Campylobacter: Developments in Detection and Control

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Campylobacter Facts

- The following four thermophilic species are clinically important because they are the dominant cause of human campylobacteriosis
  - *Campylobacter jejuni*
  - *Campylobacter coli*
  - *Campylobacter lari*
  - *Campylobacter upsaliensis*
- *Campylobacter jejuni* is the primary cause of bacterial gastroenteritis in the United States and many other countries followed by *Campylobacter coli*
- In the United States, *Campylobacter* and *Salmonella* alternate as the leading bacteria associated with foodborne illness
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Incidence / 100,000 population - 2008</th>
<th>Incidence / 100,000 population - 2007</th>
<th>% Change from 2007</th>
<th>% Change from 1996 - 1998</th>
<th>Healthy People 2010 Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>16.20</td>
<td>14.92</td>
<td>+ 8.58% (NS)</td>
<td>0%</td>
<td>6.80</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>12.68</td>
<td>12.79</td>
<td>- 0.86% (NS)</td>
<td>- 32%</td>
<td>12.30</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>6.59</td>
<td>6.26</td>
<td>+ 5.27% (NS)</td>
<td>- 40%</td>
<td>NA</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>2.25</td>
<td>2.67</td>
<td>+15.73% (NS)</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td><em>STEC O157</em></td>
<td>1.12</td>
<td>1.20</td>
<td>+ 6.67% (NS)</td>
<td>- 25%</td>
<td>1.00</td>
</tr>
<tr>
<td><em>STEC non-O157</em></td>
<td>0.45</td>
<td>0.57</td>
<td>- 0.21% (NS)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><em>Yersinia</em></td>
<td>0.36</td>
<td>0.36</td>
<td>0%</td>
<td>- 48%</td>
<td>NA</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>0.29</td>
<td>0.27</td>
<td>+ 0.07% (NS)</td>
<td>- 36%</td>
<td>0.24</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>0.29</td>
<td>0.24</td>
<td>+ 20.83% (NS)</td>
<td>+ 47%</td>
<td>NA</td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
<td>0.04</td>
<td>0.03</td>
<td>+ 33.33% (NS)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Campylobacter Facts

- The infectious dose of *Campylobacter* in humans can be as low as a few hundred cells
- Cross-contamination of food products is a major contributor to human illness
- Outbreaks of human campylobacteriosis have been associated with raw milk, untreated water, and raw poultry meat
- Poultry carcasses are frequently contaminated with the pathogen and be responsible of sporadic cases
- Contamination is thought to originate from the intestinal tract of the birds and spreads to the rest of the carcass during transport and processing
Campylobacter Facts

- The crops of broiler chickens, particularly after feed withdrawal before transport to the processing facility, can harbor large numbers of *Campylobacter*
  - Levels in the intestinal tract of broilers entering the processing plant can be $10^7$ CFU/g of cecal contents
  - When whole carcasses with feathers are rinsed, $10^6$ CFU/g of rinses can be recovered
- *Campylobacter* spp. are fastidious organisms whose culture requires a specific growth temperature, gaseous environment, and nutrient-rich medium
Campylobacteriosis Clinical Features

- Typically, a patient may present with symptoms 2 – 4 days after ingestion of contaminated food or drink
- Low-grade fever and diarrhea may accompany abdominal cramping and pain
- Bowel discharge can vary from loose stools to grossly bloody diarrhea with or without vomiting
- Less than 1% of patients manifest extraintestinal symptoms in infections caused by *C. jejuni* or *C. coli* but can be more common in infections caused by *C. fetus*
- Most *Campylobacter* infections are self-limiting, and adequate supportive treatment is usually sufficient for full recovery
Campylobacteriosis Clinical Features

- Antimicrobial therapy is needed in severe cases characterized by high fever, severe or bloody diarrhea, an prolonged duration of clinical symptoms
- Several forms of sequelae of campylobacteriosis have been reported
  - Arthritis
  - Reiter’s Syndrome (Reactive Arthritis)
  - Guillain – Barré Syndrome
- Relatively, little is known about the molecular pathogenesis of arthritis and Reiter’s syndrome following *Campylobacter* infections
Campylobacteriosis Clinical Features

• Although Guillain – Barré syndrome appears to have multiple etiologies, up to 40% of cases are associated with antecedent campylobacteriosis
  – In the United States, the costs of *Campylobacter*-associated Guillain – Barré syndrome has been estimated to be as high as $1.8 billion per year
• Guillain – Barré syndrome is an acute, autoimmune polyradiculoneuropathy involving the peripheral nervous systems and presents as motor paralysis with or without sensory abnormalities
  – Weakness of limbs and respiratory muscles is common
Campylobacter Detection and Enumeration

