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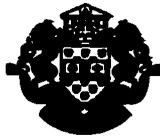

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A thesis submitted to the School of Graduate Studies

University of Ottawa

In partial fulfilment of the requirements for the degree of

Master of Science

Department of Biochemistry, Microbiology & Immunology

Faculty of Medicine

By

Diane Medeiros

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ABSTRACT

Campylobacteriosis is the most frequently reported type of acute bacterial gastroenteritis in Canada. In 2000 alone, 11,846 campylobacteriosis cases were reported in Canada. The majority of these cases are sporadic, and their causes remain unknown. An attempt was made to gain a better understanding of the epidemiology of campylobacteriosis, both by identifying foods and environments that harbor *Campylobacter* spp., and by characterizing clinical, food and environmental isolates using pulsed-field gel electrophoresis (PFGE). Spot maps were also used to determine the geographical relationship of these campylobacteriosis cases.

A variety of raw and ready-to-eat foods were tested for the presence of *Campylobacter* spp. From the 300 samples analyzed, *Campylobacter* spp. were detected in four samples, one raw beef liver sample and three raw chicken samples. An isolation rate of 9.7% was observed among the raw chicken samples tested, a significantly-reduced percentage, as compared to a 1981 Canadian survey. The prevalence of *Campylobacter* spp. in a poultry foodservice operation in Ottawa, was also determined from March to August 2001. No *Campylobacter* spp. were detected in the 125 samples tested.

Campylobacter clinical and food isolates were characterized using PFGE with two restriction enzymes, *Sma*I and *Kpn*I. *Kpn*I produced more complex banding patterns than *Sma*I, and proved to be more discriminatory. Among the 154 isolates assigned to clusters by *Sma*I, only 42% gave concordant results with *Kpn*I. In contrast, among the 53 isolates assigned to 23 clusters by *Kpn*I, 87% gave concordant results with *Sma*I. Five of the 20 concordant clusters represented isolates obtained from the same person, suggesting that some of these individuals may have become re-infected.

Spot map analysis revealed a significant clustering of campylobacteriosis cases in the former city of Ottawa, most of which, did not belong to the same postal code. In contrast, very few cases were observed in outlying regions; however, most of these cases belonged to the same postal code, suggesting the possible presence of local outbreaks.

Dedicated to my Family and Friends, and especially to Harry

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LIST OF ABBREVIATIONS

AFLP	amplified fragment length polymorphism
BSA	bovine serum albumin
bp	base pair(s)
CCDA	modified charcoal cefaperazone desoxycholate agar
CDC	Centres for Disease Control and Prevention, USA
CDT	cytolethal distending toxic
CFU	colony forming unit(s)
cm	centimetre(s)
DNA	deoxyribonucleic acid
D-value	decimal reduction value
EDTA	ethylenediaminetetra acetic acid
ESP	EDTA-sarcosine-proteinase K
FDA	Food and Drug Administration, USA
g	gram(s)
GBS	Guillian-Barré syndrome
h	hour(s)
HPFB	Health Products and Food Branch, Health Canada
INT	intestinal
kb	kilobase(s)
kGy	kiloGray(s)
L	litre(s)

LOS	lipooligosaccharide
LPS	lipopolysaccharide
M	molar(s)
mg	milligram(s)
MHA	Mueller-Hinton agar
MIC	minimum inhibitory concentration
min	minute(s)
ml	millilitre(s)
mm	millimetre(s)
mM	millimolar
MPN	most probable number
NCTC	National Type Culture Collection
O.D.	optical density
OMP	outer membrane protein(s)
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PPHB	Population and Public Health Branch, Health Canada
RAPD	random amplification of polymorphic DNA
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
rpm	revolutions per minute
TBE	Tris-borate, EDTA

TE	Tris-EDTA
U	units
USDA	United States Drug Administration
u.v.	ultraviolet
VBNC	viable but non-culturable
vol	volume
V	volts
wt	weight
µg	microgram(s)
µl	microlitre(s)
µm	micrometer(s)

1. INTRODUCTION

1.1 *Campylobacter* species

1.1.1 Historical background

Campylobacter species were first described in 1886 by Theodor Escherich. In 1913, McFayden and Stockman isolated *Vibrio*-like bacteria from aborted sheep fetuses. A few years later, Smith (1918) discovered similar bacteria in aborted cattle fetuses, and concluded that these bacteria belonged to the same species as those described by McFayden and Stockman, for which he proposed the name *Vibrio fetus* (Smith, 1918; Smith and Taylor, 1919). In the following decades, *V. fetus* continued to be isolated from the fetal fluids of aborted cattle and sheep, as well as the blood of women who aborted. In 1927, another *Vibrio*-like organism was isolated, this time from the faeces of cattle with diarrhea; this organism was named *Vibrio jejuni* (Smith and Orcutt, 1927). *V. jejuni* was subsequently isolated from clinical samples of human gastroenteritis cases, and aborted sheep fetuses. In 1944, an additional group of vibrios was isolated from the faeces of pigs with diarrhea, and named *Vibrio coli* (Doyle, 1944; Doyle, 1948). Additional species were isolated and described during the 1940s and 1950s, including *V. sputorum* (Tunicliff, 1914; Prévot, 1940). It was not until 1963, after taxonomic studies revealed that *V. jejuni* had a G+C content of 33-35 mol%, rather than the 47 mol% of other *Vibrio* spp., that the genus *Campylobacter* was proposed by Sebald and Véron (1963). As a result of subsequent taxonomic studies, four distinct species were included in the genus *Campylobacter*: *C. fetus*, *C. jejuni*, *C. coli* and *C. sputorum*.

Campylobacter spp. were not recognized as significant causes of human gastroenteritis until the 1970s (Dekeyser, 1972; Skirrow, 1977). Since then, surveillance has shown these

organisms to be the most common cause of gastroenteritis in North America and the United Kingdom, and a major cause of gastroenteritis worldwide.

1.1.2. Taxonomy

Family *Campylobacteraceae*

Campylobacter spp. are included in the epsilon division of the Proteobacteria (also known as rRNA superfamily VI), and are members of the family *Campylobacteraceae* (Vandamme et al., 1991; Vandamme and Goossens, 1992; Trust et al., 1994). Members of this family occur primarily as parasites in humans and wild and domestic animals, and include the genera *Campylobacter*, *Arcobacter*, and *Sulfurospirillium*, as well as *Bacteroides ureolyticus*. Neighboring groups include the genera *Helicobacter* and *Wolinella*, members of the recently created family *Helicobacteraceae*. Table 1 highlights some of the features of the genera *Arcobacter* and *Helicobacter*, whose members were previously classified as campylobacters, in contrast to those of the genus *Campylobacter*.

Genus *Campylobacter*

The genus *Campylobacter* now includes 16 recognized species and 8 subspecies, with *C. fetus* being the type species (Table 2) (On, 2001). *C. jejuni* and *C. coli* are the most important human pathogens among the campylobacters; *C. lari*, *C. upsaliensis*, *C. hyointestinalis* and *C. fetus* are occasionally associated with human illness. Whereas *C. jejuni* has been isolated from a wide variety of wild and domestic animals, C. *coli* is the dominant *Campylobacter* in swine and *C. lari* is mainly isolated from sea gulls.

1.1.3 Microbiology

Campylobacter cells are gram-negative, nonsporeforming, slender, S-shaped rods, 0.2-0.8 µm

Table 1. Characteristics of the genera *Campylobacter*, *Arcobacter* and *Helicobacter*. [a]

Genus	Aerobic growth	Position of flagella	Flagella sheath	Cell width (μm)	G+C content (mol %)
Campylobacter	no	Polar	absent	0.2-0.8	30-46
Helicobacter	yes	Polar and lateral	present	0.5-1.0	35-44
Arcobacter	no	Polar	absent	0.2-0.9	28-31

[a] Adapted from Wallace, 1997.

Table 2. Differentiation of *Campylobacter* species.[a]

Species	Cat[d]	25°C	42°C	Nal	Cep	Hip	NO_3	IAH
C. jejuni subsp. *jejuni*	+[b]	–	+	S[c]	R	+	+	+
C. jejuni subsp. *doylei*	+	–	–	S	S	+	–	+
C. coli	+	–	+	S	R	–	+	+
C. lari	+	–	+	R	R	–	+	–
C. fetus subsp. *fetus*	+	+	V	R	S	–	+	–
C. fetus subsp. *veneralis*	V	+	–	R	S	–	+	–
C. consisus	–	–	+	R	R	–	+	–
C. hyointestinalis subsp. *hyointestinalis*	+	V	+	R	V	–	+	–
C. hyointestinalis subsp. *lawsonii*	+	–	+	R	S	–	+	–
C. mucosalis	–	–	+	R	S	–	+	–
C. sputorum subsp. *sputorum*	–	–	+	S	S	V	+	–
C. sputorum subsp. *bubulus*	–	–	+	R	S	–	+	–
C. upsaliensis	V	V	+	S	S	–	+	+
C. curvus	–	–	+	S	S	–	+	+
C. rectus	–	–	V	S		–	+	+
C. helveticus	–	–	+	–	–	–	+	+
C. gracilis	V	–	V	V	–	–	V	V
C. showae	+	–	V	–	–	–	+	V
C. hominus	–	V	V	V	V	–	V	–
C. lanienae	+	–	+	R	R	–	+	–

[a] Adapted from Bergey's Manual and Wallace, 1997.
[b] +, positive reaction; –, negative reaction; V, variable reaction (strain-dependent).
[c] S, susceptible; R, resistant.
[d] Cat, catalase; 25°C, growth at 25°C; 42°C, growth at 42°C; Nal, susceptibility to nalidixic acid; Cep, susceptibility to cephalothin; Hip, hippurate hydrolysis; NO_3, nitrate reduction; IAH, indoxyl acetate hydrolysis.

wide and 0.5-5 μm long. Cells in old cultures may form coccoid bodies, which are considered degenerative forms, rather than a dormant stage of the organism (Hazeleger et al., 1994). Species are motile, exhibiting a characteristic corkscrew-like motility, by means of a polar unsheathed flagellum at one or both ends of the cell. Most species grow under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) at a temperature range of 25 to 42°C. *Campylobacter* spp. generally grow well in the pH range of 5.5 to 8.0, but some strains have been shown to grow between pH 4.9 and 9.5. Optimal growth occurs in the presence of 0.5 % sodium chloride (NaCl), while no growth is observed in the presence of ≥2% NaCl, or in its absence.

1.1.4 Survival and inactivation

Campylobacter spp. are highly sensitive to heat and drying. The decimal reduction values (D-values) reported amongst *Campylobacter* spp. vary considerably depending on strain and substrate differences. Blankenship and Craven (1982) reported a D-value of 4.8 min for *C. jejuni* in ground chicken heated to 53°C, while Doyle and Roman (1981, 1982) reported D-values ranging from 1.6 to 1.9 min for this organism in skim milk heated to 53°C. Although there is considerable variation in the heat sensitivity of the campylobacters, they are inactivated by pasteurization treatments currently in practice. *Campylobacter* spp. appear to be more sensitive to heat than other foodborne pathogens. For example, salmonellae exhibit a D_{57} of 1.3 min in ground chicken whereas *C. jejuni* exhibits a D_{57} of 0.8 min in this same substrate, suggesting that treatments designed to kill *Salmonella* spp., should also inactivate *Campylobacter* spp. (Ng et al., 1969).

In addition to their extreme sensitivity to heat, campylobacters are very sensitive to drying, particularly at ambient temperatures. Doyle and Roman (1982a) demonstrated that

campylobacters could not be recovered after 24 h at 25°C (at a 1% relative humidity), but survived much longer at 4°C. The lethal effect of drying has been cited as the major reason why groups, including Oosterom et al. (1983), have failed to recover *Campylobacter* from air-cooled chicken carcasses and from human hands. Coates et al. (1987) reported a 10^3 to 10^7 log reduction in *Campylobacter* levels when inoculated hands were sampled just 2 min after drying.

Campylobacters are also quite sensitive to gamma irradiation. Lambert and Maxcy (1984) reported that a dose of 0.16 kGy at 0 to 5°C, or a dose of 0.3 kGy at -30°C, led to a 10-fold reduction in numbers of *C. jejuni* in beef. It has also been shown that campylobacters in chicken paste were able to survive an irradiation dose of 0.2, but not 1kGy (National Advisory Committe on Microbiological Criteria for Foods, 1994). Consequently, a dose of 2 kGy, suggested to eliminate salmonellae from poultry, would also inactivate campylobacters (Previte et al., 1971).

Numerous disinfectants can effectively inactivate *Campylobacter* spp., including phenolic compounds, iodophors, quarternary ammonium compounds, 70% ethyl alcohol, and gluteraldehyde. Stern and Kazmi (1989) showed that under laboratory conditions with no organic matter present, a 4-log reduction within 1 min was achieved when cells were exposed to 1.25 mg/L sodium hypochlorite, 0.15 % phenol, 10 mg/L iodophor, 0.02% quarternary ammonium compounds, 0.125% gluteraldehyde or 70% ethanol. Moreover, a 7-log reduction within 15 min was achieved when cells were exposed to 5 mg/L sodium hypochlorite. Typically, campylobacters are more sensitive to disinfectants than *E. coli* (Wang et al., 1983).

Chill storage and freezing have also been shown to reduce *Campylobacter* levels. Stern et al. (1995) reported a significant reduction ($\log_{10}$ 5.8 reduced to undetectable levels) in

Campylobacter levels on fresh broiler carcasses after storage at 4°C for 10 days. Dramatic decreases in *Campylobacter* levels have also been reported as a result of freezing; however survival at -20°C is possible. In fact, Stern and Kazmi (1989) were able to recover *C. jejuni* from frozen chicken drumsticks even after three months of storage. In addition, Fernandez and Pison (1996) were able to isolate *Campylobacter* spp. from frozen commercial chicken livers.

1.2 Campylobacteriosis

Campylobacter spp. are major foodborne pathogens, capable of causing sporadic and epidemic outbreaks of gastroenteritis, particularly among the immunocompromised such as individuals with cancer, AIDS and diabetes, as well as the very young and elderly. *Campylobacter* spp. are the most common cause of gastroenteritis in North America and the UK, and a major public health concern worldwide. *C. jejuni* accounts for 99% of the reported *Campylobacter* spp. associated with human illness in North America and the UK, with *C. coli* making up the remainder, and *C. lari*, *C. fetus*, *C. upsaliensis* and *C. hyointestinalis* being occasionally isolated (Mishu et al., 1992; Tauxe, 1992).

