CAMPYLOBACTER JEJUNI & RELATED ORGANISMS

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PHR 250
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Taxonomy made simple (?)

“The Great Unknown”

\[ \text{genus Vibrio} \rightarrow \text{restricted} \]

\[ \text{genus Campylobacter} \]

\[ \text{genus Arcobacter} \quad \text{genus Helicobacter} \]

Historical aspects: the Vibrio days

- McFadyean & Stockman, British veterinarians, epizootic abortion in ewes (1909)
- Theobald Smith, investigating infectious abortions of U.S. cattle (1919): Vibrio fetus

Historical aspects: the Vibrio days (2)

- Jones, Little, & Orcutt, winter dysentery in U.S. calves (1931): Vibrio jejuni
- Doyle, swine dysentery (1944)

Historical aspects: the Vibrio days (3)

- Humans:
  - acute milkborne diarrhea, Vibrio jejuni (Levy, 1946)
  - abortion in two women, Vibrio fetus (Vinzent, 1947)
- King (1957): Vibrio fetus differentiated from "related vibrios"

Historical aspects: the new genus

- Sebald and Veron (1963): differentiation from cholera and halophilic vibrios → genus Campylobacter ("curved rod")
- C. jejuni (+ C. coli) perhaps foremost bacterial causes of diarrhea in humans; a classical zoonosis—pathogen or commensal in animals
Campylobacter species

- C. coli
- C. concisus
- C. curvus
- C. fetus
- C. gracilis
- C. helviticus
- C. hominis
- C. hyointestinalis
- C. insulae
- C. jejuni
- C. lanienae
- C. lari
- C. mucosalis
- C. rectus
- C. shueae
- C. sporerum
- C. upsaliensis

Scanning electron microscope image of Campylobacter jejuni, illustrating its corkscrew appearance and bipolar flagella (Altekruse S, 1999).

Background

- 3 Campylobacter species account for 99% of human illnesses: C. jejuni, C. coli, and C. lari
- Campylobacter species isolated from the intestinal tract of a wide variety of wild and domestic animals especially chicken, cattle, and pig (asymptomatic infection)

Campylobacter: present situation

- Most commonly reported cause of bacterial gastroenteritis in the developed world, with ~2.5 million cases per year in the U.S.
- Foodborne outbreaks mostly associated with consumption of undercooked poultry, meats, and unpasteurized milk

U.S., 1998–2002: Campylobacter spp., 61 outbreaks comprising 1,440 cases (ranked #7); leader in CA FoodNet (Bay Area)
- Largest outbreak documented in the U.S. ~3,000 cases, water (city)
- Largest milkborne outbreak in U.S., ~1,600 cases, California 2006

CAST (1994) estimates: 170,000 to 2,100,000 cases/yr, 120–360 deaths—presumably all foodborne; average medical and productivity cost/case $920, annual total near $1 billion

Cases of Campylobacter and other foodborne infections by month

Source: Altekruse S, 1999
### Incidence (/100,000) of Diagnosed Infections, 2002 (source: FoodNet, CDC)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>All Sites</th>
<th>CA FN</th>
<th>Alameda</th>
<th>Contra Costa</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>13.3</td>
<td>31.5</td>
<td>26.3</td>
<td>24.9</td>
<td>48.2</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>1.3</td>
<td>1.0</td>
<td>0.7</td>
<td>0.2</td>
<td>3.0</td>
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<tr>
<td>Cyclospora</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>E. coli O157</td>
<td>1.7</td>
<td>1.4</td>
<td>1.8</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Listeria</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Salmonella</td>
<td>16.2</td>
<td>16.1</td>
<td>17.6</td>
<td>10.8</td>
<td>19.3</td>
</tr>
<tr>
<td>Shigella</td>
<td>10.3</td>
<td>11.4</td>
<td>8.2</td>
<td>6.0</td>
<td>23.6</td>
</tr>
<tr>
<td>Vibrio</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>Yersinia</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
<td>0.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Per 100,000 population

### FoodNet data (US)

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Campylobacter (/10⁵)</td>
<td>21.7</td>
<td>12.7</td>
</tr>
<tr>
<td>E. coli O157 (/10⁵)</td>
<td>2.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Listeria (/10⁶)</td>
<td>4.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Salmonella (/10³)</td>
<td>13.5</td>
<td>14.6</td>
</tr>
<tr>
<td>Shigella (/10³)</td>
<td>7.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

### Characteristics of Campylobacter (classification)
- Small, nonsporeforming, gram-negative bacteria — curved, S-shaped, or spiral
- 0.5–8 µm long, 0.2–0.9 µm diameter
- Single polar flagellum at one or both ends — rapid, darting, corkscrew-like motility

### Characteristics of Campylobacter
- Many species and subspecies
- Many serotypes of C. jejuni, based on somatic, capsular, and flagellar antigens
- Tremendous genetic diversity (“naturally competent”): multiple molecular typing methods: PFGE, MLST, AFLP

### Survival and growth in the environment
- Labile to freezing, drying, and temperatures from 48°C up
- Stable at 4°C, dies more quickly at 25°C than at 4 or 30°C
- Some losses at atmospheric levels of O₂; optimum salt level 0.5%
- Growth above pH 4.9, good at 5.5–8, optimum at 6.5–7.5
**Infections in humans**

