Campylobacter and related infections

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1 Campylobacter jejuni

1.1 Historical aspects and current problems

The first description of a bacterium now belonging to the genus Campylobacter is attributed to Theodore Escherich, at the end of the nineteenth century (Escherich, 1886). At the beginning of the twentieth century a Campylobacter species, described as a related Vibrio, was recognized as causing abortions in sheep. Only after a suitable isolation medium was developed in the 1970s were two closely related pathogens, C. jejuni and C. coli, recognized to be common human enteric pathogens (DeKeyser et al., 1972). C. jejuni accounts for approximately 90 % of human Campylobacter infections (Kramer et al., 2000).

In 1999, the Centers for Disease Control and Prevention (CDC) estimated that there were 2.5 million cases of human campylobacteriosis in the United States each year (Mead et al., 1999). While the incidence of campylobacteriosis declined by 27 % in FoodNet surveillance sites between 1996 and 2001, Campylobacter spp. remains one of the most common bacterial foodborne infectious diseases in the United States (CDC, 2002a). Post-infectious sequelae of infection include Guillain-Barré syndrome and reactive arthritis. Current issues for the prevention of this zoonosis include implementation of pathogen reduction measures along the food chain, zoonotic infections, and infections caused by fluoroquinolone resistant Campylobacter strains.
1.2 Characteristics of *C. jejuni*, classification and virulence factors

1.2.1 Classification

*Campylobacter* and other related genera (i.e. *Arcobacter, Helicobacter*) form DNA Superfamily IV (On, 2001). *C. jejuni* and *C. coli* are closely related to one another. Like most of the characterized species in this taxonomic family, they are recognized to be human or animal pathogens and have fastidious growth requirements reflecting dependence on a warm-blooded host for replication (On and Harrington, 2001).

1.2.2 Pathogenesis, survival in the environment

The sequencing of the *C. jejuni* genome (Parkhill et al., 2000) is an important milestone for understanding *Campylobacter* pathogenesis. Already it is clear that the 1.5 million base-pair *C. jejuni* genome is small, with few homologues of the virulence determinants in other foodborne pathogens. Suspected virulence determinants of *C. jejuni* include motility, adherence, exotoxin production, iron regulation and cell invasion.

*C. jejuni* does not normally replicate outside the intestinal tract of warm-blooded animals (Nachamkin, 2003). The infectious dose is reported to be less than 1000 organisms (Black et al., 1988). Other adaptations to an intestinal niche include a single polar flagellum and the cell’s corkscrew shape (Figure 7.1). These traits facilitate motility in the viscous intestinal mucus. Requirements for growth in the laboratory (Nachamkin, 2003) also reflect this narrow ecologic niche – a micro-aerophilic nitrogen atmosphere with low oxygen (5–7 %) and high carbon dioxide tension (7–13 %). *C. jejuni* is unable to replicate at temperatures below the body temperature of warm-blooded animals (approximately 30˚C), or at a pH < 4.9. The organism is also sensitive to desiccation and osmotic stress (e.g. NaCl concentrations above 2 %).

![Figure 7.1](image_url) Scanning electron micrograph illustrating the single polar flagella and corkscrew shape of *C. jejuni*. These morphologic characteristics contribute to the characteristic ‘darting’ motility of *C. jejuni* in the viscous mucous layer of the intestinal lumen.
*C. jejuni* gradually die outside the host intestinal tract. In one study, 58 of 85 (68 %) *C. jejuni* strains could not be cultured from water after 3 weeks; however, a few strains were detected in unsterilized water after 60 days (Talibart *et al.*, 2000). Environmental factors may facilitate *Campylobacter* survival under adverse conditions. Survival times are longer in nutrient-rich water than in de-ionized water (Thomas *et al.*, 1999). Similarly, biofilms are reported to facilitate the survival of *C. jejuni* in broiler houses (Trachoo *et al.*, 2002). Some researchers postulate that campylobacters can survive in water in a viable but non-cultivable form (Cappelier *et al.*, 2000; Thomas *et al.*, 2002); however, the role of this dormant stage in the *Campylobacter* life cycle is controversial (van de Giessen *et al.*, 1996a).

### 1.3 Nature of infection in man and animals

#### 1.3.1 Man

##### 1.3.1.1 The acute clinical illness

While *C. jejuni* and *C. coli* can exist as commensal organisms of domestic poultry and livestock, they are considered human pathogens. The clinical spectrum of human *Campylobacter* enteritis ranges from loose stools to dysentery. Self-limiting acute enteritis is the most common syndrome. Prodromal symptoms are common, and include headache, low fever, and myalgia lasting from a few hours to a few days. Symptoms of acute infection often begin with abdominal cramps followed by diarrhea and high fever, peaking during the first days of illness (Blaser, 1997). *C. jejuni*-specific serum antibodies confer immunity to symptomatic infection; however, the duration of protective immunity is not known (Blaser *et al.*, 1987; Walz *et al.*, 2001).

An estimated 100 fatal *C. jejuni* infections occur each year in the United States. These fatal infections occur most often in infants, in the elderly, or in immunosuppressed individuals (Mead *et al.*, 1999). Bacteremia is most often seen in patients with underlying disease (Pigrau *et al.*, 1997), and is a potentially fatal complication of HIV/AIDS (Manfredi *et al.*, 1999). Chronic diarrhea is also a complication of HIV-associated campylobacteriosis. HIV-positive individuals who develop campylobacteriosis have shorter survival times and higher rates of bacteremia and hospitalization than HIV-positive individuals without campylobacteriosis (Angulo and Swerdlow, 1995). This aspect of campylobacteriosis has major public health significance in developing nations (Coker *et al.*, 2002).

##### 1.3.1.2 Sequelae to infection

Sequelae to infection include Guillain-Barré syndrome and reactive arthritis.

