CHARACTERISTICS OF THE ORGANISM AND TYPES OF ILLNESS

The taxonomy has changed considerably over the years and could change in the future, but to date the family Campylobacteraceae includes the genera Campylobacter, Arcobacter, and Sulfurospirillum and the generically misclassified Bacteroides ureolyticus (Vandamme, 2000). In regard to the genus Campylobacter, there are 14 species, and of these species, several are considered pathogenic to humans, causing enteric and extraintestinal illnesses. Campylobacter species are gram-negative, microaerophilic, non-spore-forming organisms with curved or small spiral-shaped cells that have characteristic rapid, darting, reciprocating motility (corkscrew-like motion by means of a single polar flagellum at one or both ends of the cell) and can occur in short or long chains. They range in width from 0.2 to 0.9 μm, and in length from 0.5 to 5 μm, and most species have an optimum temperature range for growth of 30 to 37°C, except for the thermophilic Campylobacter spp., which grow optimally at 42°C. A few strains can grow aerobically or anaerobically. An atmosphere containing increased nitrogen may be needed for optimum growth of certain strains. The cells can form spherical or coccoid bodies as cultures age, and it has been postulated that certain species can have the characteristics of a viable, but not culturable, state (Rollins and Colwell, 1986; Moran and Upton, 1987).

Campylobacter species have a chemoorganotrophic metabolism, and energy is derived from amino acids or tricarboxylic acid cycle intermediates due to their inability to oxidize or ferment carbohydrates. The majority of Campylobacter spp. reduce nitrate and nitrite. Campylobacter spp. have typical biochemical characteristics, which include the reduction of fumarate to succinate; a negative reaction to methyl red, acetone, and indole production; negative hippurate hydrolysis (except for most C. jejuni strains); and positivity for oxidase activity (Vandamme, 2000; Sellars et al., 2002). Campylobacter spp. can be either catalase positive or negative. Broadly speaking, catalase-positive Campylobacter spp. are most often associated with human disease, but not in all cases.

The pathogenic mechanisms by which campylobacteriosis occurs are not totally understood, and information on species other than C. jejuni is scarce, but some important virulence factors include motility, ability to translocate, chemotaxis, and production of toxins (Walker et al., 1986; Kelsey, 1997; Wassenaar, 1997). It appears that the virulence factors involved in the infection greatly influence the symptoms of the disease, and pathogenesis results from several different bacterial properties and host defenses. Motility, which is achieved by means of a single flagellum at one or both ends of the bacterium, has an extremely important role in virulence because it is required for the bacterium to reach the attachment sites and penetrate into the intestinal cells. If the bacterium loses its motility, its ability to colonize the gastrointestinal tract and cause infection is lost.

C. jejuni contains two flagellin genes, flaA and flaB; the wild-type bacterium expresses flaA only, but flaB can be expressed under certain conditions. The flagellum of Campylobacter spp. plays a much more important function than just motility. C. jejuni flagella may also play a role in the dissemination and internalization of the organism. In addition, flagellin has been proposed as an adhesin in the binding to culture cells. It has been shown that C. jejuni is able to become internalized within human intestinal epithelial cells and traverse monolayers of polarized human colonic carcinoma cells, allowing access to
submucosal tissue, which leads to tissue inflammation and damage (Grant et al., 1993; Ketley, 1997).

Toxin production also plays a role in pathogenicity. In regard to C. jejuni, the organism synthesizes several toxins, classified mainly as enterotoxins or cytotoxins (Wassenaar, 1997). Spikes in levels of all immunoglobulin classes have been shown with humans after infection. Even though the synthesis of several toxins has been reported, their mechanism of action and importance in regard to disease still remain unclear. The problem in determining the aforementioned is that researchers have not been able to detect toxin produced by Campylobacter spp. A more comprehensive review of the pathogenesis of Campylobacter spp. can be found in review articles by Walker et al. (1986), Ketley (1997), and Wassenaar (1997).