- Affects young adults as often as infants
- Human disease principally (~90%) from *C. jejuni*, also *C. coli*
- Infectious dose is apparently "small"
- Incubation 2–5 (1–10) days
- Duration 2–5 days, sometimes 10 days

**Pathogenesis is poorly understood:** both enteroxotic and enteroinvasive strains may exist

- Diarrhea (watery to bloody with pus & WBC), abdominal pain, malaise, fever, nausea, and vomiting
- Rarely febrile convulsions, arthritis, Guillain-Barré syndrome, or meningitis; may mimic acute appendicitis; many infections asymptomatic

**Infections in humans**

- Shedding 2–7 weeks if antibiotic treatment is not done; minor source of human infection, except for an occasional food worker contaminating food
- Lasting immunity follows infection

**Campylobacter in animals:**

**Reservoirs**

- Common in cattle, swine, sheep, and especially poultry (also companion animals and rodents)
- Carried in gall bladder and small and large intestines

**Campylobacter in animals:**

**Transmission**

- Shed in feces, which may contaminate edible portions of carcass
- Occurrence in milk may indicate shedding via the mammary gland, but mastitis is seldom involved.

**Campylobacter insulaenigrae Isolates from Northern Elephant Seals (Mirounga angustirostris) in California**

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Prevalence of *Campylobacter* in foods

- Eggs — not in outbreaks
- Poultry — common at retail; fecal cross-contamination in processing
- Meat — most common on swine carcasses; sometimes on beef and lamb

Prevalence of *Campylobacter* in foods

- Milk and milk products — readily killed by pasteurization; raw milk is a leading vehicle in U.S.
- Other foods — mainly animal products; fertilization of vegetables with manure may cause contamination

Prevalence of *Campylobacter* in feed and water

- Animal feed — subject to contamination from bird and rodent droppings
- Water — at least two drinking water-associated outbreaks (≥130 cases), U.S., 2003–2004

Foods most often associated with human *Campylobacter* infections — U.S.

- Raw milk
- Poultry
- Other foods via cross-contamination

Detection of *Campylobacter*

- Samples ideally stored at 4°C in N₂ atmosphere, with 0.01% sodium bisulfite added
- Expect low contamination levels: pre-enrichment likely to be necessary
- Slow-growing organism — isolation medium must be selective, to inhibit competitors.
Detection of Campylobacter

- Optimum atmosphere is 5% O₂, 10% CO₂, 85% N₂; candle jars are marginally useful
- Incubation generally 42°C
- Antibiotics used in some selective media may inhibit some strains of C. jejuni, also C. coli; cefaperazone is presently recommended, not cephalothin

Identification of Campylobacter

- Gram-negative, appropriate appearance, growth temperature and atmosphere; oxidase and catalase positive; hydrolyzes hippurate and indoxyl acetate; reduces nitrate; produces H₂S; some tests require special precautions
- Nonculture detection methods and epidemiologic typing systems available

Multilocus Sequence Typing

- Approach takes advantage of information from sequenced genomes of pathogenic bacteria
- Exploits the genetic variation present in 7 housekeeping loci to determine the genetic relatedness between isolates: aspA, glyA, gltA, glnA, pgm, tkt, uncA

Multilocus sequence typing

Chromosomal DNA

Multilocus Sequence Typing (MLST)

- Amplify ≈ 450-bp internal fragments of seven housekeeping genes
- Sequence the seven gene fragments
- Each different sequence at a locus is given a different allele number
- The allele numbers at the seven MLST loci gives the allelic profile of the isolate
- Compare the allelic profile of isolate to those of all isolates within a central database on the web via the internet (www.mlst.net)

Arcobacter (1991,1992)

- "Aerotolerant Campylobacter"
- Grow at 15, 25, and 30°C, but variably at 37 and 42°C
- Similar appearance: Gram-negative, curved, S-shaped, or helical; single polar flagellum, 1–3 µm long, 0.2–0.9 µm diameter
- May grow aerobically at 30°C and anaerobically at 35–37°C
**Arcobacter**
- "Frequently isolated from cattle and pigs suffering from abortion and enteritis"
- Human illnesses from two of the species include bacteremia, endocarditis, peritonitis, and diarrhea

**Arcobacter Species in Humans**
- During an 8-year study period, *Arcobacter butzleri* was the fourth most common Campylobacter-like organism isolated from 67,599 stool specimens in Belgium
- Observations suggest that *A. butzleri* displays microbiologic and clinical features similar to those of *Campylobacter jejuni*
- *A. butzleri* was more frequently associated with a persistent, watery diarrhea
- Emerging infection?

Source: Vandenberg O, 2004

**Helicobacter pylori**
- Discovered in 1982, separated from genus *Campylobacter* in 1989
- Looks like *Campylobacter*, microaerophilic, optimum growth at 37°C
- Culture characteristics, etc., need not be discussed here

**Helicobacter pylori**
- Clinically important as a probable cause of chronic gastritis and peptic and duodenal ulcer in humans
- Human infection is widespread (nonhuman reservoirs of this species unknown); shed with feces and may contaminate food, but foodborne transmission is not clearly established

**Summary: three genera**
- *Campylobacter*: a leading bacterial cause of foodborne disease in U.S.
- *Arcobacter*: also can cause foodborne disease.
- *Helicobacter*: causes ulcers; may not be foodborne.