With several thousand cases each year, *Guillain-Barré syndrome* (GBS) is the most common etiology of acute flaccid paralysis in the United States (Nachamkin, 2002). Guillain-Barré syndrome (GBS) is an acute immune-mediated disorder of the peripheral nervous system. Leg weakness is often the presenting sign, followed by ascendant paralysis. After one year, 70 % of patients make complete neurological recovery, 22 % partially recover, 8 % remain unable to walk, and 2 % remain bedridden or require ventilation. Most cases of GBS are believed to follow an infectious disease. Approximately 40 % of GBS cases are thought to follow *Campylobacter* infection, and
GBS is estimated to occur in 1 in 1000 patients infected with *Campylobacter*. Although a diverse group of strains is associated with GBS (Dingle et al., 2001), the syndrome is strongly linked to a few strains of *C. jejuni*, such as Penner serotypes HS:19 (Yuki et al., 1997) and HS:41 (Prendergast et al., 1998). *Campylobacter* strains contain sialic acid linkages to lipo-oligosaccharides resembling sialic acid moieties on the gangliosides of peripheral nerve tissues (Ho et al., 1999). Patients with GBS develop antibodies against these gangliosides, resulting in autoimmune targeting of peripheral nerve sites. Complement-mediated damage (Hafer-Macko et al., 1996) and blockage of neurotransmission (Goodyear et al., 1999) are suspected to play a role in GBS pathogenesis.

Since many individuals are exposed to *C. jejuni* strains that mimic gangliosides and only a few develop GBS, it is suspected that host factors contribute to GBS. In one study (Rees et al., 1995) *Campylobacter*-related GBS was associated with major histocompatibility antigen, HLA-DQB1*03; however, this association was not replicated in another well-designed study (Yuki et al., 1997). Proposed treatments for GBS have not been fully evaluated in clinical trials, but include corticosteroid therapy, immunoglobulin therapy and plasmapheresis (Hughes, 2002).

**Reactive arthritis**, or Reiter’s syndrome, is another sterile post-infectious sequela to acute gastrointestinal campylobacteriosis. Onset of reactive arthritis occurs 7–10 days after onset of diarrheal illness. The frequency of reactive arthritis as a sequela of campylobacteriosis has not been well described in the US. In Finland, 45 of 870 (7 %) patients with laboratory confirmed campylobacteriosis developed reactive arthritis (Hannu et al., 2002). Arthritis was oligo- or polyarticular, and in most cases mild. In the Finnish study, 37 of the 45 patients (82 %) had *C. jejuni* and 8 (18 %) had *C. coli* infections. No cases of reactive arthritis occurred in children. In a Danish study, patients with joint pain had more severe gastrointestinal symptoms and longer duration of diarrhea than those without joint pain. Anti-*Campylobacter* antibody levels were similar in both patient groups. Antibiotic treatment did not prevent reactive arthritis (Locht and Krogfelt, 2002).

### 1.3.1.3 Treatment of acute campylobacteriosis

Supportive measures, particularly fluid and electrolyte replacement, are the principal therapies for most patients with campylobacteriosis. Severely dehydrated patients should receive rapid volume restoration with intravenous fluids. For most other patients, oral rehydration is indicated. Although *Campylobacter* infections are usually self-limiting, antibiotic therapy may be prudent for patients who have high fever, bloody diarrhea or more than eight stools in 24 hours, immunosuppressed patients, patients with bloodstream infections, and those whose symptoms worsen or persist for a week or more from the time of diagnosis. When indicated, antimicrobial therapy soon after the onset of symptoms can reduce the median duration of illness from approximately 10 days to 5 days. When treatment is delayed (e.g. until *C. jejuni* infection is confirmed by a medical laboratory), therapy may not be successful (Smith et al., 1999). Ease of administration, lack of serious toxicity and a high degree of efficacy made erythromycin the historical drug of choice for *C. jejuni* infection; however, other antimicrobial agents, particularly the quinolones and newer macrolides (including azithromycin), are also widely used.
In 2005 the US Food and Drug Administration banned the use of fluoroquinolones in poultry in response to the emergence of fluoroquinolone resistant *Campylobacter* strains as a cause of human infections in the United States. The FDA partially attributed this trend to veterinary use of fluoroquinolones, concluding that use of fluoroquinolones in poultry compromises the clinical utility of fluoroquinolones in humans. An FDA risk assessment estimated that each year thousands of people infected with fluoroquinolone resistant *Campylobacter* strains are treated with a fluoroquinolone, resulting in prolonged duration of illness (Food and Drug Administration, 2000).

Fluoroquinolone resistant *Campylobacter* strains were not detected in the United States in 1986 – the year when this class of antimicrobials was first introduced for human use (Friedman *et al*., 2001). Resistance rates increased to 5% in the next few years. The proportion of *Campylobacter* isolates from humans that exhibited resistance to fluoroquinolones increased after 1995, around the time when fluoroquinolones were approved for the treatment of colibacillosis in poultry flocks. Since 1997, 14–18% of *Campylobacter* strains isolated from humans in the United States have been resistant to ciprofloxacin (Marano *et al*., 2000). A study by the Minnesota Department of Health suggested that the epidemiology of infection with fluoroquinolone resistant *Campylobacter* strains shifted, beginning in 1995 with the emergence of a domestic reservoir of fluoroquinolone resistant *C. jejuni*. In Minnesota the molecular subtypes of fluoroquinolone resistant *C. jejuni* strains isolated from humans who had not traveled outside the US matched the molecular subtypes of fluoroquinolone resistant *C. jejuni* isolated from locally purchased retail poultry products (Smith *et al*., 1999). An increase in the frequency of infections with fluoroquinolone resistant strains was also observed by the National Antimicrobial Resistance Monitoring System (NARMS). Case-control studies conducted by NARMS showed that chicken consumption is an important risk factor for infection with domestically acquired fluoroquinolone resistant strains. Infections with such strains were also associated with longer duration of diarrhea and increased likelihood of hospitalization (CDC, 2002b).