For approximately 3 decades, the genus Campylobacter has had increased focus as a threat to food safety, due to the rise in enteritis in humans caused by consumption or handling of foods contaminated with the organisms. Four species (C. jejuni, C. coli, C. lari, and C. upsaliensis) are known as “thermophilic campylobacters” and are clinically significant due to their roles as dominant causative agents of human campylobacteriosis (Blaser et al., 1982; Jacobs-Reitsma, 2000; Keener et al., 2004). C. jejuni is the predominant species that causes bacterial gastroenteritis in the United States and in many other developed countries. C. coli is responsible for the majority of other cases of illness. In the United States, campylobacteriosis and salmonellosis go back and forth as the leading cause of bacterial food-borne illness. Transmission of Campylobacter spp. to humans generally occurs by either ingestion of contaminated food or water or by direct contact with fecal material from infected animals or persons. In humans, there are two types of illnesses associated with Campylobacter infections, and they are intestinal and extraintestinal infections. Two types of diarrhea are usually observed with campylobacteriosis: (i) an inflammatory diarrhea, with slimy, bloody stools containing leukocytes and fever and (ii) noninflammatory diarrhea, with watery stools and the absence of blood and leukocytes (Wassenaar, 1997). In some cases, intense abdominal pain, headaches, cramping, and vomiting can occur. Serious complications, such as Reiter’s syndrome, Guillain-Barré syndrome (GBS), osteomyelitis, pancreatitis, nephritis, myocarditis, cystitis, septic abortion, and bacteremia in certain cases, can arise (Altekruse et al., 1999; Winer, 2001; Keener et al., 2004). Although campylobacteriosis does not usually lead to death, it has been estimated that as many as 730 people in the United States with Campylobacter infections die annually, often due to secondary complications (Saleha et al., 1998).

In the vast majority of cases, campylobacteriosis is mainly a self-limiting bacterial gastroenteritis, and recovery is completed in approximately 8 days, either spontaneously or after appropriate antimicrobial therapy. However, in some instances symptoms can persist longer than 2 weeks. The population that is most susceptible to illness includes children less than 1 year of age, young adults aged 15 to 25 years, and immunosuppressed individuals (Keener et al., 2004). A concern for those suffering from campylobacteriosis is that they will suffer from neurological sequelae months or even years afterward. Two neuropathies associated with C. jejuni infections are GBS and Miller Fisher syndrome. Both of these syndromes are characterized by being acute or subacute, immune-mediated neuropathies.

In regard to GBS, this is characterized by alexia, motor paralysis, acellular increase in the total protein content in the cerebrospinal fluid, and inflammatory demyelinating polyradiculoneuropathy. GBS occurs in approximately 1 out of 1,000 cases (Winer, 2001). GBS cases are associated with nerve roots, causing mononuclear infiltration of peripheral nerves, and this eventually leads to primary axonal degeneration or demyelination. Molecular mimicry is believed to be the cause of GBS because a few peripheral nerves of the human neurological system share molecules similar to those of antigens on the surface of C. jejuni cells (Winer, 2001). Since C. jejuni contains a lipopolysaccharide structure (LPS) attached to the outer membrane, the core oligosaccharides of its LPS contain ganglioside-like structures, which are similar to certain human gangliosides (Ang et al., 2001). The LPS structure is very antigenic, and upon exposure to C. jejuni, the immune system produces antibodies against the LPS structure as an attempt to fight the infection. Due to the similarity of the core oligosaccharides of the LPS and the gangliosides, after the infection, antibodies attack the gangliosides on the neuromuscular junction, contributing to the appearance of neurological symptoms (Lindsay, 1997; Ang et al., 2001). A more detailed review of GBS can be found in review articles by Lindsay (1997), Willison and O’Hanlon (2000), and Winer (2001).

**SOURCES AND INCIDENCE IN THE ENVIRONMENT AND FOODS**

Unlike many other enteric pathogens, Campylobacter spp. have limited spread from host to host. Campylobacter spp. may not be recovered by conventional cultural methods outside of the host if exposed to dry conditions or atmospheric oxygen levels for extended periods of time. Campylobacter...
enteritis can be classified as a zoonosis, because animals are the main reservoir of these organisms. *Campylobacter* spp. exist as commensals in many wild and domestic animals (Keener et al., 2004). This presents a risk to food safety due to the contamination of carcasses at slaughter and other foodstuffs by cross-contamination when raw or undercooked meat is handled. Contamination with this pathogen can occur at numerous stages along the food chain. This includes, but is not limited to, production, processing, distribution, handling, and preparation. *Campylobacter* spp. are fastidious organisms that are capable of existing in a broad range of environments and have been sporadically recovered from rivers, coastal waters, shellfish, and vegetables but routinely recovered from sheep, cattle, swine, rodents, and avian species (Jacobs-Reitsma, 2000; Kemp et al., 2005). Certain species of *Campylobacter* are routinely associated with certain species of animals. In poultry and cattle, *C. jejuni* is the predominant species, and *C. coli* is the common species recovered from swine. The majority of *Campylobacter* infections are sporadic, and outbreaks are rare but have been traced back to contaminated water, raw milk, poultry, beef, eggs, fruits, and contact with farm animals and pets (Altekruse et al., 1999; Friedman et al., 2004). Generally speaking, the primary source of contamination of the environment and foods is believed to be from animal feces (Brown et al., 2004).

