Reducing *Campylobacter*: an integrated approach

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Canadian Meat Council's Technical Symposium on Pathogen Reduction

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Plan

- **CRSV**: meet us!
- *Campylobacter* generalities
- *Campylobacter* at the farm
  - Risk Factors
  - Control
- *Campylobacter* and food processing
  - Where?
  - Process effect
  - Reducing further

Haddock 2010 Microbiology (2010), 156, 3079–3084
CRSV: who is this?

CRSV: Chaire de Recherche industrielle-NSERC en Salubrité des Viandes
Université de Montréal, Faculté de Médecine Vétérinaire, Saint-Hyacinthe

Mission

To do fundamental and applied research in meat safety as an answer to the problematics encountered by the meat industry in order to increase the safety of meat products offered to the consumer.

NSERC funded this year!
2.2 Millions + 5 private partners

Ongoing projects:
Diagnostic activities
Campylobacter research
Salmonella chicken and swine vaccine research
Design of control measure of foodborne pathogens
Use of ozonated water for control of bacteria
And MANY others!

http://www.medvet.umontreal.ca/crsv/
A great place for chicken studies!

Centre de recherche avicole (CRA)

Facility allowing research on foodborne and poultry pathogen or general poultry health in a level 2 biosecurity confinement

From hatching to processing!
Take Home Messages

“It’s ok to be different”, Dr E Gaynor, UBC, Canada
What works for Campy might not for every other bacteria

Different campy strains, different biologic properties, different stress reactions
Different flocks, Different strains...

Reducing campy: No silver bullet

Farm level
Risk practices
Biosecurity
Control measures still to be applied commercially, moderate effect

Processing level
Different facilities, different contexts
“Natural processing” reduce campy
Carcass decontamination: not 100% effective
New technologies underway

Lowering the initial Campylobacter charge at the farm + food processing control measures = winning situation!

A little history

•First seen in 1866 by Escherich

•Campylobacter fetus firstly identify in 1906 by McFadyean and Stockman from a sheep uterus

•First suspected human case: 1938 ➔ milk outbreak

•1963: genus Campylobacter proposed (was considered a vibrio)

•1972: first isolation from human feces by Dekyser et Butzler

•2000: first Campylobacter jejuni sequenced genome published
Generalities

• First seen in 1866 by Escherich

• *Campylobacter fetus* firstly identified in 1906 by McFadyean and Stockman from a sheep uterus

• First suspected human case: 1938 ➔ milk outbreak

• 1963: genus *Campylobacter* proposed (was considered a *vibrio*)

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Campylobacter active research still relatively young!!

Meet *Campylobacter*

Gram negative bacteria, bird like

*Salmonella* cousin who is not on Youtube or Facebook

Mobile and chemotactic ➔ flagella

Microaerobic ➔ atmosphere poor on O₂ concentration

Feeds on organic acids and amino acids

Optimal growing temperature: 37°C-42°C, min 30°C

No replication out of their animal host! So far...

Viable but non culturable state (VBNC) still infectious?

http://www.microbe-edu.org/etudiant/campylo.html
Meet *Campylobacter*

- 18 known species
  - Main human pathogen is *C. jejuni* (*Campylobacteriosis*)
- *C. jejuni* found in gastrointestinal track of animals
- Many virulence factors (Toxins, chemotaxis, bacterial membrane, adhesins)
  - No smoking gun identified
- High genetic variability ➔ many different *C. jejuni*, love to exchange
- LOW infectious dose in human (less than 500 CFU)
  (Teunis 2005, Epidemiology and Infection)

A complex ecology

Arsenault, 2010
C. jejuni main sources

Handling (consumers, workers)

Direct contact

Campylobacter and human health

• Bacteria responsible for the most foodborne infection in industrialized countries

• One of the most important pathogens associated with diarrhea in the world
  • 5-14% of all

• Causes campylobacteriosis (1 to 10 days)
  • Diarrhea
  • Fever
  • Abdominal pain

• Can cause severe disease
  • Guillain-Barré syndrome
    • Neuromuscular paralysis
  • 30% of the cases could be associated with C. jejuni infection
  • 1 out of 1000 campylobactersiosis leads to GBS