A report by Engberg and colleagues documented the emergence of fluoroquinolone resistant *Campylobacter* strains as a cause of human infection in 10 developing nations during the 1990s in relation to the approval of this class of antimicrobial drugs for use in veterinary practice (Engberg *et al*., 2001). Most fluoroquinolone resistance is caused by spontaneous point mutations in the DNA gyrase A subunit region that alter the fluoroquinolone-binding site (Hooper *et al*., 1987). Strains with this mutation have elevated minimum inhibitory concentrations (MIC). This attribute confers selective advantage to the bacterium in the presence of fluoroquinolones (McDermott *et al*., 2002).

### 1.3.1.4 Risk factors for human illness

- **Poultry consumption.** The initial epidemiologic studies of sporadic campylobacteriosis conducted in the United States (Harris *et al*., 1986; Deming *et al*., 1987; Hopkins and Scott, 1993) and western Europe (Norkrans and Svedhem, 1982; Oosterom *et al*., 1984; Kapperud *et al*., 1992) revealed robust associations with the handling
(Norkrans and Svedhem, 1982; Hopkins and Scott, 1993) or eating (Oosterom et al., 1984; Deming et al., 1987; Kapperud et al., 1992) of poultry, particularly undercooked poultry (Hopkins et al., 1984; Harris et al., 1986). More recent epidemiologic studies in the United States (Effler et al., 2001), the United Kingdom (Rodrigues et al., 2001) and New Zealand (Eberhart-Phillips et al., 1997) confirmed the association between human campylobacteriosis and poultry consumption, and added an additional nuance—an association between Campylobacter infection and eating commercially prepared poultry (Effler et al., 2001). These associations are not unexpected, given that the majority of chicken in stores is contaminated with C. jejuni (Zhao et al., 2001). Molecular subtyping studies demonstrate partial correspondence between poultry and human isolates (Smith et al., 1999; Hanninen et al., 2000). In Quebec, 20% of genotypes from humans and poultry had matching pulsed field gel electrophoresis patterns (Nadeau et al., 2002).

- **Commercially prepared foods.** Case-control studies in the United States and other developed nations indicate that eating chicken in restaurants is associated with increased risk of infection (Eberhart-Phillips et al., 1997; Effler et al., 2001; Rodrigues et al., 2001). On occasions, other foods prepared in restaurants or commercial kitchens have been implicated in outbreaks of campylobacteriosis, including tuna salad (Roels et al., 1998), sweet potatoes (Winquist et al., 2001), and lettuce (CDC, 1998). Cross-contamination during food preparation is suspected to be a contributory factor in such outbreaks, and it has been clearly shown that C. jejuni can survive on food contact surfaces and thereby cross-contaminate other foods (Cogan et al., 1999).

- **Other food items.** In addition to poultry, several types of meat have been epidemiologically implicated as sources of human campylobacteriosis in developed nations. Some of these implicated food items include pork loins, barbequed foods (Studahl and Andersson, 2000), and liver pâté (Gillespie et al., 2002).

- **Unpasteurized milk.** Drinking unpasteurized milk is the principal risk factor for outbreaks of campylobacteriosis. Between 1981 and 1990, 20 outbreaks of Campylobacter enteritis were reported in the United States (Wood et al., 1992). Of these 20 outbreaks, 14 (70%) occurred among children who drank unpasteurized milk on school field trips or other youth activities. Unlike sporadic Campylobacter infections; which peak during the summer and are also associated with activities such as eating chicken, eating at restaurants and international travel, milk-associated outbreaks have a bimodal seasonality, with peaks during the spring and fall corresponding with the peak seasons for youth activities such as school field trips. Despite regulatory efforts to address the hazard, unpasteurized milk-associated outbreaks continue to occur (CDC, 2002c). Recently, molecular typing studies have linked outbreak-associated infectious strains with unpasteurized milk from implicated dairies (Lehner et al., 2000; CDC, 2002c).

- **Water.** One of the first case-control studies of campylobacteriosis, conducted in Colorado (Hopkins et al., 1984), found an association with consumption of untreated surface water. More recently, a study conducted in England found that people with C. coli infection were more likely to report drinking bottled water than were those with C. jejuni infection (Gillespie et al., 2002). Waterborne outbreaks of
Campylobacteriosis typically involve lapses in community water sanitation (Sacks et al., 1986; Melby et al., 2000). The proportion of Campylobacter infections caused by contaminated water is likely to vary by region, and with economic development.

- **Zoonotic transmission.** Case-control studies identify contact with pet dogs and cats, and especially juvenile or diarrheic pets, as risk factors for Campylobacter infection, accounting for perhaps 5% of human campylobacteriosis (Norkrans and Svedhem, 1982; Hopkins et al., 1984; Deming et al., 1987; Kapperud et al., 1992; Saeed et al., 1993). The hazard of zoonotic campylobacteriosis may be greatest for young children, an age group with elevated rates of campylobacteriosis (Tauxe et al., 1988). In one case report, a 3-week-old girl in a household with a recently acquired Labrador retriever puppy developed bloodstream C. jejuni infection. Amplified fragment-length polymorphism (AFLP) analysis confirmed that the human and canine isolates were genetically similar (Wolfs et al., 2001). In an Australian case-control study, children less than 3 years of age who lived in a home with a pet puppy had a 17-fold increase in risk of campylobacteriosis compared to children with no puppy. Elevated risk of pediatric campylobacteriosis was also associated with pet chicken ownership (Tenkate and Stafford, 2001). Occupational risk factors for campylobacteriosis include farm residence, poultry-related occupations, and daily contact with chickens (Studahl and Andersson, 2000).

- **Foreign travel.** Foreign travel is a commonly reported risk factor for campylobacteriosis (Eberhart-Phillips et al., 1997; Smith et al., 1999; Rodrigues et al., 2001). In nations where campylobacters are uncommon in chicken (i.e. some Scandinavian nations), international travel is the dominant source of human Campylobacter infections (Norkrans and Svedhem, 1982). In the United States it is estimated that between 20 and 25% of Campylobacter infections are acquired during international travel (CDC, 2002a). Campylobacteriosis was the most frequently reported enteric bacterial infection in Austrian tourists returning from Southern Europe and Asia (Reinthaler et al., 1998). In England, travel to South Africa was associated with C. coli infection (Gillespie et al., 2002). Causal exposures (e.g. food, beverage, dining venue, antimicrobial usage, animal contact) for travel-associated infections remain to be determined.