Avian species, particularly poultry, are the most common host for *Campylobacter* spp.; therefore, poultry is considered the main source of human illness. Studies have shown that as much as 70% of human illnesses due to *Campylobacter* spp. are caused by the consumption or handling of raw or undercooked poultry or poultry products. Increased attention has been given to reducing the level of *Campylobacter* spp. in poultry pre- and postharvest to reduce the level and incidence of raw product contamination (Allos, 2001; Friedman et al., 2004; Keener et al., 2004). The ecology of *Campylobacter* spp. in poultry is not fully elucidated. Numerous studies are being conducted to determine when and how *Campylobacter* spp. gain entry into poultry flocks so that more effective intervention strategies can be employed.

*Campylobacter* spp. colonize the mucus layer of the intestinal tract but have been recovered from numerous tissues and organs within the bird, suggesting it is not limited to the digestive tract. In addition to the digestive tract, *Campylobacter* spp. have been isolated from the circulating blood, thymus, spleen, liver, gallbladder, unabsorbed yolk sacs, ovarian follicles, and reproductive tracts of commercial poultry (Cox et al., 2005, 2006, 2007; Richardson et al., 2007a). In regard to the digestive tract, levels up to 10^7 CFU/g of fecal content have been shown (Altekruse et al., 1999). Two modes of transmission of *Campylobacter* into poultry flocks occur and they are horizontal and vertical transmission (Keener et al., 2004; Byrd et al., 2007). It has been shown that if a single bird in a flock is colonized, then the spread to adjacent rearing mates is rapid, and within a week *Campylobacter* prevalence in the flock can reach 100% (Beery et al., 1988; Gregory et al., 1997; Wallace et al., 1998).

The prevalence of *Campylobacter* contamination of carcasses and poultry products can vary greatly, depending on the sensitivity of the cultural procedures utilized and by the point along the process chain at which sampling is being conducted. The type of methodology employed significantly affects prevalence rates of *Campylobacter* spp. from carcasses at the final stages of processing. For example, if a survey is being conducted on the prevalence of *Campylobacter* on poultry carcasses postchill and enrichment of the sample is utilized, then as much as 70% to 100% of the samples can be positive for *Campylobacter*. However, if a less sensitive method is utilized, such as direct plating onto selective agar, which may exclude sublethally injured cells, then the number of samples detected as positive could be greatly reduced. Including both direct plating and enrichment often allows the best probability for recovery. Even though the enrichment used is designed to be selective for *Campylobacter* spp., the organisms can be culturally fragile, to the extent that they can be overgrown by organisms that were meant to be suppressed (Musgrove et al., 2001). A question often asked is whether the injured or stressed cells could have the ability to infect humans and cause illness. This is one reason why studies on the incidence of *Campylobacter* in poultry processing plants vary, and it is critical to consider the cultural procedures utilized and the impact those choices have on sensitivity to recover or detect the organism.

A significantly high prevalence rate of *Campylobacter* spp. contamination can be found in retail poultry and poultry products is often directly related to the prevalence rate at the farm. The reported prevalence rate for the farm continuum varies between studies, but on average greater than 70% of the birds are *Campylobacter* positive (Maekin et al., 2008; Allen et al., 2007). In a study of supermarkets, *Campylobacter* spp. were isolated from 82%, 82%, and 71% of whole chickens, breast with skin attached, and pieces, respectively (Harrison et al., 2001). However, *Campylobacter* incidence within the farm continuum does not generally relate to an increase in retail positives in other types of animal products.
**Camphylobacter** prevalence rates in one study reported 47% of cattle and 46% of swine harbored the organism within the farm continuum (Nielsen et al., 1997). However, comparatively low prevalence rates (less than 2%) of **Camphylobacter** spp. have been found in these meat products at the retail level (Zhao et al., 2001). This could be due to, but is not limited to, a number of factors such as commensal level in poultry; skin removal from the carcasses of other animals during processing, unlike poultry; processing procedures utilized for poultry carcasses; and the sheer number of poultry carcasses being processed in the plant each day.