1.2.1 Clinical pathology

Black et al. (1988) and Robinson (1981) both demonstrated that a dose of 500 cells of *C. jejuni* (in milk) was sufficient to cause gastroenteritis in humans. The incubation period of this organism ranges from 18 h to 8 days (Skirrow and Blaser, 2000). The onset of campylobacteriosis often involves severe abdominal pain followed by diarrhea and a variety of other symptoms including fever, headache, vomiting, myalgia and blood in the faeces. Generally, after 1 to 2 days of diarrhea, blood may appear in the stool, indicating a progression of infection into the lower gastrointestinal tract (i.e., colon and rectum). After 3 to 4 days,

patients experience fewer bowel movements, but the abdominal pain may persist for several more days. The illness is generally self-limiting but unless treated with antibiotics, patients will continue to excrete *Campylobacter* spp. in their faeces for several weeks after they have clinically recovered. Most reports indicate that 5-10 % of patients are admitted to hospitals, and that fatalities rarely occur, except among the elderly, young and immunocompromised (Skirrow and Blaser, 2000).

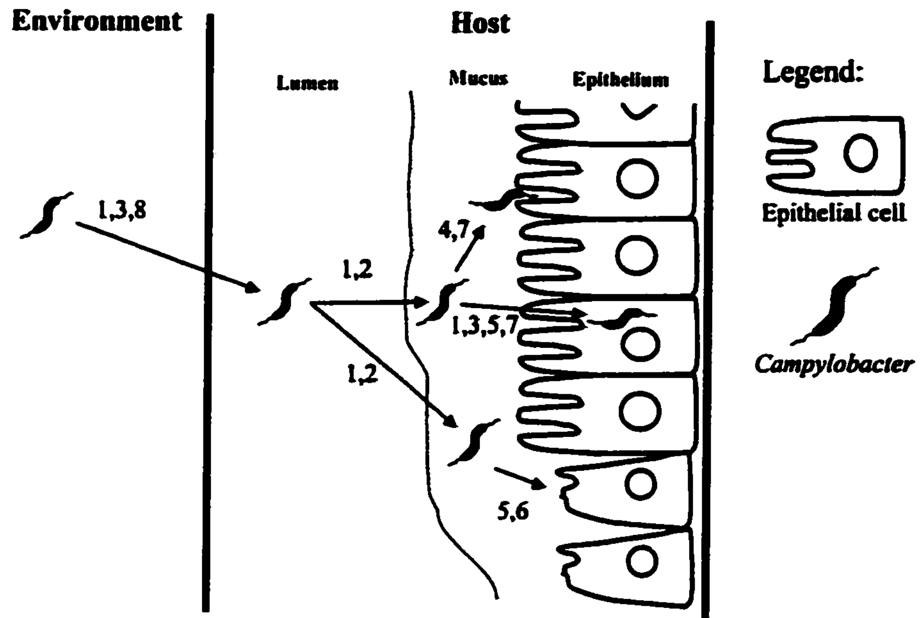
1.2.2 Pathogenesis

Although *Campylobacter* spp. are recognized as major human pathogens, relatively little is understood about the pathogenesis of campylobacteriosis. Based on the clinical symptoms associated with illness, it has been postulated that three main mechanisms of pathogenesis are employed by *Campylobacter*: adherence to the intestinal wall and production of enterotoxins (causing diarrhea), invasion and proliferation within intestinal epithelial cells (causing cell damage and inflammation; may lead to bloody diarrhea), and crossing of the intestinal mucosa and migration via the lymphatic system to a variety of sites (causing meningitis, urinary tract infection, etc.) (Figure 1) (Hu and Kopecko, 2000; Pickett, 2000). The pathogenesis of campylobacteriosis, like other foodborne illnesses, is highly dependent upon host susceptibility and strain virulence.

i) Colonization and Adhesion

Colonization of the intestine is believed to be essential for disease to occur, and is highly dependent upon motility and chemotaxis. Campylobacters are able to efficiently penetrate the mucus layer covering the intestinal cells by means of their polar flagella, which confers a "cork-screw"-like motility (Newell et al., 1985; Lee et al., 1986; Szymanski et al., 1995). Mutational

Figure 1. Interaction of *Campylobacter* with host cells. Some known and putative virulence factors are highlighted: 1, motility; 2, chemotaxis; 3, oxidative stress defence; 6, toxin production; 7, iron acquisition; 8, temperature stress response, at the various stages of infection: 4, adhesion; 5, invasion. Adapted from van Vliet and Ketley, 2001.


Environment
Host
Lumen
Mucus
Epithelium
Legend:
Epithelial cell
Campylobacter
1,3,8
1,2
4,7
1,3,5,7
1,2
5,6

analysis, such as that performed by McSweegan et al. (1987) and Yao et al. (1994) have shown that non-flagellated mutant strains colonize the intestine much less efficiently than flagellated strains, highlighting the importance of flagella to *Campylobacter* colonization. In addition to motility, chemotaxis is essential for *Campylobacter* colonization. Takata et al. (1992) showed that non-chemotactic mutants were unable to colonize the intestines of mice.

Once colonization has occurred, *Campylobacter* cells can cross the mucus layer covering the epithelial cells, and then adhere to, and subsequently invade these cells. Adhesion of *Campylobacter* spp. to epithelial cells is thought to be mediated by constitutively synthesized products, including the PEB1 (de Melo and Pechère, 1990; Pei et al., 1991; Pei and Blaser, 1993) and CadF (Konkel et al. 1997; Konkel et al., 1999) proteins. The PEB1 protein exhibits similarity to a periplasmic binding protein found in other gram-negative bacteria which is involved in nutrient acquisition (Garvis et al., 1996). The CadF protein, on the other hand, is a fibronectin-binding protein encoded by the *CadF* gene. Both the *CadF* gene and protein are highly conserved among *C. jejuni* and *C. coli* isolates (Konkel et al., 1999). Pei et al. (1998) reported a significant reduction in adherence and invasion of *C. jejuni* to HeLa cells when *peb1A* null mutants were used in mice challenge assays. Similarly, Konkel et al. (1997) observed that CadF mutants of *C. jejuni* exhibited a significant reduction in binding to fibronectin-coated coverslips. Ziprin et al. (1999) also observed that these mutants were unable to colonize newly hatched Leghorn chickens.

In addition to the known adhesins, PEB1 and CadF, some putative adhesins have also been described and include pili, flagellin (FlaA), major outer membrane proteins (OMPs) and lipopolysaccharide (LPS). Dolg et al. (1996) reported that *C. jejuni*, *C. coli* and *C. fetus*

produced pilus-like appendages in response to the bile salt deoxycholate. Recently, Gaynor et al. (2001) showed that these bile-induced pili were, in fact, artifacts of the culture media. Thus, it remains unclear as to whether *Campylobacter* spp. produce pili, or other appendages which may act as adhesins. The outer membrane proteins (OMPs), PEB2, PEB3 and PEB4, identified by de Melo and Pechère (1990), are also thought to be putative adhesins, although their function(s) remain unclear. As in colonization, flagellae play an important role in adhesion, although their function is also not clear. Lipooligosaccharide (LOS) and LPS are the major surface components of gram-negative bacteria, including *Campylobacter*. As such, they play an important role in host and environmental interactions. Accordingly, LOS/LPS are thought to be important adhesins, although there is no direct evidence to indicate that they do play a role in adhesion.

ii) Invasion

Penetration of the epithelial mucus layer is considered to be an important component of *Campylobacter* pathogenesis. Several studies have highlighted the ability of *Campylobacter* spp. to adhere to and invade different cell lines of intestinal origin, including INT-407, Hep-2, Caco-2 and A549 cells (Konkel and Joens, 1989; Wassenaar et al., 1991; Everest et al., 1992). Moreover, intracellular *C. jejuni* have been observed in colonic epithelial cells obtained from patients with *C. jejuni* colitis.

A crucial requirement for invasion is *de novo* protein synthesis, especially of proteins that bind host cell receptors. *C. jejuni* produces at least 8 to 14 new proteins in the presence of intestinal epithelial cells (Konkel and Cieplak, 1992; Konkel et al., 1997; Konkel et al., 1999a). Konkel et al. (1997) observed a significant (98%) reduction in invasion when using rabbit antiserum raised against whole-cell *C. jejuni* cultured in the presence of INT-407 cells, indicating

the importance of one or more of these proteins in host cell invasion. Recently, the *Campylobacter* invasion antigen B (CiaB) protein was identified as one of these newly synthesized proteins. CiaB exhibits a 40 to 45% similarity to the invasion ligands of other bacteria, including *Salmonella*, *Shigella* and *Yersinia*. Konkel et al. (1999a) observed that *C. jejuni* ciaB null mutants were able to bind to INT-407 cells, but were significantly less (~100-fold) invasive. The specific roles of the remaining *de novo* proteins have not been elucidated.

After invasion, *Campylobacter* cells must be able to survive and/or replicate within host cells. An important prerequisite for survival is the ability of campylobacters to mount an oxidative stress defense in response to superoxide radical production, an important host immune defense. *C. jejuni* and *C. coli* possess the same oxidative stress defense systems, which include a superoxide dismutase (SOD) protein, SodB (Purdy and Park, 1994). SodB functions in removing superoxides by converting them to hydrogen peroxide. SodB *C. jejuni* mutants exhibit significantly reduced survival within INT-407 cells, indicating the importance of SodB in intracellular survival. Like SodB, the proteins catalase (KatA) and alkyl hydroperoxide reductase (AhpC≡Tsa≡TsaA) are also important for intracellular survival, and make up the peroxidase stress defense system (Baillon et al., 1999).

iii) Translocation

Campylobacter spp. are able to cross the intestinal mucus layer and migrate to various extraintestinal sites. Everest et al. (1992) reported that translocation across Caco-2 cells occurred in the majority (86%) of *C. jejuni* strains isolated from individuals with colitis. Translocation is thought to occur via an endocytic pathway however, certain *C. jejuni* strains have been shown to translocate across cell monolayers by migrating between cells. Thus, it

appears that *C. jejuni* can translocate across host cells by an intracellular and paracellular route (Everest et al., 1992; Grant et al., 1993).

1.2.3 Environmental Regulation of Bacterial Genes

There is strong evidence that *Campylobacter* spp. regulate gene expression in response to environmental conditions, including fluctuations in pH, temperature, osmolarity and free-iron concentration. These changes in gene expression affect the adherence of *Campylobacter* to host cells. Konkel et al. (1992a) observed a significant decrease in the adherence of *C. jejuni* M129 to INT 407 cells when 72 h cultures rather than 24 h cultures were used. Similarly, Konkel et al. (1992a) observed a significant decrease (25% decrease) in adherence of *C. jejuni* M129 to INT 407 cells when bacteria were grown at 42°C instead of 37°C, and an even greater decrease (91% decrease) was with cells cultured at 30°C.

As is the case with temperature and culture age, free-iron concentration acts as a signal for gene expression in campylobacters. Since the free-iron concentration in host tissues is too low to allow bacterial growth, campylobacters must synthesize new envelope-associated proteins in order to increase free-iron uptake. However, because iron is capable of generating toxic oxygen metabolites, campylobacters must also be able to repress the uptake of iron when intracellular iron concentrations are sufficient, ensuring iron homeostasis. Chan et al. (1995) and van Vliet et al. (1999) reported that *C. jejuni* synthesizes a ferric uptake regulator (Fur) protein in the presence of eukaryotic cells. *C. jejuni* contains two genes encoding Fur homologues, *fur* and *perR*. The *C. jejuni fur* gene is the first bacterial *fur* gene discovered that does not possess its own promoter; instead, it is expressed from two upstream promoters (of housekeeping genes) (van Vliet et al., 2000). van Vliet et al. (1999) observed that a *C. jejuni fur* mutant was unable to

regulate the expression of any of the known iron acquisition systems, and grew much more slowly than the wild-type strain.

Campylobacter spp. also regulate the expression of other virulence factors, using two-component regulatory systems. These systems consist of a histidine protein kinase (HPK) sensor which, under certain environmental conditions, phosphorylates a response regulator (RR) protein which is required for initiation of transcription of specific genes (Parkinson, 1993; Wren et al., 1992). Additional regulators include the sigma factors α^{28}, α^{54}, α^{70} (Wosten et al., 1998). These regulators, like those described above, affect both the adherence and invasive abilities of *Campylobacter* spp., and are not constitutively expressed.

1.2.4 Toxins

i) Cytolethal distending toxin (CDT)

Much attention has focused on the characterization of *Campylobacter* toxin production. Several studies have reported the production of a variety of cytotoxins and/or enterotoxins. However, genes encoding these toxins, other than the cytolethal distending toxin (CDT), have not been identified. Johnson and Lior (1988) first reported *Campylobacter* CDT production. Of the over 700 *Campylobacter* strains examined, including *C. jejuni*, *C. coli*, *C. lari* and *C. fetus*, approximately 40% produced CDT. Pickett et al. (1996) isolated and characterized the cdt genes from *C. jejuni* 81-176. Recently, sequence analysis of the *C. jejuni* NCTC 11168 genome has led to the identification of the cdt genes, two genes both containing hemolysin domains and a phospholipase (pldA) (Parkhill et al., 2000). Three adjacent (or slightly overlapping) genes, namely cdtA, cdtB and cdtC, encode CDT, producing CdtA, CdtB and CdtC, with CdtB having the most conserved sequence (e.g., 60% amino acid similarity between *E. coli* and *C. jejuni*

CdtB).

CDT is a cytotoxin that causes host cells (e.g., HeLa and Caco-2 cells) to distend, leading to cell death. This distension is caused by a blockage in the G2 phase of the cell cycle; more specifically, CDT blocks the cdc2 kinase which is involved with entry into mitosis (Pickett, 2000). Although cdt genes have been identified in all *C. jejuni* and *C. coli* strains tested, CDT titers vary considerably. *C. coli* strains show mostly low CDT activity in comparison to *C. jejuni* strains.

The role of CDT in *Campylobacter* pathogenesis is unclear, but appears to be involved in the development of diarrhea. CDT may block maturation and survival of epithelial cells needed for absorptive functions, leading to loss of function and thus, diarrhea (Pickett, 2000).

ii) Non-CDT cytotoxins

A variety of non-CDT-related cytotoxic activities have been described. Wassenaar (1997) grouped these activities into five groups: 1) CHO/HeLa cell cytotoxin, 2) Vero-active and Shiga-like toxins and hepatotoxins, 3) hemolysins, 4) Cytolethal rounding toxin (CLRT) and 5) porin-lipopolysaccharide toxin. Since different strains were tested using dissimilar procedures, it is difficult to determine whether these toxins possess identical or different activities (Pickett, 2000).

iii) Enterotoxins

Several groups have hypothesized that *Campylobacter* spp. must produce an enterotoxin, since the symptoms of campylobacteriosis can include watery diarrhea. It has been suggested that *C. jejuni* produces a cholera-like enterotoxin. However, sequence analysis of the *C. jejuni* NCTC 11168 genome has not led to the identification of any cholera-like enterotoxin genes, suggesting that *C. jejuni* does not produce a cholera-like enterotoxin (Parkhill et al., 2000).