- **Antimicrobial usage.** In a Hawaiian case-control study, antibiotic use in the month before onset of illness was associated with campylobacteriosis – a unique finding in studies to date (Effler et al., 2001). One hypothesis for the observation is that antimicrobial usage lowers the infectious dose of drug resistant C. jejuni strains. Another potential explanation for the above finding is that the use of antibiotics may alter colonic flora, resulting in decreased resistance to infection even with antimicrobial susceptible C. jejuni strains.

### 1.3.2 Campylobacter ecology

#### 1.3.2.1 Wildlife reservoirs

The evidence that wildlife is a reservoir for human Campylobacter infections is somewhat equivocal. To be a substantial source of human infections, feces from wildlife would need to enter the human food or water supply. Although some wild birds are colonized with Campylobacter, a Danish study of C. jejuni isolates indicated...
that the serotype distribution in wildlife was different from the distributions in broiler chickens and humans (Petersen et al., 2001). C. jejuni contamination rates in wild bird-associated specimens vary markedly, between 0 and 50% in one US study (Craven et al., 2000). The finding of Campylobacter in wildlife may also indicate contact with food animals. In a study from Japan, 3 of 13 C. jejuni isolates from sparrows exhibited quinolone resistance, suggesting that sparrows may also acquire campylobacters from food animal populations (Chuma et al., 2000).

1.3.2.2 Poultry
Although not all flocks become colonized, C. jejuni is introduced into many broiler flocks during the production cycle (Wedderkopp et al., 2000; Denis et al., 2001; Stern et al., 2001). Risk factors for flock colonization include season, caretakers who work with other animals, and drinking-water sanitation (Denis et al., 2001; Kapperud et al., 1993; van de Giessen et al., 1996b). Associations are also reported with type of air handling system and level of beetle infestation (Refregier-Petton et al., 2001).

Infections spread rapidly within flocks after introduction. Colonization typically occurs by 3 to 4 weeks of age (Evans and Sayers, 2000; Shreeve et al., 2000). While most campylobacters do not survive in cleaned and disinfected houses (Evans and Sayers, 2000), certain strains appear to persist in successive broiler flock rotations (Petersen and Wedderkopp, 2001). Recent studies suggest that the crop is among the most frequently infected organ of broilers entering processing plants, with overall crop carriage rates in the order of 60% (Byrd et al., 1998), similar to the frequency of contamination reported on broiler carcasses after processing (Zhao et al., 2001).

1.3.2.3 Cattle, pigs, and sheep
Campylobacter species often inhabit the bovine intestinal tract, particularly of calves. In a Swiss study, the overall prevalence of C. jejuni in calves during the first 3 months of life on large cow-calf farms was 39% (Busato et al., 1999). In a Danish study, 20 of 24 cattle herds were positive and young animals had a higher prevalence than older animals (Nielsen, 2002). In 40% of positive herds, all C. jejuni isolates had the identical serotype and PFGE type. Campylobacter prevalence in a multiple herd study of adult beef cattle in California was 5% (Hoar et al., 2001). The number of adult cows on the farm was positively associated with the proportion that tested positive.

In a study of sheep raised around Lancaster, England, C. jejuni accounted for 90% of all campylobacters. Overall, 92% of sheep were carriers at slaughter. Between one-quarter and one-half shed campylobacter while on pasture. The highest rate of shedding (100%) coincided with stresses of lambing, weaning, and movement onto new pasture; and no shedding was detected among sheep fed on hay or silage (Jones et al., 1999).

C. coli is the predominant Campylobacter species of swine. In a study of fecal specimens from healthy Belgian swine, 61 of 65 (94%) Campylobacter isolates were C. coli (Van Looveren et al., 2001). A Dutch study suggests that breeding management can eliminate most C. coli by breaking the chain of transmission from sows to piglets (Weijtens et al., 2000).
1.4 *Campylobacter* in food and water

1.4.1 Food

Retail food surveillance programs in developed nations provide valuable data on foodborne hazards by type of retail meat and poultry product. In 2002, FoodNet sites in the United States began routine retail food surveys to compare genotypic and antimicrobial resistance patterns of campylobacters from human and food isolates. In an English study of nearly 500 retail specimens, chicken meat had the highest contamination rate (83 %), followed by lamb liver (73 %), pork liver (72 %) and beef liver (54 %). *C. jejuni* predominated in chicken meat (77 %), while *C. coli* predominated in pork liver (42 %) (Kramer *et al*., 2000). In metropolitan Washington, DC, 130 of 184 (70 %) packages of chicken sold at retail outlets contained *C. jejuni* or *C. coli*, followed by 4 % of 172 turkey, and less than 2 % of pork and beef (Zhao *et al*., 2001).

In a study of more than 2000 lamb carcasses from six large processing plants, less than 1 % of carcasses were contaminated with *C. jejuni* or *C. coli* (Zhao *et al*., 2001).

1.4.2 Milk and water

Surveys of bulk tank milk indicate that unpasteurized milk is a source of *C. jejuni*. In one study, approximately 10 % of unpasteurized milk specimens from dairy bulk tanks were contaminated with *C. jejuni* (Jayarao and Henning, 2001). Surface waters are often contaminated with campylobacters. In a Norwegian study, 32 of 60 water specimens from the Bo River contained campylobacters, and *C. coli* was detected more often than *C. jejuni* (Rosef *et al*., 2001). In that study, fecal coliform counts were not a reliable indicator of low-level *Campylobacter* contamination.