**C. jejuni and poultry**

Québec positive flocks *(chicken)* (Our laboratory: Arsenault 2007 and Thibodeau)
- 2003: 35%
- 2008: 19%

Québec positive flocks *(turkey)* (Our laboratory: Arsenault 2007)
- 2003: 46%

Retail *chicken* meat Ontario
- 60% positive for *C. jejuni* of 1256 fresh retail sample chicken skin (Deckert, 2010)

Retail *turkey* meat Ontario in 2004 (Cook, 2009)
- 46% of 412 fresh sample

CIPARS recovery rate in 2009 *chicken* retail meat samples
- 53% BC
- 32% SA
- 31% ON
- 20% QC
- 30% Atlantic provinces

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*C. jejuni* and poultry

*Campylobacter* is well present in Canada poultry, but the extend of its presence is unknown?
**C. jejuni and poultry**

Asymptomatic colonization  
Might be associated with vibrionic hepatitis (Jennings, 2011)  
Found in liver, spleen of animals at slaughter (Thibodeau 2011)

Horizontal contamination (rarely comes from the hatchery)

On farm colonization possible by 2-3 weeks of growth (maternal antibody and microbiota effect)  
rapid spread to most birds (coprophagy)

On-farm factors affects *C. jejuni* prevalence

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**Short Report**

Campylobacter and gastrointestinal infections

_A recipe for disaster: outbreaks of campylobacteriosis associated with poultry liver pâté in England and Wales_

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**SUMMARY**

Despite the frequency of Campylobacter as the principal cause of bacterial gastroenteritis in the UK, outbreaks attributed to this pathogen are rare. One hundred and fourteen general foodborne outbreaks of campylobacteriosis were reported to the Health Protection Agency from 1992 to 2006 with most occurring in food service establishments (644, 70/114). Poultry meat (381, 43/114) was the most commonly reported vehicle of infection, of which poultry liver pâté and undercooking were strongly associated with this pathogen. Notably, the number of outbreaks of _Campylobacter_ associated to consumption of poultry liver pâté in England and Wales increased significantly from 2007 (24% as opposed to 12%, p < 0.0001). With a predominance of these occurring in December. These outbreaks highlight the hazards associated with inappropriate culinary practices leading to undercooking of poultry liver pâté and suggest that improving catering practice is an important last line of defence in reducing exposure to _Campylobacter_-contaminated products.

(Accepted July 28 2010)  
(Online publication August 23 2010)
Campy and eggs?

_Campylobacter_ can be isolated from ovarian follicles (15%) and reproductive tract (42% lower, 48% upper) (Cox, 2009)

Egg shells (Messelhäusser, 2011)
4% of 2710 egg shell were positive, no yolk positive

Liquid eggs in Japan, unpasteurized (Satp, 2010)
28% of whole eggs, 36% of liquid yolk
Pasteurized eggs = absence of _Campylobacter_

C. jejuni and poultry

Farm Factors associated with _C. jejuni_ presence in birds

1. Biosecurity
2. Age at slaughter (the older, the more likely to be positive)
3. Thinning
4. Vectors
   1. Rodent, flies, humans, amoebas
5. Presence of other farm animals (reservoir)
6. Season
7. Transport crates
8. Private untreated water source

References
Wassenaar 2011, Letters in Applied Microbiology
Snelling 2008, Archives of Microbiology
Näther 2009, Poultry Science
Guerrin 2007, Preventive Veterinary Medicine
Recent risk analysis in Québec: Arsenault 2007, Journal of Food Protection

- Broiler caecum: rodent control program present, close manure heap, number of birds raised on the farm throughout the year

- Broiler carcass: caecum positive, slaughter end of week, digestive content

- Turkey caecum: close manure heap, untreated water

- Turkey carcass: caecum positive, bird transit >2h, visible contamination, no antibiotics feed