1.2.5 Animal models

Many species of laboratory animals have been challenged with *C. jejuni* in an attempt to better understand campylobacteriosis. Mice, rabbits and chicks are among the animals used in *in vivo* models of *Campylobacter* infection. Several mouse models have also been described, primarily because of the commercial availability and relative ease of handling and housing of these animals. However, oral dosing of mice with *C. jejuni* rarely results in diarrhea. Field et al. (1981), Blaser et al. (1983) and Abimuki et al. (1989) reported that oral dosing of mice with *C. jejuni* resulted in colonization, but did not cause diarrhea. In contrast, diarrhea and inflammatory lesions are frequently observed in the rabbit (Caldwell et al., 1983). The removal intestinal tie adult rabbit diarrhea (RITARD) and rabbit ileal loop test (RILT) are the most commonly described rabbit models. Both models involve extensive surgical procedures, limiting their usefulness. Like mice and rabbits, one-day-old chicks are routinely used as models of *Campylobacter* infection. The oral chick model has proved very useful in assessing the colonization potential of a variety of *C. jejuni* strains (Wassenaar et al., 1993, Cawthraw et al., 1996; Newell and Wagenaar, 2000). However, as in the case of mice, asymptomatic colonization is the norm.

The ferret model appears to be particularly promising in the study of campylobacteriosis. Bell and Manning (1990, 1991), as well as others, reported the development of diarrhea, accompanied by anorexia and dehydration, in ferrets after oral challenge with *C. jejuni*. Recently, Bacon et al. (2000) used this model to compare the virulence of *C. jejuni* strains, including *C. jejuni* NCTC 11168. Although the ferret model has proved promising in the study of *Campylobacter* virulence factors, its use is limited due to price, seasonal breeding and the lack

of *Campylobacter*-free ferret breeding colonies. Neonatal piglets also appear promising as a model of *Campylobacter* infection in the study of campylobacteriosis. Taylor and Olubunmi (1981), Babakhani et al. (1993), as well as Babakhani and Joens (1993) reported the development of diarrhea in neonatal pigs, lasting up to six days. However, the reproducibility of this model has yet to be determined.

1.2.6 Guillian-Barré syndrome

Although the majority of campylobacteriosis cases are self-limiting and rarely result in death, complications may include appendicitis, colitis, toxic megacolon, rashes, neonatal infection, abortion, renal and urinary tract disease as well as intestinal hemorrhage, perirectal abscess, bacteremia, pancreatitis and hepatitis. Some late-onset complications include reactive arthritis, Reiter's syndrome and Guillain-Barré syndrome (GBS).

Over the past decade, there has been increasing evidence that *C. jejuni* is associated with the development of GBS in humans. GBS is an autoimmune disorder of the peripheral nervous system which results in progressive acute flaccid paralysis. GBS cases develop weakness of the limbs and respiratory muscles. The disease is generally self-limiting, with partial or full recovery over weeks to months. However, a large proportion of cases require mechanical ventilation, and approximately 15-20 % may experience severe neurological problems (Nachamkin et al., 2000).

In 1982, Rhodes and Tattersfield reported the first case of campylobacteriosis preceding GBS. In the years that followed, numerous other reports described patients who developed GBS following infection with *C. jejuni* (Constant et al., 1983; Speed et al., 1984; Yuki et al., 1990; Allos, 1997). It is estimated that about 1 in 1000 cases of symptomatic *C. jejuni* enteritis are

followed by GBS (Nachamkin et al., 2000). Interestingly, GBS is particularly associated with *C. jejuni* serotypes that are not commonly responsible for enteritis, and include Penner serotypes O:19 and O:41, and Lior serotype 11. Yuki et al. (1997), Hao et al. (1998), and Saida et al. (1997) found that 52 to 77% of Japanese patients with *C. jejuni*-associated GBS were infected with strains of Penner serotype O:19. Lastovica et al. (1997) reported that over 50% of *Campylobacter*-associated GBS cases in South Africa were caused by strains of Penner serotype O:41, while Enders et al. (1993) observed that over 90% of *C. jejuni*-associated GBS cases in Germany were related to Lior serotype 11. These observations suggest that the onset of GBS is linked to some unique properties of these serotypes. The specific causal relationship between *C. jejuni* and GBS is further highlighted by the observation that *C. jejuni* surface polysaccharide structures (and flagella) are sialylated, i.e., they contain glycolipids that resemble gangliosides (GM1, GD1a, GD3 and GT1a) found in abundance in both the central and peripheral human nervous system (Gregson et al., 1997; Goodyear et al., 1999). This sialylation is thought to be responsible for the development of GBS through ganglioside mimicry. In fact, ganglioside structures were originally found in the LPS of Penner serotype O:19 isolated from GBS patients (Yuki et al., 1993).

1.2.7 Emerging trends

Campylobacteriosis is one of the most frequently reported types of acute bacterial gastroenteritis in many developed countries worldwide. In the US, *Campylobacter* has the highest incidence rate of all pathogens under surveillance in FoodNet, a national foodborne diseases active surveillance network (Angulo et al., 1998). This same pattern is seen in Canada, where reported campylobacteriosis cases outnumber cases of listeriosis, salmonellosis, *E. coli* and *Shigella*

combined (Figure 2). It is estimated that the true incidence of campylobacteriosis is 10 to 100 times higher than that reported (Skirrow, 1991; Kapperud, 1994).

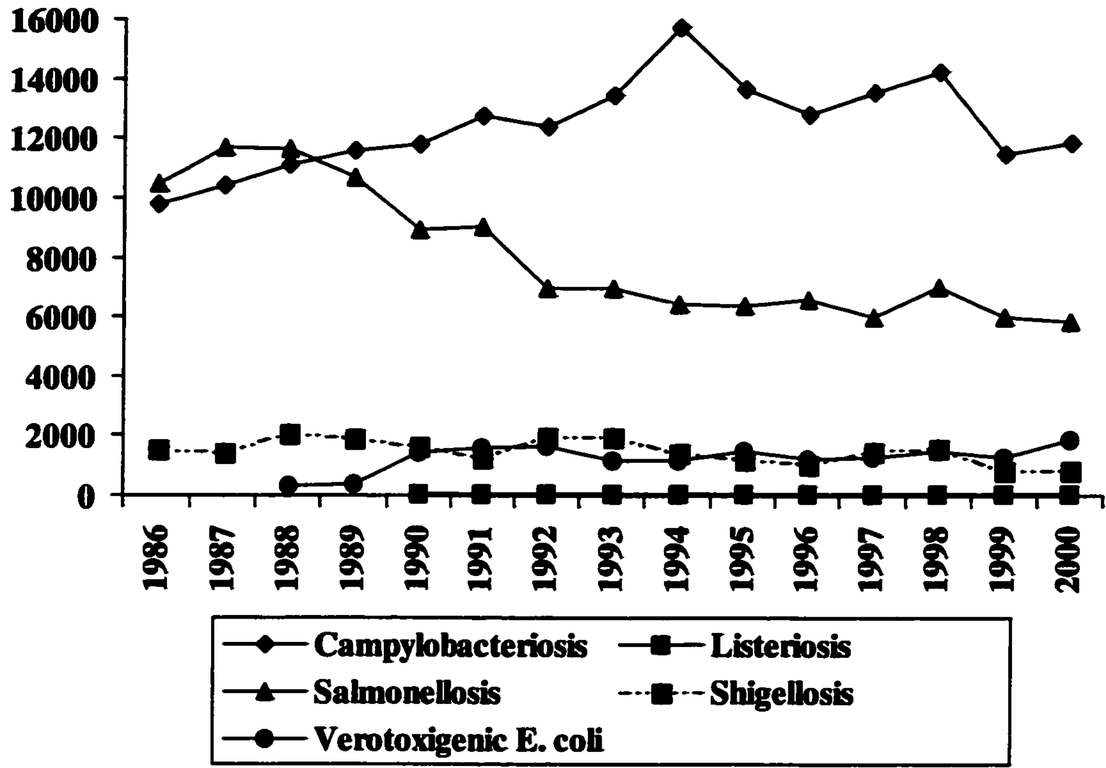
Over the past few decades, many developed countries have experienced a steady increase in the number of reported cases of campylobacteriosis. In New Zealand, for example, a nine-fold increase in incidence was observed between 1981 and 1990, followed by a three-fold increase between 1991 and 1998 (Brieseman, 1990). Similarly, Canada and the US have seen an increasingly greater number of campylobacteriosis cases. This increased incidence may be, in part, due to an increased awareness about *Campylobacter*, increased culturing by laboratories, improved detection methods and improved reporting.

Although considerable changes in the reported incidence of campylobacteriosis have been seen, the age distribution of *Campylobacter* cases has remained static. *Campylobacter* affects persons of all ages; however, there is a higher incidence in children under 4 years and adults between 15 and 44 years (Kapperud and Aasen, 1992; Kapperud, 1994). The incidence in males tends to be 1.2-1.5 times higher than in females. *Campylobacter* infection exhibits a characteristic seasonal distribution, with a peak incidence in the warmer months (Skirrow, 1991; Nylen et al., 1998).

1.2.8 Outbreaks

The vast majority of *Campylobacter* infections are sporadic, and outbreaks are generally rare, i.e., represent approximately 1% of the total reported foodborne and waterborne outbreaks (Angulo et al., 1998). However, a number of foodborne and waterborne outbreaks of campylobacteriosis have been reported over the past few decades. Between 1978 and 1996, 111 outbreaks of campylobacteriosis, affecting almost 10,000 individuals, were reported in the US

Figure 2. Incidence of campylobacteriosis as compared to the incidence of listeriosis, salmonellosis, verotoxigenic *E. coli* and *Shigella* in Canada, 1986-2000. Data were obtained from the National Laboratory for Enteric Pathogens, Population and Public Health Branch, Health Canada (http://cythera.ic.gc.ca/dsol/ndis/c_time_e.html; Ahmed, 2001).


16000
14000
12000
10000
8000
6000
4000
2000
0
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
Campylobacteriosis
Listeriosis
Salmonellosis
Shigellosis
Verotoxigenic E. coli

(Friedman et al., 2000). Interestingly, the sources of these outbreaks have changed over time. Between 1978 and 1987, water and unpasteurized milk accounted for almost 60% of outbreaks, whereas other foods accounted for 83% of outbreaks reported between 1988 and 1996 (Friedman et al., 2000). Contaminated water and milk have also been the predominant causes of campylobacteriosis in other developed nations, including the UK, Sweden and Germany (Friedman et al., 2000). In Canada, very few outbreaks of *Campylobacter* infection have been reported, and the shift in the sources of these outbreaks resembles that seen in the US.

Whereas the sources of campylobacteriosis outbreaks are generally identified, the causes of most sporadic infections remain unknown. It has traditionally been difficult to retrieve foodstuffs from the patient' s home, either because the original suspect food item was fully consumed, or because leftovers were no longer available by the time of the illness. Even when suspect foods are available, the prolonged storage of these food items makes it difficult to recover any campylobacters. Consequently, efforts have turned to epidemiological investigations which attempt to identify exposures that are most likely associated with *C. jejuni* infections. Several of these studies have consistently identified contact with and consumption of poultry, transmission from pets and other animals, and contaminated drinking water as major sources (Adak et al., 1995; Eberhart-Phillips et al., 1997; Neal and Slack, 1997; Friedman et al., 2000). Other risk factors include foreign travel and consumption of raw milk and raw milk products (Schorr et al., 1994; Adak et al., 1995; Neal and Slack, 1997; Friedman et al., 2000).

1.3 Prevalence of *Campylobacter*

1.3.1 Foods

Campylobacteriosis is considered to be primarily a foodborne illness. Consequently, a number

of studies have described the detection and prevalence of *Campylobacter* spp. in a variety of foods.

i) Detection

Generally, isolation of *Campylobacter* spp. involves a primary enrichment step which is designed to detect small numbers of cells and to recover sublethally damaged cells. Liquid enrichment media, such as Park and Sanders broth, Preston broth and Bolton broth, are typically used for this purpose (Corry et al., 1995; International Organization for Standardization, 1995). After enrichment, *Campylobacter* cells are isolated on semi-solid selective media. Commonly used selective media and their characteristics/antibiotic components are outlined in Table 3. These selective media are generally similar, in that they contain sterile blood (horse or sheep) or charcoal in order to neutralize the toxic effects of oxygen and light; as well as a variety of selective agents (cefoperazone, cycloheximide, trimethoprim lactate and vancomycin are most commonly used). A number of enrichment and selective media, and incubation conditions have been described, and some of these are outlined in the 8th edition of the Food and Drug Association (FDA)'s Bacteriological Analytical Manual (BAM) and Health Canada's Compendium of Analytical Methods (Hunt and Abeyta, 1995; Sanders, 1999).

ii) Survival of *Campylobacter* on foods

Poultry

Live poultry, including broilers, hens, turkeys and ducks, are often colonized by large numbers of campylobacters. Thus, it is not surprising that epidemiological studies have highlighted the significant association between consumption of undercooked poultry and development of campylobacteriosis (Blaser et al., 1980). *Campylobacter* spp. are typically found on the feathers

Table 3. Commonly used selective media for the isolation of *Campylobacter* spp. [a]

Selective Media	Antibiotic components
Preston	colistin, cycloheximide, rifampicin, trimethoprim
CCDA	amphotericin B, cefoperazone
Skirrow	polymyxin, trimethoprim, vancomycin
Butzler	bacitracin, cefalozin, colistin, cycloheximide, novobiocin
Blaser-Wang	amphotericin B, cephalothin, polymyxin, trimethoprim, vancomycin
Karmali	cefazolin, cycloheximide, vancomycin
CAT	amphotericin B, cefoperazone, teicoplanin

[a] Adapted from Corry et al., 1995.

and skin of these animals, and are particularly associated with their intestinal tracts (Beery et al., 1988; Achen et al., 1998; Corry and Atabay, 2001). Colonization levels in the small intestine generally range from 10^5 to greater than 10^9 CFU/g (Berndtson et al., 1992; Stern et al., 1999; Berrang et al., 2000). This means that campylobacters can be found throughout the slaughtering process, leading to contamination of equipment surfaces, processing water and air. This contamination is further exacerbated by the large amounts of water used during poultry processing. Defeathering and evisceration also contribute to contamination by causing intestinal leakage (Oosterom et al., 1983; Mead, 1989). Overall, it is estimated that the slaughtering process reduces *Campylobacter* levels in poultry by approximately 10 to 1000-fold (Newell et al., 2001). For example, Mead et al. (1995) observed a reduction in the numbers of *Campylobacter* cells on neck skin samples from of $10^{3.7}$ CFU/g after exsanguination to $10^{1.8}$ CFU/g after packaging.