1.5 Culturing

1.5.1 Specimen transport

Campylobacters are sensitive to stress and die outside their host, so stool specimens should be chilled if possible (not frozen) and submitted to a laboratory within 24 hours of collection. Storing specimens in deep, airtight containers minimizes exposure to oxygen, and desiccation. If a specimen cannot be processed within 24 hours or is likely to contain small numbers of organisms, a rectal swab placed in a specimen transport medium (e.g. Cary-Blair) should be used. Individual laboratories can provide guidance on specimen-handling procedures (Nachamkin, 2003).

1.5.2 Culture

Numerous procedures are available for recovering *C. jejuni* from clinical specimens. Direct plating is cost-effective for testing large numbers of specimens; however, testing sensitivity may be reduced. Pre-enrichment (raising the temperature from 36˚C to 42˚C over several hours), filtration, or both are used in some laboratories to improve recovery of stressed bacteria from specimens (e.g. stored foods or swabs exposed to oxygen). Isolation can be facilitated by use of a selective media containing a combination of antimicrobial agents such as cephalothin, oxygen quenching agents, and a low oxygen atmosphere (Nachamkin, 2003).
1.5.3 Polymerase chain reaction (PCR)
The polymerase chain reaction provides an important alternative to traditional microbiological culture techniques for detection (Denis et al., 2001) and characterization (Chuma et al., 2000; Wang et al., 2002) of Campylobacter strains. In one study, when PCR ELISA was performed on samples of 48-hour enrichment cultures of foods, it was 99% sensitive and 96% specific for the detection of C. jejuni and C. coli compared to selective culture (Bolton et al., 2002). Multiplex polymerase chain reaction assays can also be used to confirm the identity of a Campylobacter isolate among the five clinically most important species: C. jejuni, C. coli, C. lari, C. upsaliensis and C. fetus subsp. fetus (Wang et al., 2002). Advantages of multiplex PCR over traditional biochemical tests for characterization of campylobacter strains include rapidity, ease of use, and high sensitivity and specificity. Conversely, selective culture is less expensive than PCR and provides an isolate for additional typing (Kulkarni et al., 2002).

1.5.4 Typing schemes
The two most accepted Campylobacter serotyping schemes are the Penner scheme (Penner et al., 1983), based on heat-stable antigens, and the Lior scheme, based on heat-labile antigens (Garcia et al., 1985). Both techniques yield a high proportion of nontypable strains, and are technically demanding and costly. These limitations have led to a development of alternative subtyping schemes, and genotypic approaches are increasingly used to characterize Campylobacter isolates (Wassenaar and Newell, 2000). Options include pulsed field gel electrophoresis (PFGE), fla typing, and amplified restriction fragment length polymorphism (AFLP) analysis. Some schemes (e.g. fla typing) have advantages for use in limited situations related to ease and adequacy of discriminatory power; others (e.g. AFLP) provide the reproducibility and stability needed for large epidemiologic and taxonomic studies.

1.6 Control of Campylobacter infection
1.6.1 On-farm controls
Efforts to reduce pathogen loads at the farm increase the likelihood that pathogen reduction steps at processing and in the kitchen will enhance the safety of foods of animal origin. For this reason, intervention to reduce broiler intestinal colonization is an active field of investigation. In one study, biosecurity measures reduced Campylobacter colonization rates in flocks by 50% (Gibbens et al., 2001). Reduction in colonization was associated with use of disinfectant footbaths, daily water disinfection, and the location of ventilation units. In another study, cleaning and disinfection, change of footwear at the entrance to broiler houses, and control of vermin significantly reduced Campylobacter infections of broiler flocks on two farms (van de Giessen et al., 1996b).

Several studies have focused on interventions during broiler flock depopulation. In one study, lactic acid treatment of drinking water during the 8-hour pre-slaughter feed withdrawal period reduced carcass contamination by 15% (Byrd et al., 1998). Other studies indicate that when flocks are depopulated in batches, colonization rates increase (Wedderkopp et al., 2000; Hald et al., 2001). Recent studies suggest that the
transport crates brought to the farm at the time of depopulation may expose birds to campylobacters (Slader et al., 2002).

Competitive exclusion products have also been proposed to reduce broiler colonization. Various products containing defined poultry isolates of *C. jejuni* (Chen and Stern, 2001), *Lactobacillus* (Chang and Chen, 2000) and undefined cultures are reported to reduce colonization under experimental conditions (Hakkinen and Schneitz, 1999). Diet may alter the composition of intestinal mucus, thereby affecting the colonization potential of campylobacters (Fernandez et al., 2000).

### 1.6.2 Processing controls

Carcass processing is a promising site for pathogen reduction efforts. The microbial quality of broiler carcasses has been associated with the abattoir where processing occurred (Wedderkopp et al., 2000). Treatment of wash water is a potential processing control to reduce contamination, and the poor microbial quality of poultry wash water is thought to contribute to higher contamination rates of poultry than of red meat. The use of electrolyzed water for washing poultry carcasses reduced *C. jejuni* counts on chicken by 3 log$_{10}$ units (Park et al., 2002). Washing in 10% oleic acid significantly reduced the number of *Campylobacter* that remained attached to poultry skin (Hinton and Ingram, 2000). Campylobacters are also very sensitive to active chlorine (Yang et al., 2001). The chlorination of carcass wash water, an important component of the HACCP programs in many processing plants (Hulebak and Schlosser, 2002), may have contributed to the decline in human campylobacteriosis in the United States since the mid-1990s.

Post-processing interventions have also been investigated. Freezing poultry carcasses to –20°C resulted in a 2-log$_{10}$ reduction in *Campylobacter* counts compared to refrigeration (Stern et al., 1985). Electron beam irradiation of poultry would virtually eliminate campylobacters from poultry products; however, some consumers report that the color and texture of chicken fillets are altered by irradiation (Lewis et al., 2002). If these food quality considerations are successfully resolved, irradiation of poultry products may become among the most important technologies for the prevention of foodborne campylobacteriosis in the United States.