No industrially developed specific control measures

Target for effect on human health: minimum of 2 log reduction on caecal carriage

- Vaccination
- Feed /water additive
- Probiotic
- Bacteriocin
- Phage therapy

http://radiofreethinker.com
C. jejuni and poultry

**Vaccination**

- Not very successful but active development
- Injection or intranasal or in avirulent *Salmonella*
- 2-3 log of reduction in the best cases
- Vaccination trials tested against a low variety of strains
  - Campy highly variable

Hope:
- *Salmonella* vector achieved complete protection (Layton 2011) vs 3 field C. jejuni strain and vector not recovered

References
- de Zoete, 2007, Vaccine
- Wyszyinska, 2004, Vaccine
- Layton 2011, Clinical and Vaccine Immunology
- Wassenaar 2011, Letters in Applied Microbiology

C. jejuni and poultry

**Feed/water additive**

- Variable effects, often low number of tested strains
  - Fatty acid → pH sensitive
    - Caprylic acid
      - Best result: in feed encapsulation (-4 log) but conflicting results
  - Formic acid and sorbate
    - Elimination of campy
    - Birds performance down
  - Encapsulated organic acids
    - Reduction of 1.5 log after 7 days of colonization on some C. jejuni strains
  - Lactic acid in water (0.44%) (Food withdrawal prior to slaughter)
    - Lower bird positive incidence

References
- Hermans 2010, Poultry Science
- Metcalf 2011, Poultry Science
- Wassenaar 2011, Letters in Applied Microbiology
- Skanseng 2010, Journal of Applied microbiology
**C. jejuni and poultry**

**Probiotic**

- Primalac
  - Lower incidence (66% control vs 33% treated) at 21 day of age
  - *Lactobacillus salivarius* = no effect

- Exclusion microflora
  - Goal: replace a permissive microbiota with a resistant one
  - Colonization inhibition possible, but the right microflora must be administered to the right bird race

**References**
Chaveerach 2004, Poultry Science
Willis 2008, Poultry Science
Laisney 2004 British Poultry Science
Wine 2009, FEMS Microbiology Letters

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**C. jejuni and poultry**

**Bacteriocin** (Small antibacterial molecules secreted by bacteria)
- Great effect noticed!
- Campy elimination possible with encapsulation
- Bird performances?
- Developments coming soon

**Phage** (Bacteria virus that causes lysis)
- Highly effective to rapidly reduce colonization (-3log)
- Do not eliminate campy
- Resistant strains appear rapidly and take over
  - CRISPER (Immune system like)
- Could be use just prior to harvest

**References**
Hoang 2011, Applied and Environmental Microbiology
Svetoch 2010, Poultry Science
Carvalho 2010, BMC Microbiology
Siringan 2011, Applied and Environmental Microbiology
Where is *Campylobacter* in the food processing chain?

Can be found at all steps!
Campy and food processing

- Scalding normally lower counts
- Heat kills campy

Defeathering increases counts
Fecal matter pushed out of the cloaca
Campy and food processing

- Evisceration:
  Sometime increase, decrease or nothing change!

- Washing, cooling and packaging
  - Each lowers counts
**Campy and food processing**

Different facilities, different context, different results!

![Graph showing Campylobacter levels at different stages of meat processing](image)

**Campy and food processing**

*Campylobacter*, throughout the processing, will meet conditions aimed at reducing overall levels of bacteria on meat.

How *Campylobacter* reacts to these conditions?
Heat

Pasteurization kill *Campylobacter*

The longer the time, the higher the temperature, the more campy is killed by heat \((1,2,3)\)

Meat: 6 min at 50°C or 1 min at 60°C kill all *Campylobacter*

Low pH increase sensitivity of Campy to heat \((3)\)

Normal good cooking practices (*no cross contamination* + sufficient cooking temperature) for chicken = safest way to protect against campylobacteriosis

(1) Nguyen 2006, International Journal of Food Microbiology
(2) Alter 2006, Journal of Veterinary Medecine
(3) Sagarzazu 2010, Innovative Food Science and Emerging Technologies

Cold

In meat products, naturally or artificially contaminated

Campy can survive freezing (-20°C), but this process considerably reduce viable counts \((1,10)\)

The use of rapid cooling (at~20 °C/min) enhanced the survival of *Campylobacter* chilled to 4 °C compared to standard refrigeration.