The levels of *Campylobacter* spp. on poultry products have been examined using a variety of methods. Reported levels range from 10^2 to 10^5 CFU per carcass, or 10^1 to 10^6 CFU per 100 g of raw poultry (Hood et al., 1988; Berndston et al., 1992). The prevalence of *Campylobacter* spp. has also been examined in a variety of raw poultry products, and has been shown to vary dramatically (0-100%). For example, Kwiatek et al. (1990) detected campylobacters in only 3% of the 236 poultry carcasses examined, whereas Stern and Line (1992) reported the isolation of campylobacters from 98% of 50 poultry carcasses tested. Differences in sampling procedures and isolation methods may account for differences in isolation percentages, and make direct comparison of these various studies difficult.

Other avian species, including gulls, pigeons and parrots, also harbour *Campylobacter*

spp. Since these species are not often used for human consumption, the risk of developing campylobacteriosis from these sources is related to direct contact.

Commercial eggs have rarely been associated with the development of campylobacteriosis. In fact, only one outbreak has been reported, involving 26 individuals in the US who developed campylobacteriosis after consuming undercooked eggs (Finch and Blake, 1985). Survival of *Campylobacter* spp. on eggshells is generally rare, because of the sensitivity of these organisms to drying. Doyle (1984) and Shane et al. (1986) used artificially contaminated eggshells to demonstrate that campylobacters could not be detected after storage at room temperature for 48 h. Doyle (1984) also showed that *Campylobacter* spp. were unable to penetrate into the contents of eggs (at 4, 25 or 37°C) after artificial contamination. Jacobs-Reitsma (1994, 1995) was unable to isolate *C. jejuni* from the egg yolks of 219 recently laid eggs. Similarly, Doyle (1984) reported that *Campylobacter* spp. were not detected in the egg contents of 216 eggs examined within 12 h of being laid. Moreover, in five trials done in two commercial egg processing plants, *Campylobacter* spp. were not detected in raw eggs or in any processed egg products (Izat and Gardner, 1988). Conversely, Maruyama et al. (1995) observed the invasion of a *C. jejuni* strain into 12.5% of eggs that had been immersed into a suspension of this organism, between 0.5 h and 24 h after immersion. In addition, Pearson et al. (1996) described the transovarian transmission of *C. jejuni* into eggs, suggesting that eggs could be associated with the development of campylobacteriosis.

Dairy Products

Raw milk has been associated with a number of campylobacteriosis outbreaks, with the first one occurring in 1938. Although *Campylobacter* spp. are often isolated from the faeces of healthy

dairy cows, it is rarely isolated from bulk tank milk samples, with isolation rates in the range of 0 to 12.3 % being reported. Rohrbach et al. (1992) isolated *Campylobacter* spp. from 12.3% of the almost 300 bulk tank milk samples tested in the US, while Stone (1987) and others were unable to detect campylobacters in any of the raw milk samples tested. Doyle and Roman (1982) found that over 60% of milk-producing cows examined had *C. jejuni* in their faeces however, only 0.9% of the raw milk bulk tank samples obtained from these farms contained *Campylobacter* spp. Differences in isolation percentages amongst dairy samples are likely due to differences in sampling procedures and isolation methods used. For example, NaOH is generally added to milk samples in order to neutralize the lactoperoxidase system (which is toxic to *Campylobacter*), optimizing recovery of *Campylobacter* spp. This neutralization step is not always carried out.

Campylobacter levels in raw milk have rarely been determined. Humphrey and Beckett (1987) determined the mean contamination level in their samples to be a most probable number (MPN) of 16±30 organisms per 100 ml of raw milk. Generally, raw milk becomes indirectly contaminated from faeces or directly because of an udder infection (i.e., mastitis).

Although only a small percentage of raw milk samples harbour *Campylobacter* spp., and in small numbers, the levels present may be sufficient to cause illness. However, this risk is sufficiently eliminated by the pasteurization process. Pasteurized milk has only been implicated in one outbreak of campylobacteriosis. In this case, in the UK, birds had pecked at the foil tops of bottles delivered to the doorsteps of homes, contaminating the milk (Hudson et al., 1991).

Ordinarily, the low water activity and acidity of cheeses inhibits the growth of *Campylobacter* spp. Bachmann and Spahr (1995) were unable to detect campylobacters, at the stage of commercial ripeness, in raw milk hard and semi-hard cheeses that had been inoculated

with 10^4 to 10^6 CFU/ml. Conversely, other groups have isolated *Campylobacter* spp. from raw milk cheeses, although isolation percentages have been quite low (Barrett, 1986; Butzler and Oosterum, 1991).

Red meat

Cows and swine are frequently found to harbour *Campylobacter* spp., more specifically, *C. jejuni* and *C. coli*, respectively. Stanley et al. (1998) reported a carriage rate of 89% in samples of 360 cattle examined over the course of a year. This group also reported that, on average, the level of contamination approximated 10^2 MPN/g of fresh faeces. This is in contrast to the 10^6 to 10^9 CFU/g normally found in poultry faeces. Lambs and sheep also harbour *Campylobacter* spp., with an average of 10^4 MPN/g of fresh intestinal contents (Stanley et al., 1998a). As with poultry slaughtering, the slaughtering process of cattle, swine and sheep can lead to contamination of equipment surfaces, processing water and air. Lammerding et al. (1988) isolated *Campylobacter* spp. from a high percentage of cattle, sheep and pigs just after slaughter and before chilling. The forced-air chilling of these carcasses typically leads to a dramatic decrease in the extent of *Campylobacter* contamination. Both Oosterom et al. (1983) and Stern and Kazmi (1989) have reported significant reductions in *Campylobacter* levels after overnight forced-air chilling of swine carcasses. Adequate heating of these products has also been shown to significantly reduce the numbers of campylobacters, sufficiently eliminating the risk of developing campylobacteriosis. Campylobacteriosis cases in which cooked-meat products are implicated as the cause of illness, are typically caused by cross-contamination from raw meat products.

Seafood

Since campylobacters has been isolated from marine environments, it is not surprising to find shellfish contaminated by this organism. *Campylobacter* spp. have been isolated from different types of seafood, including clams, oysters, mussels, scallops and crab. Raw clams and oysters have also been implicated in sporadic cases of campylobacteriosis (Abeyta et al., 1993). Of the campylobacters isolated from seafood, *C. lari* appears to be most prominent, suggesting that seagulls are the primary source of contamination (Blaser et al., 1983a; National Advisory Committee on Microbiological Criteria for Foods, 1994). The prevalence of *Campylobacter* spp. in seafood varies significantly. Endtz et al. (1997) isolated *C. lari* from almost 70 and 27% of mussel and oyster batches analyzed, respectively. Conversely, Hald et al. (1998) did not detect any *Campylobacter* spp. in the almost 150 shellfish samples examined. Unlike Hald et al. (1998), Wilson and Moore (1996) reported that almost half of the 331 cockles, mussels and scallops examined shortly after harvesting, contained *Campylobacter* spp. However, only 6% of the depurated and ready-to-eat oysters examined contained *Campylobacter* spp. Since depuration does not appear to be entirely effective in eliminating *Campylobacter*, thermal processing of shellfish may be required.

Other foods

Relatively few studies have examined other foods for the presence of *Campylobacter* spp. Park and Sanders (1992) reported isolation rates ranging from 0 to 3.3% in 10 vegetable types examined. Interestingly, this group did not detect *Campylobacter* spp. in vegetables purchased from supermarkets; instead, campylobacters were only detected in vegetables purchased from farmers`outdoor markets. Odumeru et al. (1997) were unable to detect campylobacters in any of the unprocessed or ready-to-use vegetables tested, while Doyle and Schoeni (1986) isolated

Campylobacter spp. from 1.5% of the 200 packaged mushroom samples tested.

1.3.2 <u>Environment</u>

Campylobacter spp. have been isolated from various water sources, including freshwater and marine environments, as well as sewage water (Jones, 2001). In general, freshwater sources, including streams, rivers, canals, ponds, groundwater, reservoirs and drinking water, become contaminated with *Campylobacter* spp. via faecal contamination by wild birds or domestic animals, or from agricultural run-off or sewage effluent (Watson, 1985; Goss and Barry, 1995). Although campylobacters are present in these waters all-year round, their numbers decrease in the summer months, likely as a result of elevated temperatures and UV levels. Since *Campylobacter* spp. are sensitive to chlorination, if properly treated, drinking water is thought to be free of campylobacters. In fact, most water-related outbreaks have been associated with the consumption of untreated water or water contaminated after treatment (Eberhart-Phillips et al., 1997; Quick et al., 1999; Friedman et al., 2000).

Marine environments, including coastal waters and estuaries, are also thought to become contaminated with *Campylobacter* spp. via faecal contamination by wild birds and, in some cases, because of sewage effluent (Jones et al., 1990). Like campylobacters in freshwater, those in marine waters are present throughout the year, with a reduction in numbers observed in the summer months; again, thought to be due to elevated temperatures and UV levels.

Several studies have shown that campylobacters are ubiquitous in sewage, with human and animal waste being the major sources (Arimi et al., 1988; Stampi et al., 1999). Although sewage treatment greatly reduces the numbers of campylobacters, it is not until treatment with chlorine, that campylobacters are eliminated (Stampi et al., 1992).

A viable but non-culturable (VBNC) form of *Campylobacter* was first reported by Rollins and Colwell (1986), and has since been shown to be present in a variety of aquatic environments. Although much conflicting evidence exists as to the ability of VBNC cells of *Campylobacter* to cause illness, it is currently thought that these cells may be pathogenic and play a role in the transmission of campylobacteriosis (Cappelier et al., 1999; Tholozan et al., 1999).

1.3.3 Kitchen

Little information is available about the dynamics of *Campylobacter* spp. survival on kitchen surfaces, and even less is known about the prevalence of this organism on kitchen surfaces in restaurants. Dawkins et al. (1984) examined the prevalence of *Campylobacter* spp. in four kitchens, including a hospital kitchen, university kitchen, commercial roast chicken unit and a cook-freeze unit. Swabbing of surfaces in these kitchens took place before, during and after processing of chickens. *Campylobacter* spp. were not detected in any of the 58 swab samples obtained before processing, however, 21 of the 62 swab samples obtained during processing contained *Campylobacter* spp. (Dawkins et al., 1984). Moreover, this group found that only 1 of the 50 swab samples obtained after processing and cleaning, contained *Campylobacter* spp. Dawkins et al. (1984) also reported that two of the 78 swabs taken from the hands of workers, were contaminated with campylobacters, both of which were obtained during processing. Similarly, Cogan et al. (1999) found *Campylobacter* spp. in 38 of the 220 swab samples obtained from 60 domestic kitchens, during the processing of chickens.

Other groups have reported campylobacteriosis outbreaks resulting from cross-contamination of foods via contact with kitchen surfaces (Brown et al., 1988; Graves et al., 1998; Roels et al., 1998). Graves et al. (1998) described an outbreak that occurred in the US, in which

14 people developed campylobacteriosis after consumption of lettuce or lasagne at a restaurant. It was later determined that the chef of this restaurant had used the same countertop space to cut up raw chicken, and to prepare salads, lasagne, and sandwiches (Graves et al., 1998). Roels et al. (1998) and Brown et al. (1998) also reported cross-contamination of *Campylobacter* spp. as a result of processing raw and cooked foods on the same kitchen surfaces.

1.4 Subtyping of *Campylobacter*

Bacterial typing systems are generally classified into two broad categories: phenotypic and genotypic methods. Phenotypic typing methods are based on the observable characteristics of an organism, and include such methods as serotyping and phage typing. Alternatively, genotypic typing methods are based on the specific genes of an organism, more specifically, on DNA analysis of chromosomal or extrachromosomal genetic material. Commonly used genotypic methods include ribotyping, pulsed-field gel electrophoresis (PFGE) and random amplification of polymorphic DNA (RAPD). Table 4 highlights some of the properties of the most commonly used phenotypic and genotypic typing methods. Typically, phenotypic methods are less discriminatory than genotypic methods.

Over the past few decades, a variety of phenotypic and genotypic methods have been used to characterize *Campylobacter* spp. These methods vary widely, in terms of their cost, ease of performance, discriminatory power and reproducibility (Table 4). However, their principal aim remains the same; to trace sources and routes of transmission of campylobacteriosis and monitor the temporal and geographic distribution of strains.

1.4.1 Serotyping

Serotyping has typically been used for broad-scale surveillance of *Campylobacter* cases. Two

Table 4. Characteristics of bacterial typing systems. [a]

Typing System	Proportion of strains typeable	Reproducibility	Discriminatory power	Ease of interpretation	Ease of performance
I. Phenotypic methods:					
Biotyping	All	Poor to fair	Poor	Excellent	Excellent
Antimicrobial susceptibility testing	All	Fair	Poor	Excellent	Very good to excellent
Serotyping	Most	Good	Fair	Good to excellent	Fair to good
Bacteriophage typing	Variable	Fair	Fair	Fair to good	Poor to fair
Multilocus enzyme electrophoresis	All	Excellent	Good	Excellent	Fair to good
II. Genotypic methods:					
Plasmid profile analysis	Variable	Fair to Good	Good	Good	Excellent
Restriction endonuclease analysis	All	Very good	Good	Poor	Excellent
Ribotyping	All	Excellent	Fair to good	Very good to excellent	Fair to Good
Pulsed-field gel electrophoresis	All	Excellent	Excellent	Excellent	Fair to Good
PCR ribotyping	All	Very good to excellent	Good	Excellent	Very good to excellent
PCR restriction digest	All	Excellent	Good	Excellent	Very good to excellent
RAPD	All	Good	Very good to excellent	Very good	Very good to excellent
Nucleotide sequence analysis	All	Excellent	Excellent	Excellent	Fair
Amplified fragment length polymorphism	All	Excellent	Excellent	Very good	Fair to Good

[a] Adapted from Farber, 1996.

serotyping schemes, the Penner and Lior scheme(s), were both developed in Canada in the 1980s. The Penner scheme is based on soluble heat-stable (HS) antigens, while the Lior scheme is based on heat-labile antigens (HL). The latter scheme has been more widely used, especially in Europe. The Health Canada Laboratory of Enteric Pathogens (LEP) has made several modifications to the Penner scheme in order to address existing problems. Despite these changes, a large percentage of human and animal strains remain untypeable. Frost et al. (1998) found that 19% of human isolates proved untypeable, while Newall (unpublished data) reported that up to 40% of the poultry isolates from the UK were nontypeable. This is not very surprising since this serotyping scheme, like the original Penner scheme, is based on antisera that were raised against strains prevalent in Canada in the 1980s. Currently, the LEP scheme defines 48 and 17 serotypes in *C. jejuni* and *C. coli*, respectively (Newell et al., 2000a).

1.4.2 Phage-typing

Phage typing represents an alternative phenotypic typing method which addresses some of the disadvantages of serotyping, in that it can discriminate between strains of the same serotype. This method relies on the susceptibility of *Campylobacter* spp. to lyse in the presence of a series of bacteriophages. Differences in lysis patterns are used to differentiate between strains. A variety of *Campylobacter* phage typing schemes have been described over the years. The UK scheme, for example, has defined a total of 76 phage types. Although phage-typing has proven to be reproducible and discriminatory, it is limited because of its high level of untypeability. It is now routinely used as an adjunct to serotyping (Newell et al., 2000a).