“The number of formulations proposed for the isolation of thermophilic campylobacters probably exceeds that for any other group of bacteria…”

Corry et al., 1995, Int’l J. Food Microb.
Campylobacter: Detection vs. Enumeration

• Yes or no answer is OK for ready-to-eat products

• Yes or no is not ideal for raw product (because the answer all too often is “yes”)

• Total eradication from raw meat product is not yet possible (except for irradiation)

• Enumeration is necessary to evaluate progress
Important to remember:

Any enumeration method provides only an estimation of the number of bacteria present...
To date there is no universally accepted “standard” method of isolating *Campylobacter* from food or environmental samples.
Why cultural methodology?

**Advantages**

- Selective culture is cheap, practical and as good as PCR for identification of common species (*C. jejuni*, *C. coli*).

- Reliability, novelty, cost, and scale-up practicality may limit use of DNA or antibody based assays.

- Isolate is available for further testing or typing.

**Disadvantages**

- May miss less common species or injured cells.

- Results take 48h.

- Identification is only to genus level.
Ideal cultural method for *Campylobacter* would be:

- Fast
- Reproducible
- Accurate
- Sensitive
- Inexpensive
- Quantitative
- Sensible
Campylobacter methods

• Qualitative
  – Enrichment + selective plating

• Quantitative
  – Direct plating on selective media
Isolation of *Campylobacter*

- Sampling and handling important

- Enrichment
  - NECESSARY – low infectious dose, low numbers of potentially injured cells in food (microflora)
  - nutritionally rich media
  - microaerophilic environment
  - antimicrobial ‘cocktail’ needed to suppress competitors
Enrichment Media - Cannot Enumerate

• Older and stressed cells gradually become coccoidal and increasingly difficult to culture

• Enrichment may be necessary for environmental or processed samples that may contain stressed cells

• Delayed addition of antibiotics beneficial for recovery of injured cells

• Bolton’s Broth

• 3M™ Tecra™ Broth
Several studies suggest that direct plating is superior to enrichment for some sample types and enumeration is also achieved.

Beuchat, 1987, chicken meat
Monfort, et al., 1988, feces
Musgrove, et al., 2001, ceca

Large numbers of non-Campy species may out-compete Campy during enrichment.
Selective Plating Media
Choice of media will bias selection of Campy

- Rich basal medium such as *Brucella* agar, blood agar base
- Control toxic effects of O₂ by adding blood, charcoal or chemicals
- Antibiotics to inhibit competing microflora
- Incubation temperature 37 – 42°C
- Campy Cefex
- mCCDA
- Skirrow
- Campy-Line
- Müller-Hinton
- Many others
## Typical Antibiotics Found in Campy Selective Media

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Effective against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin</td>
<td>G(-) except <em>Proteus</em></td>
</tr>
<tr>
<td>Trimethoprim/colistin</td>
<td><em>Proteus</em> spp.</td>
</tr>
<tr>
<td>Vancomycin/rifampicin</td>
<td>G(+)/G(+ and -)</td>
</tr>
<tr>
<td>Cephalothin/cefaperazone</td>
<td>G(+)</td>
</tr>
<tr>
<td>Cycloheximide/Amphotericin B/Nystatin</td>
<td>Yeasts and Molds</td>
</tr>
</tbody>
</table>
Microaerophilic atmosphere is necessary for growth of most species of *Campylobacter* including *jejuni* (10% CO2, 5% O2, 85% N2)

Atmosphere generation methods include:

- Tri-gas incubators
- Flush/fill bags using gas tank
- Gas generating envelopes (bags, jars)
- Oxyrase
- Steel wool (copper sulfate) + sodium bicarbonate
- Candle jar
Campylobacter Detection and Enumeration

Campylobacter do not ferment carbohydrates; therefore, typical pH indicators cannot be used to demonstrate acid or alkali production resulting from utilization of particular substrates.

Triphenyltetrazolium chloride (TTC) added to give contrasting color to colonies.
(CLA, Line 2001, JFP 64:1711-1715)
Correlation Between Plating Agars

Mean Campylobacter cfu/ml

Sample Number

Cefex agar  Campy-Line agar

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Correlation Between Direct Plating Agars and MPN Methods

Sample Number

Mean Log Campylobacter/ml

FSIS-MPN

ARS-Direct Plating
NARMS *Campylobacter* Isolation Method

Carcass rinse

10 ml

Concentrate by centrifugation

Resuspend pellet in 2 ml Bolton’s enrichment broth

42°C, 48h, microaerophilic

Streak 75 µl onto Campy-Cefex Agar

Sub-culture positive samples onto Blood agar plates for further analysis (PCR, susceptibility testing, and freezing)
ARS FSIS Cooperative Study:

Can *E. coli* be used as a measure of process control?