### 1.6.3 Food handling

Epidemiologic studies indicate that restaurants (Effler et al., 2001) and home kitchens (Hopkins et al., 1983) are both important venues for *C. jejuni* infection (Eberhart-Phillips et al., 1997; Rodrigues et al., 2001). Surveys indicate that safe food-handling skills could be improved in demographic groups of the US population, including males and young adults (Altekruse et al., 1999). Kitchen sanitation guidelines should emphasize cleaning and disinfection of food contact services, hands and utensils following contact with raw meat and poultry. In addition, raw meat and poultry should be stored separately from foods that are served without subsequent cooking. Meat thermometers are recommended to measure the internal temperature of meat and poultry when it is cooking; poultry should be heated to an internal temperature of 82°C (180°F) to kill *Campylobacter*.
1.6.4 Zoonosis prevention

Handwashing after animal contact is a sensible step to prevent zoonotic campylobacteriosis in both household and occupational settings. Additional sanitary precautions are recommended with juvenile or diarrheic pets. It is particularly important to ensure that children wash their hands after animal contact (Friedman et al., 1998). If sufficient attention is given to hygiene, many immunocompromised patients can safely enjoy animal companionship (Angulo et al., 1994).

1.7 Conclusion

It is humbling that a bacterium as sensitive to physiologic stress as C. jejuni remains a common cause of foodborne infection in the new millennium. Well-defined hazards for the transmission of campylobacters exist in the environment and food chain (Figure 7.2). Because no single intervention will eliminate these hazards, a combination of prevention efforts is needed – on farms, in processing plants, in kitchens, and related to animal contact. When considering options for pathogen reduction, it is important to balance cost and effectiveness; however, cost should not be an excuse for inaction.

2 Related organisms

2.1 Other Campylobacter species

Campylobacter species other than C. jejuni and C. coli include C. cervus, C. concisus, C. fetus subspecies fetus, C. hyointestinalis, C. lari, C. mucosalis, C. gracilis, C. rectus, C. showae, C. sputorum and C. upsaliensis. Many are suspected to be human or animal pathogens (Cardarelli-Leite et al., 1996). Many of these related species are also inhibited by the antibiotics in selective media used for routine isolation of C. jejuni and C. coli, and
several species (e.g. C. concisus, C. sputorum, C. cervus, C. rectus and strains of C. hyointestinalis) require different micro-aerophilic incubation conditions than C. jejuni for growth. Furthermore, procedures for the accurate identification of Campylobacter organisms to the species level are time consuming and difficult. Thus the true prevalence of human infections with these other Campylobacter species is unknown.

2.1.1 C. lari
Campylobacter lari was first isolated from mammalian and avian species (Waldenstrom et al., 2002). In 1984, the first case of human disease related to C. lari was reported – fatal bacteremia in an immunocompromised patient with multiple myeloma (Nachamkin et al., 1984). Soon after, sporadic cases of enteric infection were also described (Tauxe et al., 1985). Although C. lari bacteremia is most often reported in patients with underlying disease (Martinot et al., 2001), cases of C. lari bacteremia in immune-competent individuals have also been described (Krause et al., 2002; Werno et al., 2002).

2.1.2 C. fetus subspecies fetus
Until recently C. fetus subspecies fetus was regarded as an animal pathogen, causing bovine and ovine abortion and sterility. Between 1980 and 1995, C. fetus was implicated in at least four reported outbreaks of human disease in North America; three were associated with foods – raw milk, a supplement containing raw calf liver, and cottage cheese (Blom et al., 1995). In addition to being isolated from stools of patients with gastroenteritis, it is recognized to cause invasive infections and has been isolated from human blood, spinal fluid, abscesses, and cellulitis associated with bacteremia (Briedis et al., 2002). Bacteremia is usually seen in patients with underlying disease, such as metastatic malignancy or HIV infection (Blom et al., 1995).

2.1.3 C. hyointestinalis
Between 1979 and 1985, two of four laboratory confirmed cases of C. hyointestinalis that were reported to CDC were from stools of homosexual men (Edmonds et al., 1987). Stool isolates were also obtained from an 8-month-old girl who lived on a farm with livestock, and a 79-year-old woman who had traveled to Egypt. A small outbreak among family members in Canada may have been associated with drinking raw milk (Salama et al., 1992). C. hyointestinalis, with or without C. mucosalis, has been implicated as a causative agent of proliferative ileitis in swine (Boosinger et al., 1985) and diarrhea of calves (Diker et al., 1990).

2.1.4 C. upsaliensis
Since the first isolation of C. upsaliensis was reported in 1983, from the stools of healthy and diarrheic dogs (Sandstedt et al., 1983), pet animals have continued to be suspected as a principal source of human infection (Bourke et al., 1998). In Los Angeles, for example, C. upsaliensis was isolated from pet dogs in the households of two of six patients with C. upsaliensis infection (Labarca et al., 2002).

Initially, C. upsaliensis infections were associated with extremes of age or with underlying disease. Of 11 human isolates reported by the CDC between 1980 and 1986, 8 originated from blood (Patton et al., 1989). Blood isolates originated from
two infants with fever and respiratory symptoms, a woman with an ectopic pregnancy, three elderly men with underlying diseases, and two immunocompromised adults. Of the three stool isolates, one originated from an immunocompromised patient with persistent diarrhea.

It is suspected that there is underreporting of enteric *C. upsaliensis* infections because the antibiotics that are used in selective media for isolation of *C. jejuni* (e.g. cephalothin) inhibit the growth of *C. upsaliensis* (Walmsley and Karmali, 1989). In a Swedish study, *C. upsaliensis* was the second most common *Campylobacter* species in children with diarrhea, after *C. jejuni* (Lindblom et al., 1995); and in Los Angeles County in 1998, *C. upsaliensis* accounted for 4% of fluoroquinolone resistant *Campylobacter* isolates from human patients (Labarca et al., 2002). Regional differences in the prevalence of *C. upsaliensis* infection are suspected, with low prevalence of human infection reported in the United Kingdom (Aspinall et al., 1996) and Denmark (Engberg et al., 2000) compared to Sweden and Los Angeles.