**INTRINSIC AND EXTRINSIC FACTORS THAT AFFECT SURVIVAL AND GROWTH IN FOOD PRODUCTS AND CONTRIBUTE TO OUTBREAKS**

Cross-contamination of food products is a major factor that contributes to human illness. **Camphylobacter** spp. can be sensitive to environmental conditions outside of an animal’s intestinal tract. Even though **Camphylobacter** spp. are sensitive to drying, high oxygen concentration, and low pH (less than or equal to 4.7), they are still one of the biggest causes of gastroenteritis. Several studies have shown that strong acids, such as formic, acetic, ascorbic, and lactic acids, rapidly inhibit the growth and survival of **Camphylobacter** spp. **Camphylobacter** spp. are sensitive to sodium chloride, with an optimum growth concentration of 0.5% (Doyle and Roman, 1982a). Sensitivity to salt also shows a temperature-dependent effect. At 4°C, **C. jejuni** was sensitive to 1.0% or more NaCl, but the rate of death at this temperature was much less than that at 25°C. A 3 log<sub>10</sub> decrease of cells occurred in 4.5% NaCl after 1.25 to 2.1 days at 25°C, but a similar reduction took about 2 weeks at the same salt concentration when a temperature of 4°C was maintained.

The decimal reduction time for **Camphylobacter** spp. varies, depending on the type of food product, but survival kinetics generally follow a rapid decline in numbers, which is followed by a slower rate of inactivation. This may explain the high survival rate of **Camphylobacter** spp. on poultry carcasses due to the high levels of the organisms in the bird’s digestive tract at the time of processing. Studies have shown that the potential for survival decreases to a few hours at temperatures of 37°C and increases to a few days at temperatures of 4°C. However, **Camphylobacter** spp. have been shown to survive for several weeks in groundwater (Buswell et al., 1998).

When environments become unfavorable for growth, **C. jejuni**, it is postulated, can enter into a viable but nonculturable (VBNC) state. The cells are metabolically active and show signs of respiratory activity but are unable to be cultured through conventional methodology procedures. The VBNC stage was first described by Rollins and Colwell (1986), who postulated that it could play a role in human infection and illness. The VBNC state arises from exposure to sublethal adverse environmental conditions, and recovery occurs by passage of the organism to a susceptible host. Several studies have explored the recovery of VBNC forms of **Camphylobacter** cells (Jones et al., 1991; Saha and Sanyel, 1991; Chaveerach et al., 2003; Richardson et al., 2007b). Nonculturable **C. jejuni** and **C. coli** after subjection to acid stress were shown to be viable by injecting the cultures into the amniotic fluid and yolk sac of fertilized eggs (Chaveerach et al., 2003). In a separate study, **Camphylobacter** cells were subjected to dry stress on filter and chick paper pads and after becoming nonculturable were determined to be viable utilizing a chick bioassay (Richardson et al., 2007b). In addition, freeze-thaw-injured **C. jejuni** cells that were nonculturable were converted back to culturable after passage through the rat gut (Saha et al., 1991). The significance of the VBNC state remains unclear and controversial, but as the understanding of this phenomenon unfolds, this could shed light on how **Camphylobacter** spp. survive and persist in certain food commodities and go undetected in dry environments.