1.4.3 Ribotyping

Unlike serotyping and phage-typing, ribotyping relies on the genetic composition of an organism,

more specifically, its ribosomal genes. Ribotyping involves probing restricted chromosomal DNA with ribosomal genes (e.g., *E.coli* 23S, 16S and 5S rRNA sequences). Although this method can be highly discriminatory, especially as the number of rRNA (*rrn*) operons increases, *rrn* operons cover only ~ 0.1% of chromosomal DNA. Moreover, most *Campylobacter* strains possess three ribosomal gene copies, which is fewer than many other bacteria; therefore, limiting the discriminatory power of this method (Newell et al., 2000a). Fayos et al. (1992) were able to maximize the discriminatory power of this method by using a 16S scheme involving a combination of *Hae*III and *Pst*I digestion; over 80 types were identified within *C. jejuni*.

Ribotyping is generally not used for routine subtyping of *Campylobacter* spp., both because of its limited discriminatory power and tedious nature. However, the recent automation of this method by E.I. Dupont, in the form of the RiboPrinter™ Microbial Characterization System, has addressed some of the disadvantages of traditional ribotyping. A major advantage of this system is that it facilitates the exchange of data between laboratories. Unfortunately, because of the substantial equipment costs associated with the RiboPrinter™ system, it is unlikely that this technology will be commonly used.

1.4.4 RAPD

Unlike serotyping, phage-typing and ribotyping, random amplification of polymorphic DNA (RAPD) is a PCR-based typing method. The RAPD is a form of PCR using short (8-10 mer) primers of random sequence. Amplification occurs when annealing sites are ~ 200-2000 bp from each other and primers are in the correct orientation. This method has been applied to the typing of numerous microorganisms, including *Campylobacter* spp., and is advantageous since no sequence information is required. RAPD typing has been used to characterize a variety of

Campylobacter spp., including *C. jejuni*, *C. coli* and *C. lari*, from a range of clinical, animal and environmental sources (Hernandez et al., 1995; Madden et al., 1996). Although this method produces highly discriminatory profiles, its degree of reproducibility is of concern. Reproducibility problems have been related to a number of factors, including the type of thermocycler used, as well as the source of Taq DNA polymerase. In addition to differences in reproducibility, several groups have described variations in the degree of typeability of this method. Madden et al. (1996) found that all 276 *Campylobacter* isolates, including 200 *C. coli* porcine isolates and 76 *C. jejuni* isolates, were typeable, whereas Hernandez et al. (1995) found that only 178 out of 208 (86%) strains were typeable. Overall, the significant reproducibility problems associated with RAPD typing have precluded the widespread use of this technique for typing of campylobacters.

1.4.5 *fla typing*

The *fla* typing method is becoming a widely used PCR-based typing method for *Campylobacter* spp. This technique involves amplification of the conserved and variable regions of the flagellin gene, *fla*, and subsequent digestion of the PCR product. *Fla* typing has proven to be a very valuable tool for characterizing *Campylobacter* spp., and has been successfully applied to the typing of clinical, food, animal and environmental isolates (Koenraad et al., 1995; Stern et al., 1997; Lorenz et al., 1998).

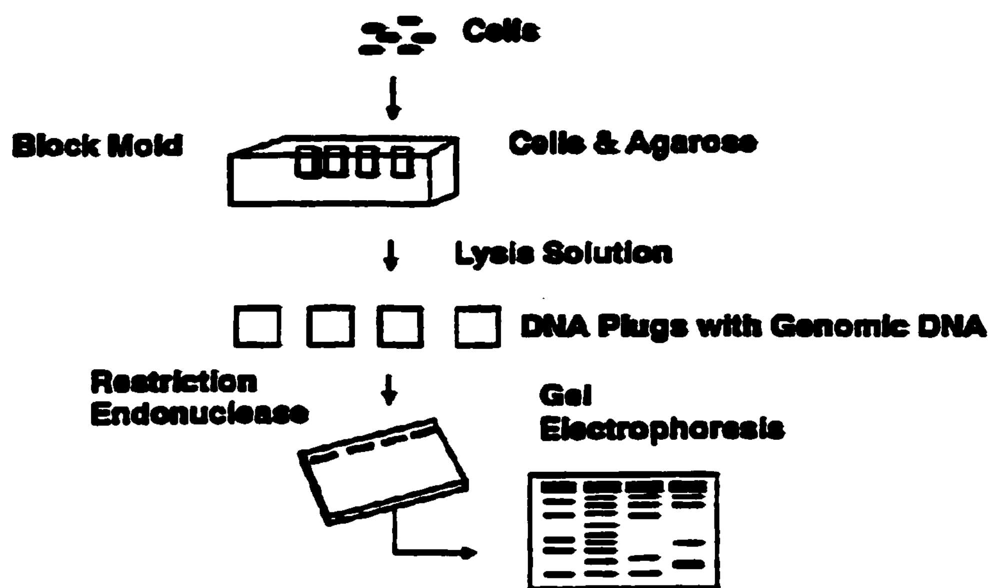
Campylobacter spp. possess two flagellin genes, *flaA* and *flaB* (both of approximately 1.7 kb). In *C. jejuni*, these flagellin genes are arranged in tandem and are separated by approximately 170 nucleotides (0.17 kb). These genes are highly (~92%) conserved in individual isolates but vary between isolates, providing the basis for a typing scheme. A variety

of different primers and restriction enzymes have been described for *fla* typing of *Campylobacter* strains. There appears to be little variation in the level of discrimination when using different primers however, much greater discrimination has been achieved when using different restriction enzymes. Overall, the level of discrimination of *fla* typing is much greater than that of serotyping, but much lower than that of PFGE. In addition to its limited discriminatory power, *fla* typing is highly affected by genetic instability. Recombination within the *fla* genes have been demonstrated among *C. jejuni* and *C. coli* strains using *in vitro* techniques. More recently, Harrington et al. (1997) compared *flaA* sequences from different *C. jejuni* strains, providing strong evidence for intergenomic recombination between *flaA* genes of different strains. There is also evidence that intragenomic recombination occurs between the *flaA* and *flaB* genes of individual strains. Although it is not clear how frequently these recombinations take place under natural conditions, *fla* typing cannot be considered a stable typing method and thus, should be used in conjunction with an alternative typing method.

1.4.6 PFGE

Pulsed-field gel electrophoresis, PFGE, is a form of macrorestiction in which infrequently cutting restriction enzymes are used in order to generate relatively few fragments of large size. This typing method involves embedding of bacterial cells in agarose (in order to prevent DNA shearing), lysis of embedded cells, extensive washing of agarose plugs (in order to remove contaminants) and subsequent restriction using "rare-cutting`` (i.e., 6-bp recognition site) enzymes (Figure 3). *Sma*I is the most commonly used restriction enzyme for PFGE typing of campylobacters; while *Sal*I and *Kpn*I have also been used (Gibson et al., 1997; Fujita et al., 1995; On et al., 1998). Restriction fragments are then separated using specialized

Figure 3. Schematic illustration of PFGE. Adapted from Farber, 1996.


Cells
Block Mold
Cells & Agarose
Lysis Solution
DNA Plugs with Genomic DNA
Restriction
Endonuclease
Gel
Electrophoresis

electrophoresis equipment which applies coordinated pulsed electric fields from different directions, allowing for a more extensive separation of fragments. Although PFGE analysis involves a relatively lengthy preparation process and requires an expensive electrophoresis apparatus, it is considered to be one of the most discriminatory and reproducible typing methods currently available. PFGE analysis has been used successfully to type a number of bacterial pathogens, including *Campylobacter* spp. (Suzuki et al., 1994; Fujita et al., 1995; Gibson et al., 1995; Gibson et al., 1997; On et al., 1998).

Several studies have shown that PFGE analysis of *C. jejuni* and *C. coli* results in highly discriminatory and reproducible profiles (Suzuki et al., 1994; Fujita et al., 1995; Gibson et al., 1995; Gibson et al., 1997; On et al., 1998). In fact, only amplified fragment length polymorphism (AFLP) appears to equal its discriminatory ability (Kokotovic and On, 1999). Studies have also demonstrated that PFGE typing is twice as discriminatory as ribotyping and anywhere from two to three times more discriminating than *fla* typing (Santesteban et al., 1996; Lorenz et al., 1998). Moreover, PFGE typing also appears to be as discriminatory, if not better than PCR-based typing methods such as RAPD.

PFGE typing has been used to characterize *Campylobacter* isolates of human, animal, food and environmental origin, worldwide. Recently, On et al. (1998) used PFGE to characterize *C. jejuni* isolates from humans, poultry and cattle. Analysis of these PFGE profiles identified groups of strains from humans, poultry and cattle that shared common PFGE patterns, suggesting a possible source of infection. This typing method has also been used to examine sporadic campylobacteriosis cases worldwide, e.g., Finland, Denmark and the UK; and is currently the basis of the Centers for Disease Control and Prevention (CDC)'s PulseNet program,

a national molecular subtyping network for foodborne bacterial surveillance in the US. In fact, the CDC have developed standardized PFGE methods for a number of foodborne pathogens, including *E. coli*, *Salmonella* and, most recently, *Campylobacter*.

When some *Campylobacter* strains are typed using PFGE, they produce smeared profiles. These strains are considered to be untypeable. Untypeable strains tend to have relatively high levels of DNases, causing degradation of DNA samples before electrophoresis. Formaldehyde has traditionally been used to deactivate DNases during PFGE plug preparation. However, because of its toxicity, and the extensive washing required to remove it, this practise has been stopped. Thiourea treatment is currently being recommended by the CDC for use with "difficult" strains (CDC, unpublished data). The addition of thiourea to the running buffer prevents DNA degradation during PFGE. Strains whose DNA cannot be digested by the restriction enzyme used, are also untypeable. A more significant problem seen with PFGE typing is the fact that, like *fla* typing, it is susceptible to genetic instability. Genetic rearrangements can lead to minor or major changes in PFGE patterns.

1.4.7 AFLP

Like RAPD, amplified fragment length polymorphism (AFLP) is a PCR-based typing method. This method involves complete digestion of chromosomal DNA with two restriction enzymes, one of these being a frequently cutting enzyme and the other an infrequent cutter. Subsequently, PCR amplification of the digestion products is initiated using radioactively or fluorescently labelled primers designed to amplify only those fragments flanked by both restriction sites. The labelled PCR products are then analysed on denaturing polyacrylamide gels.

AFLP analysis has only recently been used for typing of campylobacters. Only two

AFLP methods have been described. These methods involve either the restriction enzymes *Hind*III and *Hha*I, or *Bgl*I and *Csp6*I. Duim et al. (1999) and Kokotovic and On (1999) found that both of these methods produced highly discriminatory banding profiles when used to characterize a variety of poultry and human isolates. In this case, AFLP analysis appears to have provided the same level of discrimination as PFGE analysis. Unfortunately, like PFGE, AFLP requires specific equipment (i.e., automated DNA sequencers) and software needs, which prove quite costly. However, because preliminary studies have shown this method to be highly discriminatory and reproducible when analysing *Campylobacter* spp. (including *C. jejuni*, *C. coli*, *C. lari* and *C. fetus*), it is likely that it will be applied more widely for investigating the molecular epidemiology of *Campylobacter* spp.

1.4.8 MLST

Multilocus sequence typing (MLST) was first described by Maiden et al. (1998), and has been successfully used in the characterization of several bacteria, including *Campylobacter* spp. MLST is based on multilocus enzyme electrophoresis (MLEE), a method used to examine the electrophoretic mobilities of housekeeping enzymes. Unlike MLEE, MLST is based on the nucleotide sequence of housekeeping genes. As a result, MLST reveals all the variation at a particular locus, and is much more discriminatory than MLEE. MLST typically involves the sequencing of 450-500 bp internal fragments of housekeeping genes (usually seven genes). The different sequences for each gene are assigned as alleles, and the alleles at the various loci represent the allelic profile, or sequence type (ST) of each isolate.

MLST has recently been used to characterize *Campylobacter jejuni* isolates of human, animal and environmental origin. Dingle et al. (2001) described a MLST system based on seven

genes/loci involved in intermediary metabolism, including the following: *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt* and *uncA*. This system defined 155 sequence types among the 194 *C. jejuni* isolates examined, highlighting the genetic diversity of these isolates. Suerbaum et al. (2001) also described a high level of genetic diversity, with 31 sequence types identified, when this MLST system was used to examine 32 *C. jejuni* clinical isolates.

Although MLST is a relatively expensive typing method, it possesses several advantages. A major benefit of MLST is that it relies on the accumulation of nucleotide changes in housekeeping genes, a relatively slow process. This means that the allelic profile of an isolate will remain quite stable over time, making this method ideal for phylogenetic and population genetics/global epidemiology studies; providing important information about the source and mechanisms of transmission of *Campylobacter* spp. Another advantage of this method is that (MLST) protocols and data can be stored and accessed via the Internet. Moreover, MLST reduces the need for transport of live bacteria, since sequencing can be carried out on killed-cell suspensions, clinical material or purified DNA (using PCR). Also, unlike MLEE which can be quite subjective when it comes to gel analysis, MLST data is easily analysed (i.e., nucleotide differences) (Maiden et al., 1998; Enright and Spratt, 1999). A summary of the disadvantages and advantages of some of these genotypic methods can be seen in Table 5.

1.4.9 Genome sequencing and analysis

Recently, Parkhill et al. (2000) completed sequencing the genome of *C. jejuni* NCTC 11168. The genome of this strain was approximately 1,641 kb in size, with a G+C content of 30.6%. Sequence analysis revealed that an estimated 1,654 genes code for proteins, representing 94.3% of the genome. Interestingly, this organism is the most gene-dense bacterium sequenced to-date.

Table 5. Advantages and disadvantages of some common genotypic typing methods.[a]

Method	Advantages	Disadvantages
Plasmid Typing	Relatively quick and easy method	Plasmids usually unstable
	Results can be standardized using known markers	Some organisms contain few or no plasmids
		Different plasmids can appear to be the same size
Chromosomal DNA restriction endonuclease analysis (REA)	Universally applicable	Genomic restriction fragments are usually too numerous and too closely spaced
	Rapid, inexpensive, and relatively easy to perform	Have to screen a number of restriction endonucleases (RE)
Ribotyping	Single probe can be used to subtype all eubacteria	A little more tedious and time-consuming (multiple steps) than other molecular typing methods
	Reproducible patterns obtained with reasonable number of fragments after probing	Not as discriminating as some of the newer molecular methods (rrn operons cover only about 0.1% of chromosomal DNA)
	Can be automated	May not be useful for some bacteria that contain only 1 or 2 *rrn* loci
Pulsed-field gel electrophoresis	A tool for both taxonomic and epidemiological studies	More tedious and time-consuming than other molecular typing methods
	Highly reproducible and discriminatory	RE tend to be expensive
	Produces around 10-15 easily visible bands	Cost of equipment
RAPD	Rapid, easy to perform	Reproducibility, if not well standardized (see text)
	Does not require isotopic labelling nor use of restriction endonucleases	Only looking at possible difference from a small % of total genome when using 1 primer
	Does not require prior knowledge of DNA sequence	Comparison of different intensity bands of the same size may be problematic
	Oligo-primers can be made in unlimited amounts anywhere, so can develop standard RAPD typing procedure without need for exchanging research materials	

[a] Adapted from Farber, 1996.