*Campy* can survive well at 4°C for a very long time period (up to 7 months!), with little viable counts reduction, -1 log of bacteria and it survives better than at room temperature \((1,4,5,7,9)\)

(1) Sampers 2010, International journal of Food Microbiology
(2) Sanchez 2002, Journal of Food Protection
(3) Berrang 2008, Poultry Science
(4) Boucher, 1994, Journal of Applied Bacteriology
(5) Larazo, 1999, Applied Environmental Microbiology
(6) Moen 2005, Applied Environmental Microbiology
(7) Bhaduri 2004, Applied Environmental Microbiology
(8) Kusumaningrum, 2003, Journal of Food Protection
(9) OYARZABAL 2010, Journal of food protection
(10) El-Shibiny 2009, International Journal of Food Microbiology
**Campy and food processing**

**Cold**

Campy is sensitive to desiccation (8)

Air chilling might reduce Campy viable counts better then water chilling (2)

sometimes less then 1 log reduction (3)

sometimes less efficiently then water (11)

Some genes are up regulated when *C. jejuni* is in a cold environment (6)

*C. jejuni* retain metabolic activity at 4°C (6)

So *Campylobacter* is not much affected by cold

**But sensitive to freezing**

(1) Sampers 2010, International journal of Food Microbiology
(2) Sanchez 2002, Journal of Food Protection
(3) Berrang 2008, Poultry Science
(4) Boucher, 1994, Journal of Applied Bacteriology
(5) Larazo, 1999, Applied Environmental Microbiology
(6) Moen 2005, Applied Environmental Microbiology
(7) Bhaduri 2004, Applied Environmental Microbiology
(8) Kusumaningrum 2003, Journal of Food Protection
(9) OYARZABAL 2010, Journal of food protection
(10) El-Shibiny 2009, International Journal of Food Microbiology
(11) Berrang 2008, Poultry Science

**Campy and food processing**

**Acid and salt**

**In vitro**

Optimal growth pH: 6.5 - 7.5, survives in pH 4 to 9 (1)

• Growth prevented by acetic, scorbic, formic and lactic acid

Long term survival is best at neutral pH (2)

More resistant to acidic pH then alkaline pH (2)

Lower pH render *C. jejuni* more sensitive to salt, (1,5% NaCl max, growth) (2)

(1) Chaveerach 2003, Applied Environmental Microbiology
(2) Kelana 2003, Journal of Food Protection
**Campy and food processing**

Growth in 0.5 -1.5 % NaCl (1)

At 4.5% NaCl, survives best at 4°C (1,2,3)

Survival in Marinade! (4°C, pH 4.5, NaCl 5.9%) (5)

- Without meat:
  - Decrease of 2.4 log after 24H
  - No campy recovered after 48H

- With meat:
  - Survival up to 9 days!

Sodium polyphosphate increase *C. jejuni* survival In chicken exudates (6)

Fresh fruit extract (ex: whole lime) eliminates *C. jejuni* inoculated on chicken meat after 48hrs (7)

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**Packaging**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 2</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice pack, whole (3°C)</td>
<td>2.63 ± 0.51 A</td>
<td>1.49 ± 0.30 BCD (8/10)</td>
</tr>
<tr>
<td>Chill pack, whole (–1°C)</td>
<td>1.31 ± 1.05 BCD (7/10)</td>
<td>1.60 ± 1.13 ABCD (7/10)</td>
</tr>
<tr>
<td>Chill pack, parts (–1°C)</td>
<td>2.16 ± 0.62 AB (10/10)</td>
<td>1.63 ± 1.15 ABCD (7/10)</td>
</tr>
<tr>
<td>Vacuum pack (5°C)</td>
<td>1.96 ± 1.00 ABC (10/10)</td>
<td>1.22 ± 1.00 BCD (6/10)</td>
</tr>
<tr>
<td>Modified atmosphere, O2 (3°C)</td>
<td>1.65 ± 1.12 ABCD (8/10)</td>
<td>0.80 ± 0.90 BCD (6/10)</td>
</tr>
<tr>
<td>Modified atmosphere, mix (3°C)</td>
<td>1.99 ± 0.88 ABC (9/10)</td>
<td>1.83 ± 1.40 ABC (7/10)</td>
</tr>
<tr>
<td>Frozen</td>
<td>1.74 ± 0.80 ABC (9/10)</td>
<td>1.09 ± 0.52 BC (4/10)</td>
</tr>
</tbody>
</table>