**FOOD PROCESSING OPERATIONS THAT INFLUENCE THE NUMBERS, SPREAD, OR CHARACTERISTICS**

While outbreaks of human campylobacteriosis have been associated with raw milk and untreated water, poultry meat, which is frequently contaminated with the organism, may be responsible for as much as 70% of sporadic campylobacteriosis (Skirrow, 1991). Contamination is thought to originate from the intestinal tract of primarily avian species (mainly poultry) and then spread to the meat during transport and processing, though it has also been demonstrated that broiler crops, particularly after a feed withdrawal prior to transport to the processing facility, may harbor large numbers of **Camphylobacter** bacteria (Musgrove et al., 1997; Berrang et al., 2001). Crops burst more often than cecal pouches or other parts of the gut and can contaminate previously
Campylobacter-free carcasses. As birds enter the plant, levels of Campylobacter in the intestinal tract can be as high as $10^7$ CFU/g cecal contents, and when whole carcasses with feathers are rinsed, $10^6$ CFU/ml of rinse can be recovered (Berrang et al., 2004; Northcutt, 2005). External contamination often increases during transport from grow-out houses to the processing plant. Generally, Campylobacter counts decrease in the scalding tank, increase during removal of feathers (picking), and are at their highest immediately following evisceration (Berrang and Cason, 2000). In most commercial processing facilities, carcasses are chilled in a series of tanks using recirculated cool water. This part of the process has a tendency to dilute Campylobacter numbers, especially on those carcasses that are highly contaminated. Since passage of hazard analysis and critical control points, the amount of water used during processing has increased dramatically, and many researchers report a decreased incidence of Campylobacter contamination on many poultry products.

Campylobacter is a culturally fastidious organism. Recovering this organism from samples which are low in biologically available moisture can be especially challenging. This has contributed to the perception that Campylobacter contamination of egg may not be important. Doyle (1984) reported that only 8.1% of layer hens shed Campylobacter chronically and found ~1% of shell-contaminated table eggs. Izat and Gardner (1988) sampled two commercial egg processing facilities. Samples analyzed included eggs, egg products, egg wash water, and surfaces within the facilities. Campylobacter was never recovered from any of the sample types. However, cultural media for Campylobacter have seen many changes in the last 15 to 20 years. Cox sampled spent hens and a small number of eggs from a commercial shell egg washing facility. While Campylobacter spp. were not recovered from either egg contents or shells, most of the hens were positive for the organism, including the reproductive tract (Cox et al., 2006). Musgrove and Jones (2006) sampled packer head brushes at two commercial shell egg processing facilities. These brushes assist in gently transferring eggs from the weighing/grading equipment to a series of belts, some of the last surfaces to touch the eggs before they reach retail packaging. Though low rates of recovery were observed with packer head brushes (1.5%) and pooled crushed egg shells/membranes (4.2%), the presence of the bacteria bears consideration, particularly since an egg-borne outbreak of campylobacteriosis has been reported (Finch and Blake, 1985).

**RECENT ADVANTAGES IN BIOLOGICAL, CHEMICAL, AND PHYSICAL INTERVENTIONS TO GUARD AGAINST THE PATHOGEN**

In developed countries worldwide a great deal of effort is being expended on developing interventions in Campylobacter contamination of poultry and poultry products. Preharvest efforts include improved biosecurity, training of farm personnel, drinking water amendments, and even treatment with other microorganisms or their products (Humphrey et al., 1993; Gibbens et al., 2001). Competitive exclusion products can be supplied to hatching chicks to prevent enteric colonization by human pathogens. Generally, more success has been achieved in suppressing Salmonella than Campylobacter, but some degree of protection has been observed for various formulations. Commercial products, many of which are of a defined nature, are now available. However, undefined cultures made from mucosal scrapings have been the most effective. Truly effective vaccines and application strategies have yet to be developed for prevention of avian intestinal colonization. A formalin-inactivated C. jejuni whole-cell vaccine was shown to reduce colonization in immunized chickens by 16% to 93%, and purification of flagellin antigens by preservation of conformational epitopes may enhance their use as vaccines (Widders et al., 1998; Muir et al., 2000). Other interventions have included use of other microorganisms, such as Pseudomonas aeruginosa or excretory-secretory products of Trichuris suis to affect attachment to skin or intestinal epithelial cells (Abner et al., 2002). Campylobacter colonization of broilers has been affected by application of bacteriophages (Carillo et al., 2005; Wagenaar et al., 2005).