2.2 *Arcobacter*

2.2.1 Historical aspects and current problems

The genus *Arcobacter* was proposed in 1991 to include several aerotolerant campylobacter-like organisms that grow at 15˚ to 30˚C, which is lower than the temperature range used for incubation of *Campylobacter*. Arcobacters have been recovered from livestock and from humans with enteritis (Kiehlbauch et al., 1991) as well as bacteremia (On et al., 1995; Hsueh et al., 1997). Two *Arcobacter* species are most suspected to cause human disease: *A. butzleri* and *A. cryaerophilus*. *A. butzleri* infection was reported in an outbreak of gastroenteritis in schoolchildren (Vandamme et al., 1992), in two patients with chronic disease who developed severe diarrhea (Lerner et al., 1994), and in a neonate with bacteremia (On et al., 1995). *A. cryaerophilus* has been recovered from the blood of a uremic patient with pneumonia (Hsueh et al., 1997). The burden of human illness remains uncertain. In a Danish study of 3267 patients with diarrhea, one stool specimen tested positive for *A. butzleri* and *A. cryaerophilus*, respectively (Engberg et al., 2000).

2.2.2 Environmental sources

*A. butzleri* and *A. cryaerophilus* have been found in various environments, and a focus of research has been the development of protocols for their isolation (Ohlendorf and Murano, 2002). *A. butzleri*-associated diarrheal illness has been reported in non-human primates (Anderson et al., 1993), and *A. cryaerophilus* and *A. butzleri* have each been recovered from late-term aborted porcine and equine fetuses (Wesley et al., 1995). In a study conducted in Denmark, of 10 broiler carcasses obtained from supermarkets and 15 from abattoirs, all carcasses were confirmed to carry *Arcobacter butzleri* (Atabay et al., 1998). Three supermarket and 10 abattoir carcasses also carried *A. cryaerophilus*, and two abattoir carcasses also carried *A. skirrowii*. In the Netherlands, *A. butzleri* was present in 53 of 220 (24%) poultry meat specimens, as well as in some beef and pork meat specimens (de Boer et al., 1996). In Germany, the pathogen was detected over
several months in a drinking water reservoir (Jacob et al., 1993). To the extent that
*A. cryaerophilus* and *A. butzleri* are found to be human pathogens, the findings from
environmental microbiological studies such as these may have significance for public
health protection.

### 2.3 *Helicobacter pylori*

#### 2.3.1 Historical aspects and current problems

Even though *Helicobacter pylori* organisms were cultured for the first time in the early
1980s, descriptions of their presence in the gastric mucosa of humans date from the
beginning of the twentieth century. The organism was first named *Campylobacter*-like
organism (CLO) because of its resemblance to the *Campylobacter* species. Then it was
named *C. pyloridis*, later changed to *C. pylori*, and finally in 1989 it was placed in the
new genus *Helicobacter*, and renamed *H. pylori* (Goodwin et al., 1989; Versalovic and
Fox, 2003).

*H. pylori* colonizes approximately 50% of the adult population in the world;
however, clinical disease occurs in less than 10% of those who are colonized (Torres
et al., 2000; Blaser and Berg, 2001). The diagnosis and treatment of the upper
gastrointestinal disease have changed with the recognition of *H. pylori* as a pathogen
of the gastric mucosa. Superficial gastritis is now considered the histopathological
confirmatory sign of gastric mucosal infection. *H. pylori* infection is associated with
chronic gastritis, duodenal and gastric ulcers, gastric carcinoma, and gastric mucosa-
associated lymphoid tissue. The causal role of *H. pylori* in gastric cancer is almost
universally accepted (Peek and Blaser, 2002).

#### 2.3.2 Characteristics and virulence factors of *H. pylori*

*H. pylori* is a spiral, Gram-negative rod that grows well at 37°C in a micro-aerobic
atmosphere (between 5% and 10% CO₂). The organism prefers enriched media such
as blood agar, or media supplemented with 5% newborn calf serum, starch, fetal
bovine serum or others (Perez-Perez, 2000). Colonies may be observed after 48 hours
of incubation, but in primary cultures from gastric biopsies the incubation should be
prolonged for up to 7 days. The organism is oxidase and catalase positive, and has a
potent urease activity that produces large amounts of NH₃ which allows the neutral-
ization of the normal acid pH of the stomach. The genomes of representative
*H. pylori* strains have been sequenced (Tomb et al. 1997; Alm et al., 1999). The
genome encodes for approximately 1500 proteins. Initial studies indicated that
urease production and motility were essential for the first steps of the infection
(Eaton et al., 1991, 1996). Later studies showed that *H. pylori* binds tightly to epithe-
lial cells using adhesins such as BabA and other members of the Hop protein family
(Ilver et al., 1998). Almost all *H. pylori* strains express a 95-kDa vacuolating
cytotoxin (VacA). Since *in vitro* vacuolating activity is detected in only 60% of the
isolates, the pathogenic role of this toxin is debated. Although VacA is not essential
for colonization, mutant strains of *H. pylori* that are VacA negative are outcompeted
by wild-type bacteria in mouse models (Atherton et al., 1997; Salama et al., 2001).
Another important virulence factor of *H. pylori* is the Cag pathogenicity island
(cag-PAI), which is present in 60% of all isolates (Censini et al., 1996). The cag-PAI is a 37-kb genomic fragment containing 29 genes that most likely was acquired by horizontal gene transfer (Akopyants et al., 1998). Some of the encoded components belong to a type-IV secretion apparatus that injects the 120-kDa protein CagA into host cells where the CagA protein is phosphorylated and induces a strong response of cytokine production by the host cells (Higashi et al., 2002).

2.3.3 Epidemiology and transmission

*H. pylori* infection is present in every country where it has been investigated, but the prevalence varies from country to country, and even among different population groups in the same country. The presence of *H. pylori* is mainly associated with socioeconomic level and age (Malaty and Graham, 1994; Perez-Perez et al., 2002). Prevalence among adults ranges from greater than 80% in many developing countries to between 20% and 40% in developed countries.