*Campylobacter* Enumeration

Performed by J. Stan Bailey and Mark Berrang
USDA, Agricultural Research Service
Materials and Methods

- 20 randomly selected plants
- 4 seasons
- FSIS collected samples (and survey information) and sent refrigerated to ARS in Athens, GA
- 10 carcass rinses post-pick and post-chill
Materials and Methods

- Quantitative: *E. coli*, coliform, *Campylobacter*

- Qualitative: *Salmonella*

- 3M™ Petrifilm™ for *E. coli* and coliforms and direct plating on Campy Cefex for *Campylobacter*

- BAX PCR with cultural back-up for *Salmonella*
Campylobacter Methodology

- Carcasses rinses (100 mL) were shipped on ice packs by overnight freight to the laboratory
  - Rinse temperature had to be less than 10°C on arrival

- Rinse samples were serially diluted in physiological saline and surface spread plated onto Campy Cefex agar plates in duplicate
  - Zero dilution is obtained by plating 0.25 mL onto each of four well dried plates
Campylobacter Methodology

- Plates were sealed in ziploc bags in a gas mixture of 5% O₂, 10% CO₂, and 85% N₂ and incubated at 42°C for 48 hr

- Presumptive colonies were counted and a representative sampling of the colonies examined for typical morphology and tumbling motility under phase contrast microscopy and confirmed with latex agglutination

- Cultures were stored frozen for further characterization if necessary
Number of Campylobacter grouped by seasons

- **Fall**: Pre-hang: 2.81 + 1.55, Post-chill: 0.37 + 0.65
- **Winter**: Pre-hang: 2.46 + 1.91, Post-chill: 0.35 + 0.56
- **Spring**: Pre-hang: 2.86 + 1.90, Post-chill: 0.65 + 0.88
- **Summer**: Pre-hang: 2.51 + 1.95, Post-chill: 0.35 + 0.67
Campylobacter in poultry processing
“The value of enumeration using the zero dilution (4 plates)”

Post-Pick
Post-Chill

> 10
< 10

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Comparison of *Campylobacter* Levels and *Escherichia coli* Levels on Poultry Carcass Rinse Samples

Mean *E. coli* and *Campylobacter* Counts and *Salmonella* prevalence for Poultry Carcass Rinse Samples

<table>
<thead>
<tr>
<th>Rehang Rinse Data</th>
<th>Postchill Rinse Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Rinses</td>
<td>E. coli CFU/ml</td>
</tr>
<tr>
<td>800</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Final Thoughts – Cultural Detection

• All methods have inherent biases which will favor one subpopulation over another

• The Campy Cefex direct plate method with the four plate zero dilution has been demonstrated in numerous laboratories and in a large national study to effectively be able to provide *Campylobacter* enumeration

• Whatever biases any method has will be consistent over time and location and will allow comparison of data

• With limited resources, MPN enumeration limits the number of samples that can be included in a large scale study
A Plethora of Campy Methods and Media

- Direct Gram stain
- Conductimetric methods
- Antigen/Antibody based assays
  - ELISA
  - Latex agglutination
  - Immunomagnetic separation
  - Specific colony-lift immunoassay
- DNA based assays
  - PCR
  - Real-time Quantitative PCR
  - rtPCR
- Cultural methodology
  - Enrichment
  - MPN
  - Direct Plating
Direct Gram Stain Microscopy

- Adapted for clinical microbiology
- Low cost
- Relatively high sensitivity (89%)
- Works for samples containing large campy populations

Conductimetric Methods

Conductimetric instruments monitor microbial metabolism inside a growth medium by measurement of changes in electrical activity.
Conductimetric Methods

Detection of *Campylobacter* by monitoring capacitance changes in broth media.
Enzyme-Linked Immunosorbent Assays (ELISA)

- Most prevalent antibody assay used for pathogen detection in foods
- Quantitative if serial dilutions analyzed
- Automatable
- Typical detection limit of $10^4$ cfu/ml

3M™ Tecra™ Campylobacter Visual Immunoassay ELISA
Recovery of *Campylobacter* from chicken rinse samples with the 3M™ Tecra™ VIA kit and cultural procedures using 3M™ Campylobacter broth or Bolton’s broth to Campy Cefex