Postharvest efforts include making plants sanitary, ensuring clean water for processing, maintaining increased water during processing, adding new equipment, evaluating chill tank water sanitizers, providing carcasses frozen instead of fresh, irradiating, and antimicrobial packaging. In order to reduce the chances of cross-contamination, amendments such as sodium hypochlorite, acidified sodium chlorite, and trisodium phosphate may be added to the inside-outside bird washer or to the chill tank (Kemp et al., 2001; Chantarapagant et al., 2002). Prior to further processing or transport to a retail market, carcasses and other poultry products may be stored under refrigerated or frozen conditions. Survivability of the organism is favored at refrigerated temperatures but may also occur under frozen conditions. Oosterom et al. (1983) reported that freezing affected C. jejuni only during the first few hours. They detected an initial drop but determined
that the organism could survive on chicken carcasses and chicken livers at −20°C for more than 64 and 84 days, respectively. *Campylobacter* spp. die faster at 25°C than at either 4 or 30°C. Heat injury of *C. jejuni* occurs at 46°C, and death occurs at 48°C. Doyle (1982b) determined rates of thermal inactivation for five strains of *C. jejuni* in a skim-milk heat menstrum, and at 48°C, $D$ values ranged from 7.2 to 12.8 min, while at 55°C they ranged from 0.74 to 1.00 min. These data indicate that ordinary cooking temperatures should be sufficient to destroy *campylobacters* contaminating poultry or other meat samples.

*Campylobacter* spp. are sensitive to certain spices and food ingredients, such as sodium chloride. Deibel and Banwart (1994) conducted a study on the effects of various concentrations of oregano, sage, and ground cloves on the growth of *C. jejuni* in a liquid growth medium incubated at 4°C, 25°C, and 42°C over a 48-h period. At 25°C, more than a 3 $\log_{10}$ decrease in cell numbers was observed with the suspensions containing sage or oregano. At 4°C, less than a 1 $\log_{10}$ reduction was observed for any of the three spices. Koidis and Doyle (1983) analyzed the inhibitory effects of garlic, onion, black pepper, and oregano on *C. jejuni* in an enrichment nutrient broth stored at 4°C. A 3.9 $\log_{10}$ decrease was noted for the broth containing onion, and 3.0 $\log_{10}$ reductions below the respective controls were noted for the maximum concentrations of garlic, pepper, and oregano. This may suggest that further processing, particularly marination, may create a more hostile environment for *Campylobacter* spp.

**DISCRIMINATIVE DETECTION METHODS FOR CONFIRMATION AND TRACE-BACK OF CONTAMINATED PRODUCTS**

Isolation, identification, and confirmation of *Campylobacter* isolates by traditional methods require plating enrichment broth samples after incubation onto selective agar plates. Typical colonies are smooth, convex, and glistening, with a distinct edge, or flat, shiny, translucent, and spreading, with an irregular edge. They are colorless or grayish or light cream and may range from pinpoint to 4 to 5 mm in diameter. Growth may be confluent without distinct colonies, particularly on wet agar. Presumptive *Campylobacter* isolates should be observed using dark-field or phase-contrast microscopy for characteristic morphology and motility; however, cells from cultures older than 24 h may appear coccoidal and nonmotile (Mugrove et al., 2001). An isolated colony can be useful in further characterization, in terms of antimicrobial resistance, speciation, relatedness to other isolates, or pinpointing sources for epidemiological purposes, though some information can be obtained without obtaining a specific isolate (Lu et al., 2003). In addition, the method used for isolation can affect genotyping results (Newell et al., 2001).

*Campylobacter* spp. can be confirmed using latex agglutination assays, which are commercially available. A variety of other automated kits and systems based on either immunological or molecular criteria are now available, and some methods combine various identification approaches (Cloak et al., 2001; Padungtod et al., 2002). Immunomagnetic separation of *Campylobacter* spp. coupled with PCR-based detection systems are being developed for detection with food samples (Docherty et al., 1996).

Typing of *Campylobacter* strains is needed for epidemiological purposes. By 1982, the most widely used serological schemes were published and in use (Penner and Hennessy, 1980; Lior et al., 1982). Typing methods can be divided into categories, one of which is typing based on phenotypic characteristics, such as heat-stable or heat-labile antigens or antimicrobial susceptibility patterns. A second category consists of the various genotyping analyses. Included in this category are restriction fragment length polymorphism (RFLP) of selected genomes, such as done with ribotyping or other genomes; bacterial restriction endonuclease DNA analysis (BRENDA); and pulsed-field gel electrophoresis. The usefulness of any of these approaches depends upon availability of resources and personnel time and reproducibility and discriminatory capability of the method, as well as the openness of management to embrace new and sometimes daunting technologies. In the last 15 years, strain differentiation has shifted to highly specific genotyping analyses, including flagellin typing, randomly amplified polymorphic DNA, pulsed-field gel electrophoresis of chromosomal DNA, multiplex PCR-RFLP, and ribotyping. Genotyping techniques can be used to determine sources of infection and routes of transmission in humans and animals, though the variety of approaches published in studies worldwide make interlaboratory comparisons difficult (Wassenaar and Newell, 2000).