Sequence analysis also revealed that there are very few repeat sequences and even fewer insertion sequences. Another interesting observation made by Parkhill et al. (2000) is that there is a high level of genetic variation, in the form of homopolymeric runs of nucleotides, in genes encoding the biosynthesis or modification of sugar molecules. These hypervariable sequences may play an important role in the pathogenesis of *C. jejuni*.

2. RATIONALE AND OBJECTIVES

Campylobacteriosis is the most frequently reported type of acute bacterial gastroenteritis in Canada, accounting for more cases than listeriosis, salmonellosis, *E. coli* and *Shigella* combined (Figure 2). In 2000 alone, 11,846 campylobacteriosis cases were reported to the National Laboratory for Enteric Pathogens, Health Canada. The majority of these cases are sporadic, and their causes remain unknown.

The overall objective of this study was to gain a better understanding of the epidemiology of campylobacteriosis in Ontario, both by identifying foods and environments that harbor *Campylobacter* spp. (and thus, could possibly cause illness) and by characterizing clinical, food and environmental isolates using PFGE.

The specific objectives of this study were:

1. To characterize *Campylobacter* clinical isolates, obtained from campylobacteriosis cases in the Ottawa-Carleton region, using PFGE.
2. To determine the prevalence of *Campylobacter* spp. in raw and ready-to-eat foods, and to type any food isolates using PFGE.
3. To determine the prevalence of *Campylobacter* spp. in a retail, chicken foodservice operation, and to type any environmental isolates using PFGE.
4. To determine the genetic relatedness of *Campylobacter* clinical, food and environmental isolates by analyzing PFGE profiles.
5. To relate the geographical distribution of campylobacteriosis cases in the Ottawa-Carleton region to PFGE profiles.

3. MATERIALS & METHODS

3.1. Bacterial strains and growth conditions

The *Campylobacter* clinical (C) and food (F) isolates analyzed in this study and their relevant properties are listed in Table 6, and Tables 1A and 2A (Appendix A). Clinical strains were all of faecal origin, and food strains were isolated mainly from raw chicken. All strains were obtained from the Bureau of Microbial Hazards (BMH) culture collection in the Sir F.G. Banting Research Center, Health Canada in Ottawa. Pure cultures of these *Campylobacter* strains were maintained in Brain Heart Infusion broth (BHI) (Difco Laboratories, Detroit, MI) containing 20% glycerol at -80°C. They were routinely subcultured onto Preston agar (Oxoid Inc., Nepean, ON) or modified charcoal cefaperozone desoxycholate (CCDA) (Oxoid) agar and grown at 37°C in a 10% CO_2 incubator for 24-48 h. The reference strain, *Staphylococcus aureus* NCTC 8325, was obtained from Dr. Sophie Michaud of the Department of Microbiology, Université Sherbrooke. This strain was routinely subcultured onto Tryptic Soy agar (TSA; Difco) containing 0.6% yeast extract, and incubated at 37°C for 24 h.

3.2. Isolation of *Campylobacter* spp.

3.2.1. Foods

i) Sampling:

The incidence of *Campylobacter* spp. in a variety of raw and ready-to-eat foods was determined. Foods were purchased from local supermarkets, on a weekly or biweekly basis, over the course of 2 years (September 1999 to August 2001). Raw milk samples were also tested for the presence of campylobacters. These samples were collected by government inspectors and shipped under refrigeration by overnight courier. Samples were collected from raw milk cheese

Table 6. Clinical and food isolates used for PFGE analysis.

Species	**Clinical isolates** No. (%)	**Food isolates** No. (%)
C. jejuni	208 (88%)	40 (98%)
C. coli	19 (8%)	1 (2%)
C. lari	10 (4%)	0
Total	237	41

manufacturers in New Brunswick, Nova Scotia, Quebec, Manitoba, Alberta and British Columbia at two-week or monthly intervals over the period of January 2000 to March 2001.

ii) Sample preparation and enrichment:

Isolation and identification of *Campylobacter* spp. from foods was carried out according to a modification of the procedures outlined in Health Products and Food Branch (HPFB) method MFLP-46 (Sanders, 1999).

3.2.1.1 Raw chickens

Whole raw chickens were placed in a large sterile plastic bag to which 400-500 ml of 0.1% (wt/vol) peptone water was added. The bag was twisted and tied to create a tight seal, and the contents were shaken in an automated paint shaker for 2 min. Chicken parts (e.g., thighs, breasts) and chicken hearts were manually massaged for 5 min in a bag containing 100-200 ml of 0.1% (wt/vol) peptone water. Twenty-five ml of chicken rinse was added to 100 ml of Park and Sanders (P-S) enrichment broth (Brucella broth, lysed horse blood, sodium cefaperazone [0.032 g/L], cycloheximide [0.01g/L], vancomycin [0.01g/L] and trimethoprim lactate [0.01g/L]).

3.2.1.2 Raw milk

Raw milk samples were tested as shown in Figure 4. In some cases, milk samples were tested in duplicate, with 25 ml of the sample being added directly to 100 ml of P-S enrichment broth, and the other portion (100 ml) being centrifuged and the pellet added to 100 ml of P-S enrichment broth.

3.2.1.3 Raw meat

One hundred g of meat (ground pork, ground beef, beef liver) was placed into 100 ml of 0.1 % (wt/vol) peptone water in a Stomacher™ bag and pummelled for 2 min. A volume of 25 ml was

Figure 4. Method used for isolation of *Campylobacter* spp. from raw milk (Sanders, 1999).

Test pH using Litmus paper

Adjust pH to 7.6 (using 1-2 N NaOH), if necessary

Centrifuge 100 ml of raw milk sample
(16, 000 × g , 20 min at 4°C)

Discard supernatant and fat layer

Suspend pellet in 100 ml of
Park and Sanders enrichment broth

Incubate broth at 37°C for 4 h, and then at 42°C for 24-48 h,
under shaking conditions (100-120 cycles/rpm)
(microaerophilic [5% O_2, 10% CO_2, 85% N_2] conditions)

Plate onto Preston and CCDA plates after 24 and 48 h of enrichment; incubate under
microaerophilic [5% O_2, 10% CO_2, 85% N_2] conditions for 24-48 h

removed and introduced into 100 ml of P-S enrichment broth.

3.2.1.4 Paté, cheeses, BBQ foods

Samples were cut into small pieces (0.3-0.5 cm^3) and 25 g was placed into P-S enrichment broth.

3.2.1.5 Ready-to-eat salads

Salads (100 g) were rinsed with 200 ml of 0.1% (w/v) peptone water by manually massaging or pummelling for 5-10 min. The resulting salad rinse was either centrifuged (16,000 × g for 20 min at 4°C) or introduced, at a volume of 25 ml, directly into 100 ml of P-S enrichment broth. In the case of centrifugation, the remaining pellet was retrieved and suspended in 100 ml of P-S enrichment broth.

iii) Incubation conditions:

Enrichment broths were placed in an anaerobic jar filled with a microaerophilic gas mixture (5% O_2, 10% CO_2, 85% N_2), and then incubated at 37°C for 4 h, followed by 42°C for 24-48 h under shaking conditions (100-120 cycles/rpm).

iv) Isolation and identification:

After 24 and 48 h of enrichment, a few loopfuls of each broth were streaked onto Preston and CCDA plates. These plates were incubated at 37°C in a CO_2 incubator (5 % O_2, 10 % CO_2, 85 % N_2) for 24-48 h. Suspect colonies were presumptively identified by phase-contrast microscopy, then purified by streaking onto CCDA. Confirmatory testing included Gram staining, biochemical tests (catalase, oxidase, hippurate hydrolysis) and antimicrobial susceptibility tests (nalidixic acid and cephalothin), and were performed according to the procedures described by Sanders (1999).

3.2.2 Faeces

Faecal samples from campylobacteriosis cases, primarily from the Ottawa-Carleton region, were received from Gamma-Dynacare Laboratories (Ottawa, ON). Samples were accompanied by a service report which generally included the patient and physician's name, complete address, as well as the date when the sample was tested (i.e., service date). Two loopfuls of faeces were streaked onto Preston and CCDA agar and then incubated for 48 h at 37°C in a 10% CO_2 incubator. From each positive agar plate, one typical *Campylobacter* colony was subcultured onto CCDA and tested for Gram-staining, motility, production of oxidase and catalase, and hippurate hydrolysis; antimicrobial susceptibility tests (nalidixic acid and cephalothin) were also performed. In some cases, a *Campylobacter* isolate could not be retrieved, primarily because too much time had elapsed between the initial testing at Gamma-Dynacare Laboratories to receipt of samples.

3.2.3 Environment

The prevalence of *Campylobacter* spp. in a retail poultry foodservice operation in Ottawa (ON, Canada) was determined from March to August 2001. Samples were collected on a weekly or biweekly basis, at different times of the day; and visits were unannounced. Various kitchen surfaces, at different stages of the preparation process (Figure 5), were tested using a modification of the procedures outlined by Humphrey et al. (1995). Surfaces were sampled using a sterile swab which was moved 5 times horizontally and 5 times vertically over the surface. The swab was immediately introduced into a sterile 15 ml screw cap Falcon tube containing 5 ml of Maximum Recovery Diluent (MRD) (Oxoid) and 0.05 % (wt/vol) of sodium thiosulphate (Difco). A second swab was subsequently used to sample the same area, and was

Figure 5. Sampling sites in the kitchen of a foodservice operation.

OVENS
C
RAW CHICKEN PREPARATION AREA
B
PREPARATION OF GRAVY
COOKING AREA
STORAGE OF RAW POTATOES (IN BROWN BAGS)
REFRIGERATOR (STORAGE OF RAW CHICKEN AND CHICKEN FAT)
A
COOKED CHICKEN (PLATE PREPARATION)
D
STORAGE OF COOKED CHICKEN (HEATED DRAWERS)
E
REFRIGERATOR (STORAGE OF READY-TO-EAT FOODS – E.G., SALADS, DESSERTS)
H
F
TAKE-OUT
G
PREPARATION OF READY-TO-EAT FOODS (E.G., SALAD WITH COOKED CHICKEN)
WASH-UP AREA (DISHWASHER)

introduced into the same MRD tube. Tubes were labelled and immediately placed in a freezer box for transport back to the laboratory.

The MRD was introduced into 225 ml of Exeter selective enrichment broth (Mast Nutrient broth, *Campylobacter* growth supplement [FBP], *Campylobacter* Enrichment supplement [Exeter]; Mast Diagnostics, Merseyside, UK) within an hour of sampling, and incubated under microaerophilic (5% O_2, 10% CO_2, 85% N_2) conditions at 37°C for 120 h under shaking conditions (100-120 rpm). In some cases, the swab was also tested by being introduced into a separate flask containing 225 ml of Exeter selective enrichment broth. A positive control was included with each set of environmental samples, and consisted of a loopful of isolate C134 being introduced into 225 ml of Exeter selective enrichment broth. In some cases, the MRD and/or swab was introduced into 225 ml of Exeter selective enrichment broth and 100 ml of P-S enrichment broth, in order to compare the recovery potential of these two broths. A few loopfuls of the enrichment broth were streaked onto Preston (Oxoid), Muëller-Hinton (MHA) plus 5% lysed horse blood (Oxoid), and Exeter (Mast Diagnostics) agar after 24, 48, 72 and 120 h of enrichment. These plates were incubated at 37°C for 24-48 h under microaerophilic conditions. Presumptive colonies were examined as described above (section 3.2.1, iv).

Detection limits of environmental sampling method

A modification of the method of Humphrey et al. (1995) was first tested using stainless steel chips. The chips (2 cm x 2 cm) used were of the same grade (410) of steel as that used to construct restaurant counter-tops, thereby simulating the type of surface that *Campylobacter* spp. would be in contact with in the environment of a foodservice operation. Five *Campylobacter* strains (C72, C127, C134, F26, F34) were introduced onto these chips and then recovered, as

outlined in Figure 6. A number of factors were varied, in order to determine the detection limit of this method, as well as the maximal drying time for recovery (Table 7, Tables 1B and 2B, Appendix B).

3.3 Characterization of *Campylobacter* isolates

The 237 clinical and 41 food isolates shown in Table 6, and Tables 1A and 2A (Appendix A) were typed by pulsed-field gel electrophoresis. Strains were typed twice (i.e., plug preparations on separate days) in order to determine the reproducibility of the method.

3.3.1 Optimization of PFGE methods

A variety of *Campylobacter* PFGE protocols have been described in the literature. These methods use different procedures for plug preparation, lysis, washing, restriction and electrophoresis, making it difficult to compare data. In an attempt to facilitate interlaboratory comparisons, the CDC have and continue to develop standardized PFGE protocols for a number of foodborne pathogens. CDC's *Campylobacter* PFGE protocol was not available until recently (Ribot et al., 2001). As a result, it was necessary to test and optimize existing *Campylobacter* PFGE methods before typing the *Campylobacter* isolates described above. The PFGE method of Chang and Chui (1998) was optimized by incorporating some elements of CDC's standardized PFGE method for *E. coli* O157:H7 and *Salmonella* Typhimurium (CDC, unpublished data; Swaminathan et al., 2001). Table 8 highlights some of the parameters tested and optimized.

3.3.2 PFGE analysis

i) *Sma*I

Strains were typed using a modification of the PFGE method described by Chang and Chui (1998), as follows (Figure 7):

Figure 6. The recovery of *Campylobacter* spp. from artificially-inoculated stainless steel chips using a modification of the environmental sampling method of Humphrey et al. (1995).