Great! O2 rich atmosphere is detrimental to *C. jejuni* as opposed to O2 poor atmosphere which is not

Byrd 2011 Poultry Science
**Campy and food processing**

Forms biofilm to increase resistance (1)

Campy in biofilm can be killed by (3)
- Chlorine, 50ppm, 45s
- May survive: quaternary ammonia, peracetic acid (PAA) and a PAA/peroctanoic acid mixture at 50ppm or 200ppm, 45s treatment

Campy can be recovered after disinfection during cleaning (2)
- May recontaminate subsequent lot (4)

(1) Alter 2006, Journal of Veterinary Microbiology
(2) Peyrat 2008, International Journal of Food Microbiology
(3) Trachoo 2002, Journal of Food Protection
(4) Kudirkienė 2010, International Journal of Food Microbiology

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**Campy and food processing**

Starved Campy have better heat resistance and remain virulent (1,3)

Most stress cause VBNC state in *C. jejuni*... (2)

VBNC state impossible to assess by standard culture methods.

VBNC resuscitation = controversy (4)

VBNC still infectious! (5)

There may be more campy than it seems that survive harsh conditions!

(1) Klancnik 2009, Research in Microbiology
(2) Jackson 2009, Antonie van Leeuwenhoek
(3) Gaynor 2005, Molecular Microbiology
(4) Murphy 2006, Journal of Applied Microbiology
(5) Chaisowwong 2011, Journal of Veterinary Medical Science
**Campy and food processing**

Different campylobacter types have different ability to survive stresses (1,2,6) and to survive food processing (7)

- Acid stress increase stress resistance (3)
- Cold increase stress resistance (4)
- Stress increase virulence (5,8)

(1) Habid 2010, Food Microbiology  
(3) Ma 2009, Journal of Food Safety  
(5) Gaynor 2005, Molecular Microbiology  
(6) Reezal, 1998, Applied Environmental Microbiology  
(7) Alter 2006, Journal of Veterinary Medicine  
(8) Haddad 2010, Current Microbiology

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**Campy food processing control**

Golden rules for a successful disinfection or decontamination

1. Have the right molecule or method
   1. Adapted to the microorganism
2. Absence of organic matter
3. Appropriate contact time
4. Drying
5. No undesirable by-products, by-effects

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www.thymox.com
Campy and food processing

In media free of organic matter, campy sensitive to many disinfecting agents: chlorine, acidified sodium chlorite, and peracetic acid (1) when used on chicken skin contaminated by C. jejuni.

- 100 ppm, 15 minutes \(-1 \text{ log}\)
- 40 ppm, 2 minutes \(\text{no difference}\)

Chlorine carcass rinse (50 ppm, at 21°C, 43°C, or 54°C) had no effect on campy (2).

But in most studies, a modest effect (3,4,5) the longer the contact time, the better the decontamination.


Campy and food processing

Hydrostatic pressure (1)
- Heat sensitive C. jejuni
- Cold exposure increased resistance
  - 2–3 log units reduction at 300–325 MPA and completely killed at 400 MPA (2, 3)

Pulse light
- 3 Hz, maximum of 505 J=pulse, and a pulse duration of 360 ms (4)
- Reduction of 3.5 log on inoculated package for 30 sec
- Reduction of 1.22 log on skin and 0.96 on breast for 5 sec through plastic

30 seconds on meat affects color through plastic (but two studies state the opposite)

[References: (1) Sagarzazu, 2010, Journal of Applied Microbiology; (2) Solomon 2004; (3) Martinez-Rodriguez 2005; (4) Haughton 2011, Foodborne Pathogens and Disease]
Take Home Messages

“It’s ok to be different”, Dr E Gaynor, UBC, Canada
What works for Campy might not for every other bacteria

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Thank you!