Grajewski et al. (1985) originally described a set of 14 phage types which discriminated about 90% of 255 human isolates tested. Therefore, to be of value, it appears that phage typing should be used together with another typing method to confidently discriminate among isolates. Another phenotypic assay used to discriminate between isolates involves the analysis
of the outer membrane protein contents of *Campylobacter* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Although discrimination is possible, limitations of sodium dodecyl sulfate-polyacrylamide gel electrophoresis to discriminate between isolates have also been reported (Derclay et al., 1989). In addition, considerable technician time is required for such analyses, and interlaboratory reproducibility has been poor.

An early comparison of 10 methods for sub-grouping *Campylobacter* strains was published in 1991 (Patton et al., 1991). The genotyping methods studied included multilocus enzyme electrophoresis (MEE), BRENDA, restriction digests of selected bacterial chromosomal DNA (ribotyping), and plasmid profile analysis. For the MEE approach, isolates were assayed for enzymatic activity and the patterns were then statistically analyzed to determine the genetic distances between strains. BRENDA entailed electrophoresing DNA fragments, and observing patterns were revealed by UV illumination following ethidium bromide staining. Ribotyping analysis required characterizing the strains for restriction patterns resulting from the digest of the ribosomal DNA. The authors concluded that MEE, BRENDA, and ribotyping were the most sensitive methods that were capable of identifying nine types among 22 strains. The authors observed that MEE and ribotyping had several advantages over the other methods because they measured relatively stable and significant chromosomal differences and were applicable to other species and genera.

Hernandez et al. (1996) analyzed 39 strains of *Campylobacter* by ribotyping and confirmed the above favorable conclusions. Modification to the method by Patton et al. (1991) employed use of the HaeIII enzyme to digest the isolated chromosomal DNA. Upon electrophoresis of the digest product, they were able to distinguish 32 different band profiles that allowed discrimination between the strains analyzed. Each ribotyping pattern comprised between 3 and 11 fragments of 1 to 10 kb.

A typing scheme for *Campylobacter* spp. has been described (Nachamkin et al., 1996) and entails probing the flagellin gene, flaA, for differences or similarities between strains. The method uses a PCR and RFLP. Meinersmann et al. (1997) have described a system that allows differentiation of strains based on differences in flaA gene sequencing. Methods such as these are now the standard means for determining outbreak strains and for more in-depth analysis of epidemiological data. Techniques such as these allow for a greater level of discrimination than was previously possible. However, for many methods, genotype instability has also been demonstrated. Multilocus sequence typing has shown that *C. jejuni* is partly nonclonal, having a natural ability to take up DNA. This genome plasticity, allowing for recombinations within loci used in typing, makes for complex population genetics and further complicates interpretations of typing data (Wassenaar, 2004).

**CONCLUDING REMARKS**

Commercial poultry have been shown to be a major source of *Campylobacter* gastroenteritis in humans. *C. jejuni* and *C. coli* are the species most often associated with human illness and isolated from poultry. *Campylobacter* spp. are widespread in poultry, and in order to reduce the contamination level of poultry products, reductions within the preharvest continuum will have to be accomplished in order to reduce the level of contamination entering processing facilities. In order for this to be accomplished, a large portion of microbiologists will have to stop arguing whether *Campylobacter* is transmitted via the egg from parent birds to progeny. Many continue to say that there is no evidence to suggest egg transmission and hatchery contamination when the evidence is not only present, but in recent years overwhelming (Clarke and Bueschken, 1985; Lindblom et al., 1986; Maruyama and Katsube, 1990; Maruyama et al., 1995; Chuma et al., 1994, 1997; Pearson et al., 1996; Cox et al., 2002; Hiett et al., 2002, 2003; Acevedo, 2005; Byrd et al., 2007). It has never been said that vertical transmission is the only source of contamination of a flock, but it is definitely a source, and additional evidence continues to mount. If the research community continues to ignore published facts, then this source will always be present and the level of contamination of commercial poultry will never be eliminated in reference to *Campylobacter*.