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Cultural Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M™ Tecra™ Campylobacter VIA</td>
<td>3M™ Campy broth for Tecra™ kit</td>
</tr>
<tr>
<td>317/398</td>
<td>328/398</td>
</tr>
<tr>
<td>4 FP</td>
<td></td>
</tr>
<tr>
<td>15 FN</td>
<td></td>
</tr>
<tr>
<td>3M™ Tecra™ Campylobacter VIA</td>
<td>Bolton’s broth</td>
</tr>
<tr>
<td>313/398</td>
<td>280/398</td>
</tr>
</tbody>
</table>
**Campylobacter spp.** Prevalence from Poultry Rinse Samples from Eight Facilities

<table>
<thead>
<tr>
<th>Facility</th>
<th>3M™ TECRA™ Broth</th>
<th>Bolton Broth</th>
<th>Direct Plating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10/10 A</td>
<td>1/10 B</td>
<td>0/10 B</td>
</tr>
<tr>
<td>2</td>
<td>10/10 A</td>
<td>10/10 A</td>
<td>10/10 A</td>
</tr>
<tr>
<td>3</td>
<td>9/10 A</td>
<td>10/10 A</td>
<td>10/10 A</td>
</tr>
<tr>
<td>4</td>
<td>20/20 A</td>
<td>15/20 A</td>
<td>20/20 A</td>
</tr>
<tr>
<td>5</td>
<td>26/30 A</td>
<td>21/30 B</td>
<td>30/30 A</td>
</tr>
<tr>
<td>6</td>
<td>16/20 A</td>
<td>16/20 A</td>
<td>18/20 A</td>
</tr>
<tr>
<td>7</td>
<td>20/20 A</td>
<td>20/20 A</td>
<td>20/20 A</td>
</tr>
<tr>
<td>8</td>
<td>20/20 A</td>
<td>10/20 B</td>
<td>4/20 C</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>131/140 A</strong></td>
<td><strong>103/140 B</strong></td>
<td><strong>112/140 B</strong></td>
</tr>
</tbody>
</table>

Conclusions

- 94% of rehang carcasses were positive for *Campylobacter* spp. with the 3M™ TECRA™ enrichment broth vs. 74% with Bolton enrichment broth.
- Overall, 3M™ TECRA™ enrichment broth significantly suppressed non-*Campylobacter* microflora (P < 0.05) compared to Bolton enrichment broth.
- Overall, 3M™ TECRA™ enrichment broth yielded an 11% higher total number of *Campylobacter*-positive samples compared to Bolton enrichment broth.
- *Campylobacter* spp. detection in postchill rinse samples was significantly greater (P < 0.05) by enrichment (84%) than by direct plating (19%).
Polymerase Chain Reaction (PCR)

**Advantages**
- Extremely sensitive for ID of pure cultures
- Can identify campy to species level
- Rapid - results obtained on the same day
- May be automated for high throughput

**Disadvantages**
- Inhibitors in food/environmental samples can prevent primer binding and diminish sensitivity
- Some enrichment may be necessary
- Expensive, labor intensive
- Cannot distinguish living from dead cells
- PCR does not provide isolate for further identification
U.S. Government Activity

• In May, 2010 the USDA announced the publication of new performance standards to reduce the incidence of *Salmonella* and *Campylobacter* in broilers and turkeys

• The new performance standard for *Campylobacter* is based on two percentages
  – One specifying the percentage of 1 ml portions that are positive
  – The other specifying the percentage of total sample-specific positive results counting either the 1 ml or the 30 ml rinsate portions as positive
U.S. Government Activity

- In the new USDA baseline sampling, 51 samples will be taken for a set and analyzed for both \textit{Salmonella} and \textit{Campylobacter}.
- Each portion of sample rinsate used for \textit{Campylobacter} testing will be subdivided into two portions:
  - A 1 ml portion that is plated for both qualitative (presence/absence) and quantitative (enumeration) results.
  - A 30 ml portion which is enriched and then plated for qualitative (presence/absence) results only.
U.S. Government Activity

• The 30 ml enrichment-based test laboratory procedure increases the practical sensitivity (0.03 CFU / ml of sample)
• To meet the new *Campylobacter* performance standard, a broiler plant will have no more than
  – 8 positive samples in the 1 ml portion (10.4%)
  – 27 total positive samples out of 51 samples in either the 30 ml or 1 ml portion tests (46.7%)
• More information is available at
Acknowledgement

• Special thanks to Dr. J. Stanley Bailey, formerly with USDA – Agricultural Research Service, Russell Research Center, Athens, GA for providing content for this presentation
Thank You!

Questions?

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