Streak *Campylobacter* isolate (C72, C127, C134, F26, F34) onto Preston agar; incubate at 37°C for 48 h under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2)

Introduce a pure colony from this plate into 50 ml of Brucella broth, in a 250 ml flask; incubate at 37°C for 24-48 h under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2); swirl the contents at 100-120 cycles/min

Prepare dilutions ranging from 10^{-1} to 10^{-7} in peptone water (or Brucella broth or negative chicken rinse) after 48 h of incubation

Smear 10 µl (or 100 µl) of each dilution onto the stainless steel chip (2 cm^2); allow to air dry for 5 min (15 min, 30 min, 45 min, 1 h, 2 h, or 4 h) at room temperature or at 4°C

Swab the surface of the stainless steel chip with a sterile swab - move 5X horizontally and 5X vertically

Introduce the swab into Maximum Recovery Diluent (MRD) containing 0.05% sodium thiosulphate; include a positive control (i.e., MRD spiked with ~10^7 *Campylobacter* cells/ml)

Use a second swab to swab the same region, and introduce it into the same Maximum Recovery Diluent (MRD) containing 0.05% sodium thiosulphate

Introduce the MRD into modified Exeter broth (or plate directly); incubate under microaerophilic (5% O_2, 10% CO_2, 85% N_2) conditions at 37°C for 120 h, and shake the contents at 100-120 cycles/min

Streak two loopfuls of the culture onto Preston, Exeter plates and Mueller-Hinton (plus 5% horse bllod) media after 24, 48, 72 and 120 h enrichment; incubate plates at 37°C for 24-48 h under microaerophilic conditions

Table 7. Factors varied when testing a modification of the the environmental sampling method of Humphrey et al. (1995).

Factor	Variation(s)
Diluent	Peptone H_2O, Brucella broth, Negative chicken rinse
Drying time	5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h
Inoculum concentration	10^2 to 10^8 cells/ml
Inoculum size	10 μl, 100 μl
Isolates	Clinical (C72, C127, C134), Food (F26, F34)
Drying temperature	Room temperature, 4°C
Enrichment broth	Exeter, no enrichment
Enrichment time	24 h to 5 days

Table 8. Parameters varied in order to optimize the discriminatory power and reproducibility of existing *Campylobacter* PFGE methods.

Parameter	Variation(s)
Optical density (cell suspension)	1.0 - 2.0
Cell lysis solution	• ESP buffer (0.25 M EDTA (pH 9.5), 1% (w/v) sodium lauroyl sarcosine, 0.5 mg/ml proteinase K) • 50 mM Tris: 50 mM EDTA, pH 8.0, 1% (w/v) sodium lauroyl sarcosine plus 25 μl of proteinase K (20 mg/ml)
Washes	• Four TE washes at room temperature • Two ddH_2O washes followed by four TE washes at 54°C
Digestion conditions	
• Enzyme concentration	• 20 U, 40 U
• Incubation time	• 2 h, overnight
Electrophoresis conditions	
• Switch times	• Initial switch time (2.16 s, 54.17 s), final switch time (5.3 s, 35 s)
• Run times	• 19.5 - 23 h

Figure 7. Method used for PFGE typing of *Campylobacter* clinical and food isolates using the restriction enzyme *Sma* I.

Remove cells from Preston plate using a sterile loop or swab; adjust to an O.D. of 1.8 in 1.5 ml of 12 mM Tris-HCl buffer, pH 7.6

Transfer 100 μl of cell suspension into a 1.5 ml Eppendorf tube containing 10 μl of proteinase K (20 mg/ml) and incubate for 5 min at 50°C

Add 450 μl of 1% (w/v) Seakem Gold® agarose (incubated at 55-65°C) to the cell suspension; mix gently and transfer into the wells of a plug mold; allow to solidify at room temperature for 10-15 min

Transfer each plug into a 50 ml Falcon tube containing 1.5 ml of ESP solution [0.25 M EDTA (pH 9.5), 1% (w/v) sodium lauroyl sarcosine, 0.5 mg/ml proteinase K]; incubate in a 50°C waterbath for 1.5 h

Cut a 1-mm thick slice with a single-edge razor blade using a gel-cutting template and transfer into a 1.5 ml tube containing 1 ml of ddH_2O; incubate in a 50°C waterbath for 15 min (gentle shaking); repeat once more

Wash 4 times with TE buffer (10 mM Tris, 10 mM EDTA, pH 7.6) in a 50°C waterbath for 15 min/wash

Replace the TE buffer with a pre-restriction mixture (100 μl of 1X restriction buffer for *SmaI*) and incubate at room temperature for 15 min

Replace the pre-restriction mixture with 100 μl of the same restriction enzyme mixture containing 20 U of *Sma* I and incubate at 25°C overnight

Prepare 1% (w/v) agarose gel in 0.5X M TBE; transfer a slice into each well and overlay the well with this same agarose

Transfer the gel into the electrophoresis cell of a CHEF Mapper (or CHEF-DRII or CHEF-DRIII) unit and run as follows: <u>Temperature</u>: 14°C; <u>Initial switch time</u>: 5.3 s; <u>Final switch time</u>: 35 s; <u>Included Angle</u>: 120; <u>Cell voltage</u>: 6V/cm; <u>Run time</u>: 22 h

Plug preparation

Fresh (48 h) *Campylobacter* cells grown on Preston agar were harvested using a sterile loop or swab and suspended in a glass round-bottom test tube containing 1.5 ml of 12 mM Tris-HCl buffer, pH 7.6. Each cell suspension was adjusted to an optical density of 1.8 using a Dade MicroScan turbidity meter (Baxter Diagnostics, Mississauga, ON). This optical density is equivalent to an absorbance value of 2.931 when using a spectrophotometer adjusted to a wavelength of 610 nm. A 10 μl volume of 20 mg/ml proteinase K (Roche Diagnostics, Laval, QC) solution was added to 100 μl of this cell suspension, mixed gently, and incubated for 5 min in a 50°C water bath. A 450 μl volume of melted 1% (wt/vol) Seakem® Gold (SKG®; Mandel, Guelph, ON) agarose in TE (10 mM Tris, 10 mM EDTA, pH 7.6), that had been incubated in a 55-65°C water bath, was added to the treated cell suspension. After gently mixing the agarose by pipetting the mixture up and down a few times, a 350-μl volume of the cell-agarose mixture was dispensed immediately into the wells of a reusable plug mold (Bio-Rad Laboratories, Hercules, CA), and allowed to solidify at room temperature for 10-15 min.

Lysis of cells in plugs

Each agarose plug was transferred into a 50 ml Falcon tube (polypropylene tubes; 30 by 115 mm; VWR CanLab, Mississauga, ON) containing 2 ml of ESP lysis solution [0.25 M EDTA (pH 9.5), 1% (wt/vol) sodium lauroyl sarcosine, 0.5 mg/ml proteinase K]. This was followed by incubation in a 50°C water bath for 1.5 to 2 h, with constant and vigorous agitation (175-200 rpm). After this incubation period, plugs were either stored in ESP at 4°C or washed.

Washes

A 1 mm-wide slice from each plug was cut with a single-edge razor blade using a gel-cutting

fixture/template (S&S Service Company, Georgia, US) and transferred into a 1.5 ml Eppendorf tube containing 1 ml of 50°C-preheated ddH_2O, and incubated in a 50°C water bath for 15 min. Slices were washed once more with 1 ml of preheated ddH_2O and then four times (15 min/wash) with 1 ml of preheated TE buffer (10 mM Tris, 10 mM EDTA, pH 7.6) in a 50°C water bath.

Restriction digestion

After discarding the TE buffer, the slices were incubated in 100 μl of 1X restriction buffer solution (SureCut buffer A; Roche) at room temperature for 15 min. This pre-restriction mixture was removed and replaced with 100 μl of the restriction enzyme mixture containing 20 U of *Sma*I (Roche). The plug slices were incubated overnight at 25°C. The plug slices were then loaded into the wells of a 1% SKG® agarose gel (prepared in 0.5X TBE [0.045 M Tris-borate, 1mM EDTA, pH 8.0]), and overlaid with this same agarose.

Electrophoresis conditions

Electrophoresis was performed using a CHEF Mapper (or CHEF-DR III, CHEF-DR II) unit (Bio-Rad Laboratories). Gels were run for 22 h in 0.5X TBE at 14°C, and were subjected to an initial switch time of 5.3 s and a final switch time of 35 s (gradient of 6 V/cm and an included angle of 120°). Gels run using the CHEF-Mapper were adjusted to an AutoAlgorithm of 50-400 kb. After electrophoresis, gels were stained with 400 ml of ethidium bromide solution (0.5 mg/ml) for 30 min at room temperature, de-stained for 1h, and then photographed under u.v. illumination using the Bio-Rad Gel Doc system.

ii) *Kpn*I

Strains were typed using a modification of CDC's rapid PFGE protocol for subtyping of *Campylobacter jejuni* (Ribot et al., 2001), as follows:

Plug preparation

Fresh (48 h) *Campylobacter* cells grown on Preston agar were harvested using a sterile loop or swab and suspended in a glass round-bottom test tube containing 2 ml of saline (0.85% NaCl, pH 7.2). Each cell suspension was adjusted to an optical density of 1.5 using a Dade MicroScan turbidity meter. This optical density is equivalent to an absorbance value of 2.443 when using a spectrophotometer adjusted to a wavelength of 610 nm. A 400 μl aliquot of this suspension was transferred to a 1.5 ml Eppendorf tube containing 20 μl of proteinase K (20 mg/ml) and mixed gently by inverting the tube. An equal volume of melted 1% SKG® agarose in TE (10 mM Tris, 1 mM EDTA, pH 8.0) was added to the cell suspension and mixed gently by pipetting up and down a few times. A 450-μl volume of the cell-agarose mixture was immediately dispensed into the wells of a reusable plug mold. The agarose plugs were allowed to solidify at room temperature for 10-15 min.

Lysis of cells in plugs

Each agarose plug was transferred into a 50 ml Falcon tube containing 5 ml of cell lysis buffer (50 mM Tris: 50 mM EDTA, pH 8.0, 1% sodium lauroyl sarcosine), and 25 μl of proteinase K (20 mg/ml). This was followed by incubation in a 54°C water bath for 30 min, with constant and vigorous agitation (175-200 rpm).

Washes

After lysis, the plugs were washed once with 10 ml of 54°C-preheated ddH_2O for 15 min. in a 54°C shaking water bath. Slices were then washed four times (15 min/wash) with 10 ml of 54°C- preheated TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) under the same conditions. After the final wash, the TE buffer was replaced with 5 ml of fresh TE buffer at room temperature.

Restriction digestion

A 1 mm-wide slice from each plug was cut with a single-edge razor blade using a gel-cutting template and transferred into a 1.5 ml Eppendorf tube containing 200 μl of 1X restriction buffer solution (Buffer #1, bovine serum albumin (BSA); New England BioLabs, Mississauga, ON) and incubated at 37°C for 15 min. This pre-restriction mixture was removed and replaced with 200 μl of the restriction enzyme mixture containing 20 U of *Kpn*I (New England BioLabs). The plug slices were incubated overnight at 37°C. The plug slices were then loaded into the wells of a 1% SKG® agarose gel (prepared in 0.5X TBE [0.045 M Tris-borate, 1mM EDTA, pH 8.0]), and overlaid with this same agarose.

Electrophoresis conditions

Electrophoresis was performed using a CHEF Mapper (or CHEF-DR III, CHEF-DR II) unit. Gels were run for 22 h in 0.5X TBE at 14°C, and were subjected to an initial switch time of 4.0 s and a final switch time of 20 s (gradient of 6 V/cm and an included angle of 120°). Gels run using the CHEF-Mapper were adjusted to an AutoAlgorithm of 30-700 kb. After electrophoresis, gels were stained with 400 ml of ethidium bromide solution (0.5 mg/ml) for 30 min at room temperature, de-stained for 1h, and then photographed under u.v. illumination using the Bio-Rad Gel Doc system.

iii) Reference/standard strain

*Sma*I digests of *Staphylococcus aureus* NCTC 8325, the reference strain, were prepared according to the method described by Michaud et al. (2001, 2001a) as follows:

Plug preparation

Staphylococcus aureus strain NCTC 8325 was first grown for 24 h at 37°C on TSA containing

0.6% yeast extract, harvested using a sterile loop or swab and then suspended in a glass round-bottom test tube containing 1.5 ml of saline (0.85% NaCl, pH 7.2). Each cell suspension was adjusted to an optical density of 1.9 to 2.2 using a Dade MicroScan turbidity meter. This optical density corresponds to an absorbance value of 3.094 to 3.583 when using a spectrophotometer adjusted to a wavelength of 610 nm. A 100 μl aliquot of this suspension was transferred into a 1.5 ml Eppendorf tube. A 450 μl volume of melted 1% SKG® agarose in TE (10 mM Tris, 1 mM EDTA, pH 8.0) was added to the cell suspension and mixed gently by pipetting up and down a few times. A 450-μl volume of the cell-agarose mixture was immediately dispensed into the wells of a reusable plug mold. The agarose plugs were allowed to solidify at room temperature for 10-15 min.

Lysis of cells in plugs

A 1 mm-wide slice from each plug was cut with a single-edge razor blade using a gel-cutting template and then transferred into a 1.5 ml Eppendorf tube containing a 1 ml solution of lysozyme (1 mg/ml) and lysostaphin (25 μg/ml). This was followed by incubation in a 37°C heating block for 3-3.5 h. Slices were subsequently incubated in 1ml of cell lysis buffer (50 mM Tris: 50 mM EDTA, pH 8.0, 1% sodium lauroyl sarcosine), and 25 μl of proteinase K (20 mg/ml), and incubated at 54°C for 1-1.5 h.

Washes

After lysis, the plugs were washed once with 1 ml of 54°C-preheated ddH_2O for 15 min in a 54°C water bath. Slices were then washed four times (15 min/wash) with 1 ml of 54°C-preheated TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) in the same water bath. After the final wash, the TE buffer was replaced with 1 ml of fresh TE buffer at room temperature.

Restriction digestion

After discarding the TE buffer, the slices were incubated in 100 μl of 1X restriction buffer solution (SureCut buffer A; Roche) at room temperature for 15 min. This pre-restriction mixture was removed and replaced with 100 μl of the restriction enzyme mixture containing 20 U of *Sma*I. The plug slices were incubated overnight at 25°C. The plug slices were then loaded into the wells of a 1% SKG® agarose gel (prepared in 0.5X TBE [0.045 M Tris-borate, 1mM EDTA, pH 8.0]), and overlaid with this same agarose. *Sma*I digestion of the reference strain resulted in 16 fragments of the following sizes: 673.7, 361, 324, 262, 257, 208, 175, 135, 117, 80, 76, 44, 36, 10 (doublet) and 9 kb.

3.4 Analysis of PFGE patters and campylobacteriosis cases

3.4.1 PFGE patterns

The PFGE patterns were analyzed using the Molecular Analyst Fingerprinting Plus software package (version 1.61; Bio-Rad). The TIFF images were normalized by aligning the peaks of the reference strain (*Staphylococcus aureus* NCTC 8325), which was routinely loaded in three lanes (lanes 1, 8 and 15) of each gel, with the database standard. Matching and database UPGMA (unweighted pair group method with averages) analysis of PFGE profiles was performed using the Dice coefficient with a 1.0 to 1.5 % tolerance window, as described by Ribot et al. (2001). Unique profiles were defined as varying from each other by a minimum one-band difference. A number was assigned to each profile, with numerical closeness reflecting genetic similarity. For example, isolates assigned the profile number 1 are more similar to isolates assigned the profile number 2, than profile number 5. The discriminatory power of *Sma*I and *Kpn*I PFGE patterns were compared using Simpson's Index of Diversity (Hunter and Gaston, 1988).