There is conflicting evidence in the published literature as to whether *Campylobacter* can pass from breeder hen to progeny through the fertile egg, whether it is vertical or horizontal contamination of the egg and its developing embryo. It is easy to take issue with some of the studies which support the hypothesis that *Campylobacter* is not transmitted by the egg. The number of samples tested in some studies is very small, and then strong conclusions, such as "*Campylobacter* cannot be transmitted through the egg," are made. For example, Acuff et al. (1982) stated that no *Campylobacter* could be found in turkey eggs or young turkey pouls; however, only 20 samples of each were tested, with sampling methods that were not very sensitive. Callicott et al. (2006) stated that they could not find any evidence for vertical transmission of *Campylobacter* when only 13...
pooled fluff samples were tested out of 60,000 parent breeders. Studies such as these leave the impression that transmission via the egg is highly improbable; however, the majority of the published data support just the opposite conclusion.

For example, when fertile chicken eggs were inoculated with Campylobacter by pressure differential, 11% of these chicks at hatch had the inoculated microorganism in their intestinal tract (Clarke and Bueschkens, 1985). Also, Campylobacter could be transmitted to the offspring via the egg following oral inoculation of Japanese quails (Maruyama and Katsube, 1990). Chickens raised in a laboratory environment without exposure to any farm environment still became colonized by Campylobacter (Lindblom et al., 1986). Studies using a sensitive detection method (colony DNA hybridization) indicated the carrier rate of Campylobacter in the cecal contents of newly hatched chicks to be as much as 35%, suggesting that the chicks were already contaminated with Campylobacter before they were delivered to the farm (Chuma et al., 1994). More recently, Campylobacter isolates from two independent commercial broiler breeder flocks, as well as from their respective progeny, were shown to be clonal in origin using both ribotyping and DNA sequencing analysis (Cox et al., 2002). Through molecular testing, Campylobacter has been found in hatchery fluff, intestinal tracts of developing embryos, and newly hatched chicks (Chuma et al., 1994, 1997; Hiett et al., 2002, 2003). Also, epidemiological surveys have traced the source of broiler flock infections to hatcheries (Pearson et al., 1996).

However, even in light of these numerous peer-reviewed published studies demonstrating the transmission of Campylobacter from breeders and hatcheries to broiler flocks, obvious deficiencies in the standard cultural methodologies prevent this hypothesis from being universally accepted. The actual samples may be called negative utilizing traditional cultural detection methods, when Campylobacter actually is present, but in low numbers or in a VBNC state. Some recent studies support this hypothesis. Byrd et al. (2007), using a modified methodology procedure, were able to culture Campylobacter from three different commercial hatchery chick pads, and the breeders providing eggs to these hatcheries were also positive for Campylobacter. In addition, Campylobacter was cultured from a commercial incubator and from the interior egg content and egg surfaces of fertile commercial breeder eggs (Acevedo, 2005).

These studies conclusively prove that Campylobacter is present in the hatchery, breeder flocks, and chicks prior to exposure to any possible environmental source. As we continue to improve our laboratory methods to detect Campylobacter, both viable and dry-stressed viable, but presently nonculturable, it will be accepted as fact that the fertile egg is a significant source of Campylobacter spp. Given that other microorganisms, such as E. coli and Salmonella spp., have been demonstrated to transmit from one generation of chickens to another via fertile eggs (Gordon and Tucker, 1965; Humphrey et al., 1991; Petersen et al., 2006), it seems strange that some scientists refuse to consider that Campylobacter is also doing the same. Perhaps, the main reason is the inability of routine cultural methods to consistently recover these organisms from many dry types of samples (e.g., egg shells, hatching debris, etc.). Modifications in standard laboratory procedures have led to recent discoveries. As methodology procedures improve and our understanding of the Campylobacter ecology improves, vertical transmission will not be debated, but will become part of a more focused and effective set of intervention strategies. Due to the array of environmental conditions in the poultry continuum, this provides an excellent means of studying the ecology of Campylobacter spp., and with knowledge, vision, and persistence, numerous advances and discoveries can be achieved.

REFERENCES


