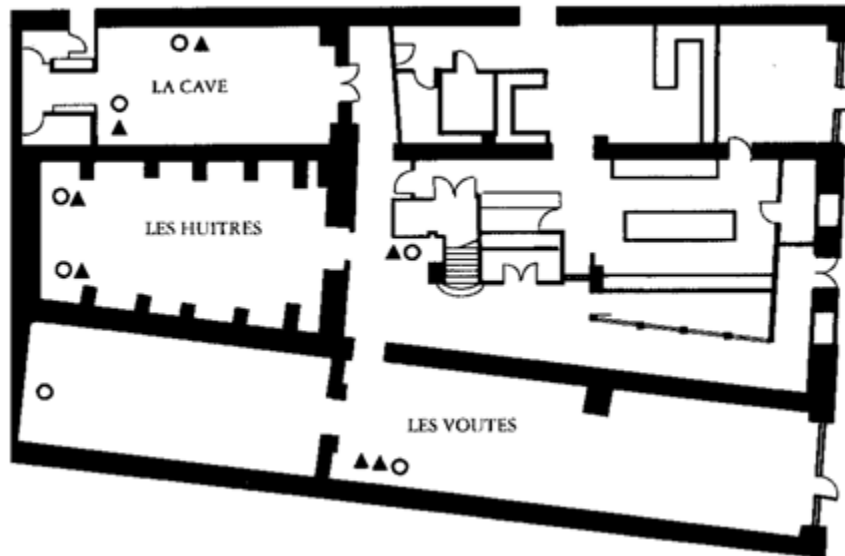
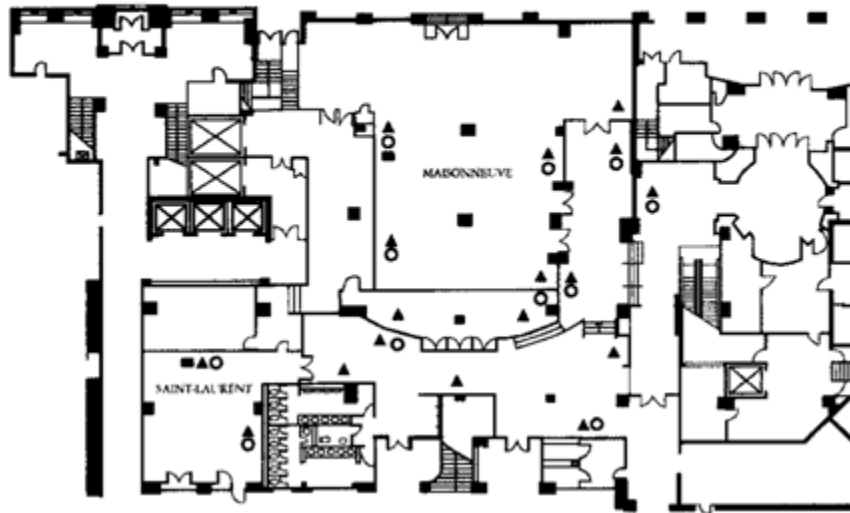
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