It is generally accepted that the organism is acquired by the oral route, and that person-to-person transmission within the family during early childhood is important in the natural history of infection. Food and water are not established vehicles for transmission of the bacterium. There is also no conclusive evidence of animal reservoirs for the transmission of *H. pylori* to humans; although the organism has been found in non-human primates and other animals such as cats (Handt et al., 1994). Once acquired, infection with *H. pylori* is usually chronic, and spontaneous elimination of the bacterium is relatively uncommon (Perez-Perez et al., 2002).

2.3.4 Clinical outcome of *H. pylori* colonization in man

Disease expression as a consequence of *H. pylori* colonization is unpredictable, and is influenced by bacterial and host factors. As was mentioned before, histological gastritis is universally present in patients infected with *H. pylori*. However, the pattern and distribution of gastritis are associated with particular disease outcomes. Patients with antral-predominant gastritis are more likely to develop a duodenal ulcer, whereas patients with corpus-predominant gastritis are more likely to have a gastric ulcer and possibly gastric carcinoma (Suerbaum and Michetti, 2002). The role of *H. pylori* in the development of peptic ulcer disease has been clearly demonstrated. Evidence of the role of *H. pylori* in the pathogenesis of peptic ulcer disease includes the recognition that antimicrobial therapy to eradicate infection dramatically reduces the recurrence rate of *H. pylori*-associated peptic ulcer disease (Marshall et al., 1988). Some of the principal lines of evidence that chronic *H. pylori* infection increases the risk of gastric cancer were derived from large seroepidemiologic cases-control studies (Parsonnet et al., 1991; Nomura et al., 1995), studies using experimental animal models (Fox, 1998), and prospective clinical data from Japan (Uemura et al., 2001). Another impressive line of evidence of the important causal role of *H. pylori* in the development of gastric cancer is the parallel decline in *H. pylori* infection rates and gastric cancer rates in most industrialized countries (Parsonnet, 1995). Eradication and reduced rates of acquisition of *H. pylori* are associated with several clinical advantages, including the cure of peptic ulcer disease and the decrease of gastric cancer; however, the decline of *H. pylori* prevalence has also been suggested as being
the main reason for the increase of gastro-esophageal reflux disease (GERD) and esophageal cancer (Meining et al., 1998; Blaser, 1999). Data from case-control and cohort studies have, for example, suggested that *H. pylori* infection protects against GERD (Chow et al., 1998; Vicari et al., 1998). This hypothesis remains controversial. The high prevalence of *H. pylori* and the interest in its potential role in the etiology of other chronic diseases have prompted epidemiologic studies that suggest that *H. pylori* may be a risk factor for extra-gastrointestinal chronic diseases, including coronary arteriosclerosis (Perez-Perez et al., 1999) and pancreatic cancer (Stolzenberg-Solomon et al., 2001).

### 2.3.5 Diagnostic methods for *H. pylori* infection

Methods for the diagnosis of *H. pylori* can be divided into two categories – invasive and non-invasive – based on whether endoscopic biopsies are performed. Diagnostic options vary depending on the clinical setting and purpose of the test. When endoscopy is clinically indicated, invasive tests are preferred. The diagnostic screening test of first choice is the urease test, because it is relatively simple to perform and less expensive than other diagnostic tests. A histologic diagnosis is definitive, and provides information on the stage of disease. A third option, bacterial culture, permits evaluation of antimicrobial susceptibility patterns with potential implications for therapy. The selection of an invasive diagnostic method is based largely on cost. Cultures to detect *H. pylori*, for example, are not routinely requested, but are recommended when antimicrobial therapy failures occur (Perez-Perez, 2000).

Non-invasive tests are useful for screening large numbers of patients, and have positive applications for epidemiological studies. The non-invasive diagnostic methods include the urea breath test, serological test, and stool antigen detection. The sensitivity of these methods is high, because in theory they are sampling the entire stomach while biopsy samples only a minuscule portion of it. Under certain circumstances, the sensitivity of serologic tests may be diminished. For example, serology is not the optimal test to follow eradication of infection, since the patient’s antibody titers may persist after treatment (Cutler et al., 1995; Perez-Perez et al., 1997).

### 2.3.6 Treatment of *H. pylori* infection

Once eradication of *H. pylori* has been achieved, reinfection is a rare event. For this reason, the benefit of treatment is enormous. *H. pylori* eradication regimens using antibiotics alone have cure rates of less than 80%. Most eradication regimens combine antibiotics with proton-pump inhibitors or other agents that inhibit gastric acid secretion (Lind et al., 1999; Suerbaum and Michetti, 2002). The use of a proton-pump inhibitor in combination with two or three antimicrobial agents for 14 days is often effective in eradicating *H. pylori* infection; however, non-compliance is a concern with this regimen. The use of a combination of antimicrobial agents reduces the risk of selecting resistant *H. pylori* strains. Although most *H. pylori* are susceptible to amoxicillin and tetracycline, resistance to clarithromycin and metronidazole is common, particularly in developing countries (Torres et al., 2001).
3 Summary

C. jejuni is a common bacterial cause of human gastroenteritis in the US and elsewhere. Sequelae of campylobacteriosis add to the burden of disease. Mishandled or improperly prepared poultry products are commonly implicated vehicles for transmission of C. jejuni infections to humans, and restaurants are increasingly recognized as being important venues for infection. Each link in the food chain from producer to consumer has a role in preventing illnesses caused by this pathogen. For organisms related to C. jejuni (e.g., C. upsaliensis, Arcobacter butzleri), a need exists to determine the reservoirs, the clinical syndromes, and the burden of the illnesses they cause, so as to better define prevention priorities and strategies. H. pylori colonizes approximately 50% of the adult population worldwide. H. pylori infection is the principal cause of chronic gastritis, gastric ulcer disease and gastric cancer; however, these diseases only occur in a fraction of people who are colonized. The diagnosis and treatment of upper gastrointestinal diseases have changed with the recognition of H. pylori as a major gastrointestinal pathogen.

Bibliography