3.4.2 Campylobacteriosis cases

The geographical distribution of campylobacteriosis cases in the Ottawa-Carleton region was determined by preparing spot maps. The campylobacteriosis cases data set was provided to GIS Analysts at the Center for Surveillance Coordination's Geographic Information Systems (GIS) Infrastructure, Health Canada, through their Map and Data Exchange collaborative website. The GIS Analyst produced maps in ArcView 3.2a using data sets retrieved from their Spatial Data Warehouse and in return posted the maps in the Map and Data Exchange in jpeg format. Complete methodology and the list of data sets used are included in Appendix D, as provided by the GIS Infrastructure. Fifty isolates were excluded from the data set, either because there was insufficient address information, or because they represented cases outside of the Ottawa-Carleton region (e.g., strain C308 was obtained from a case residing in Langley, British Columbia). As a result, 187 of the 237 cases were plotted.

4. RESULTS AND DISCUSSION

4.1. Prevalence of *Campylobacter* spp.

4.1.1 Raw and ready-to-eat foods

Relatively little is known about the foods responsible for causing sporadic cases of campylobacteriosis. In the majority of cases, the sources of infection are rarely identified, either because the suspected food item is no longer available, or because *Campylobacter* spp. cannot be recovered after prolonged storage of the suspected food item.

Epidemiological studies have demonstrated a significant association between the handling and consumption of raw or undercooked poultry and the development of campylobacteriosis. However, the extent to which poultry consumption is responsible for sporadic campylobacteriosis cases is not known. In addition to poultry, other foods must be considered as potential sources of infection, since *Campylobacter* spp. have been isolated from red meats, milk and dairy products, seafood, vegetables and fruits.

A variety of raw and ready- to-eat foods were analyzed for the presence of *Campylobacter* spp. Of the 55 raw foods tested, only four samples, chicken pieces (3) and beef liver (1), contained *Campylobacter* spp. (Table 9). This was not surprising, since poultry parts have been shown to be major sources of *Campylobacter* spp., especially *C. jejuni*. In fact, poultry consumption trends throughout the late 1980s and mid 1990s strongly mirrored the number of campylobacterioris cases reported in Canada (Figure 1E, Appendix E). However, somewhat surprising was the low isolation percentage among the raw chicken samples, i.e., out of the 31 samples analyzed, *Campylobacter* spp. were found in only three (9.7%) samples. This prevalence is much lower than that observed in a 1981 Canadian survey of fresh whole market

Table 9. Prevalence of *Campylobacter* spp. in raw foods.

Food	No.[a] (55)	*Campylobacter* spp.[b] (No. of positives;%)
Whole chicken	5	-
Chicken pieces	16	(3; 19%)
Ground pork	8	-
Ground beef	8	-
Chicken hearts	10	-
Beef liver	8	(1; 12.5%)

[a] No., Number of samples tested.
[b] -, *Campylobacter* spp. not detected.

chickens, where Park et al. (1981) detected *Campylobacter* spp. in 62% of whole raw retail chickens examined, but correlates well with the findings of an on-going study conducted by Michaud et al. (2001) in which *Campylobacter* spp. were detected in 12% of whole raw retail chickens examined in Sherbrooke, Quebec. These differences in incidence may be related to changes in the chicken slaughtering process. For example, Stern et al. (1999) recently reported a 30% prevalence of *Campylobacter* spp. in whole raw chickens from US plants using chlorinated water chilling. This is in comparison to the 80 to 90% prevalence reported in an earlier study, involving chickens from plants that did not rely on chlorinated water chilling (Stern and Line, 1992). In Canada, significant modifications have occurred in the chicken processing industry over the past decade, and include the incorporation of several water showers, complete detachment of the viscerae, and air chilling of carcasses during the slaughtering process (Thiessen, 2001). The use of showers helps to remove cells on the chickens' surface while lubricating the intestine, thereby preventing intestinal tearing and leakage. Air chilling also helps to reduce the level of cross-contamination that occurs during the slaughtering process because of its drying effect. Overall, these procedures aid in reducing the levels of *Campylobacter* spp. and, possibly, the prevalence of this organism in raw whole retail chickens. Reduced isolation rates may also be the result of improved rearing conditions within broiler houses, as well as increasingly stringent hygienic practices both at the farm and processing levels. Differences in isolation percentages may also, in part, be related to differences in sampling and detection methods. This would account for the wide variation in isolation percentages (0 to 100%) described worldwide. Interestingly, *Campylobacter* isolates were obtained from chicken samples analyzed over the summer months (i.e., July and August), suggesting the possibility of a seasonal

effect.

Although cattle and swine harbor campylobacters, the incidence rate is much lower than that found in poultry. In fact, the overnight forced-air chilling of pig and beef carcasses has been shown to significantly reduce the number of *Campylobacter* spp., with isolation rates ranging from 0 to 23% being reported for beef and pork (Fricker and Park, 1989; Hald et al., 1998; Madden et al., 1998; Jacobs-Reitsma, 2000). In the present study, no *Campylobacter* spp. were detected in the 16 raw ground beef and pork samples analyzed, and *Campylobacter* spp. were detected in only one of the 8 beef liver samples tested (Table 9).

In addition to the raw foods shown in Table 9, over 100 ready-to-eat foods were analyzed for the presence of *Campylobacter* spp. (Table 10). Among these foods, were two varieties of raw milk cheese, Brie and Camembert. *Campylobacter* spp. were not detected in any of the 34 raw milk cheese samples examined. Although it is generally thought that raw milk cheeses pose a higher risk of contamination than pasteurized milk cheeses, very few researchers have been able to detect *Campylobacter* spp. in raw milk cheese.

A variety of ready-to-eat poultry products were also tested for the presence of *Campylobacter* spp. These products included both preserved (e.g., cold-cuts) and freshly prepared poultry items. *Campylobacter* spp. were not detected among the 33 poultry products analyzed, suggesting that these food items had been properly cooked and had not come into contact with contaminated foods or surfaces. Similarly, *Campylobacter* spp. were not detected among any of the seafood products tested, including the seafood paté and pre-cooked shrimp (Table 10). While *Campylobacter* spp., especially *C. lari*, are frequently isolated from seafood shortly after harvesting, very low isolation percentages have been reported among ready-to-eat

Table 10. Prevalence of *Campylobacter* spp. in ready-to-eat foods.

Food	No.[a] (119)	*Campylobacter* spp.
Raw milk cheeses		
• Brie	20	-[b]
• Camembert	14	-
Ready-to-eat salads		
• Caesar	10	-
• Greek	10	-
• Garden	10	-
Paté		
• Seafood	7	-
• Liver	8	-
Cold-cuts		
• Turkey cold cuts	7	-
• Chicken cold cuts	16	-
BBQ chicken	5	-
BBQ pork	5	-
BBQ duck	5	-
Shrimp	6	-
Stir-fry vegetables	5	-
Alfalfa sprouts	7	-

[a] No., Number of samples tested.
[b] -, *Campylobacter* spp. not detected.

seafood products (Hald et al., 1998; Jacobs-Reitsma, 2000).

Few studies have examined the prevalence of *Campylobacter* spp. in vegetables, even though there exists the possibility of contamination by the application of natural fertilizers, contaminated surface waters, and/or by wild birds and animals. In this study, a variety of vegetables were analyzed for the presence of *Campylobacter* spp. Alfalfa sprouts, stir-fry vegetables and ready-to-eat salads were among the items tested. *Campylobacter* spp. were not detected on the 30 ready-to-eat salads, 5 ready-to-use stir-fry vegetables or 7 alfalfa sprouts samples analyzed in this study. Park and Sanders (1992) were unable to detect *Campylobacter* spp. in any of the 1,031 vegetable samples they obtained from local supermarkets. Similarly, Fricker and Park (1989) did not detect *Campylobacter* spp. in any of the 106 ready-to-eat salads examined as part of a British retail study, nor did Odumeru et al. (1997) detect *Campylobacter* spp. in the 65 unprocessed and 296 fresh-cut and packaged ready-to-use vegetables examined. The overall low prevalence of *Campylobacter* spp. in these commodities may be due to the inability of campylobacters to survive and/or grow on vegetables, primarily because of drying effects.

4.1.2 Raw milk

Raw milk has been associated with a number of campylobacteriosis outbreaks. Among these, was a large outbreak in the UK in 1979 in which over 2,500 children became ill after the consumption of raw milk (Jones et al., 1981). *Campylobacter* spp. were not detected in any of the 126 raw milk samples examined in this study (Table 11). A number of other groups have either been unable to detect *Campylobacter* spp. in raw milk (Stone, 1987; Hahn et al., 1992), or have found it to be present in a low percentage of samples tested (Doyle and Roman, 1982; Gomolka

Table 11. Prevalence of *Campylobacter* spp. in raw milk.

Animal origin/source	No.[a] (126)	*Campylobacter* spp.
Cow	59	-[b]
Sheep	39	-
Goat	28	-

[a] No., Number of samples tested.
[b] -, *Campylobacter* spp. not detected

and Uradzinski, 1996). Steele et al. (1997), for example, isolated *Campylobacter* spp. from only 0.5% of the 1,720 raw milk bulk tank samples obtained from Ontario farms.

4.1.3 Environment

Very little is known about the dynamics of *Campylobacter* spp. survival in the environment of a foodservice operation. Environmental studies have focused primarily on *Campylobacter* spp. survival within abattoirs, and only a few studies have studied the dynamics of *Campylobacter* survival in a restaurant (Dawkins et al., 1984; Brown et al., 1988; Graves et al., 1998; Roels et al., 1998). Thus, much remains unknown about this organism's potential niches and how it is transferred from one location to another in a home and restaurant environment. The prevalence of *Campylobacter* spp. in a major chicken foodservice operation was determined by swabbing a variety of surfaces inside the foodservice establishment (Figure 5). Before the restaurant sampling began, a modification of the method of Humphrey et al. (1995) was evaluated by inoculating five *Campylobacter* test isolates onto the surface of stainless steel chips. Based on these experiments, the detection limit of this method was determined to range from 10^2 to 10^3 cells/ml (Table 1B, Appendix B). The maximal drying time after which cells could still be recovered, ranged from 45 min to 1 h (Table 2B, Appendix B).

Campylobacter spp. were not detected in any of the 125 restaurant samples collected (Table 12). This may be due to routine cleaning of preparation surfaces, adequate preparation and storage of foodstuffs, good worker hygiene, and physical separation of raw foods from cooked foods, limiting cross-contamination. The lack of campylobacters in these samples may also be reflective of the sensitivity of *Campylobacter* spp. to drying conditions and heat. In fact, as mentioned above, some *Campylobacter* strains used to test the environmental sampling method

Table 12. Prevalence of *Campylobacter* spp. in a retail foodservice operation.

Surface (sampling area/size)	No.[a] (125)	Site[b]	*Campylobacter* spp.
Fridge wall (raw chicken storage); 1.5' × 1.5'	11	A	-[c]
Stainless steel cooking spits	11	C	-
Salting area (raw chicken preparation.); 1.5' × 1.5'	11	B	-
Plastic cutting board (large); 5 cm × 5 cm	11	F	-
Plastic cutting board (small); 5 cm × 5 cm	11	F	-
Potato bags (splash marks of chicken fat)	5	C	-
Salad preparation area (using cooked chicken); 1.5' × 1.5'	8	G	-
Heated drawer (top); 5 cm × 5 cm	11	E	-
Cutting knife (entire length)	11	F	-
Fridge floor (raw chicken storage); 1.5' × 1.5'	11	A	-
Worker's hand (gloved, left); entire palm and fingers	8	D	-
Tile wall (behind potato bags); 1.5' × 1.5'	5	C	-
Spit rack (raw chicken juices accumulated at the base of the rack); kept in fridge	11	A	-

[a] No., Number of samples tested.
[b] Please refer to Figure 5 for site locations.
[c] -, *Campylobacter* spp. not detected.

of Humphrey et al. (1995), could not be recovered from stainless steel chips after only 45 min of drying (Table 2B, Appendix B). Other groups have also described the harmful effects of drying on *Campylobacter* survival and recovery. For example, Cogan et al. (1999) observed a significant decrease in the isolation rate of *Campylobacter* spp. from surfaces, if sampling did not occur within 3 h of contamination. Humphrey et al. (1995) also reported a decrease in the ability to isolate *Campylobacter* spp. from blood droplets on kitchen surface materials, once the droplets had begun to dry (i.e., in less than 2 h); and was unable to recover *Campylobacter* spp. once the drops had dried completely. Exposure to drying conditions, as on the various restaurant surfaces, may render *Campylobacter* cells sublethally injured, and perhaps viable but non-culturable (VBNC). It is also possible that there were *Campylobacter* spp. present in some of these samples, but that they could not be detected using the method employed in this study (i.e., below the limit of detection).

4.2 Characterization of *Campylobacter* isolates

4.2.1 Discrimination (Simpson's Index) and Reproducibility

In order to elucidate possible sources of sporadic campylobacteriosis infections, PFGE typing was used to characterize *Campylobacter* clinical and food isolates. The clinical and food isolates shown in Table 6, and Tables 1A and 2A (Appendix A) were analysed using PFGE with the restriction enzymes *Sma*I and *Kpn*I. PFGE analysis using *Sma*I resulted in 171 profile types (or pulsotypes), while restriction with *Kpn*I resulted in 248 pulsotypes (Table 13). Both enzymes produced completely reproducible banding patterns. *Kpn*I profiles were typically more complex than *Sma*I profiles, with an average of 10 to 17 bands per profile as compared to 8 to 10 bands for *Sma*I (Figure 8, 9), which is in accordance with the findings of other groups (Yan et al., 1993;

Table 13. Discriminatory indices of PFGE using *Sma*I and *Kpn*I.

Restriction enzyme	No. of profiles[a]	No. of unique profiles	Size (%) of largest group	Discriminatory index[b]
*Sma*I	171	124	6.4	0.993
*Kpn*I	248	225	1.8	0.999

[a] A total of 237 clinical and 41 food isolates were pulsotyped.
[b] As determined by using Simpson's Index of Diversity (Hunter and Gaston, 1988).

Figure 8. *Sma* I PFGE banding patterns. Lanes: 1, *Staphylococcus aureus* NCTC 8325 (reference/standard strain); 2, strain no. C236; 3, strain no. C141; 4, strain no. C139; 5, strain no. C234; 6, strain no. C239; 7, strain no. C228; 8, *Staphylococcus aureus* NCTC 8325; 9, strain no. C230; 10, strain no. C246; 11, strain no. C297; 12, strain no. C240; 13, strain no. C163; 14, strain no. C191; 15, *Staphylococcus aureus* NCTC 8325.

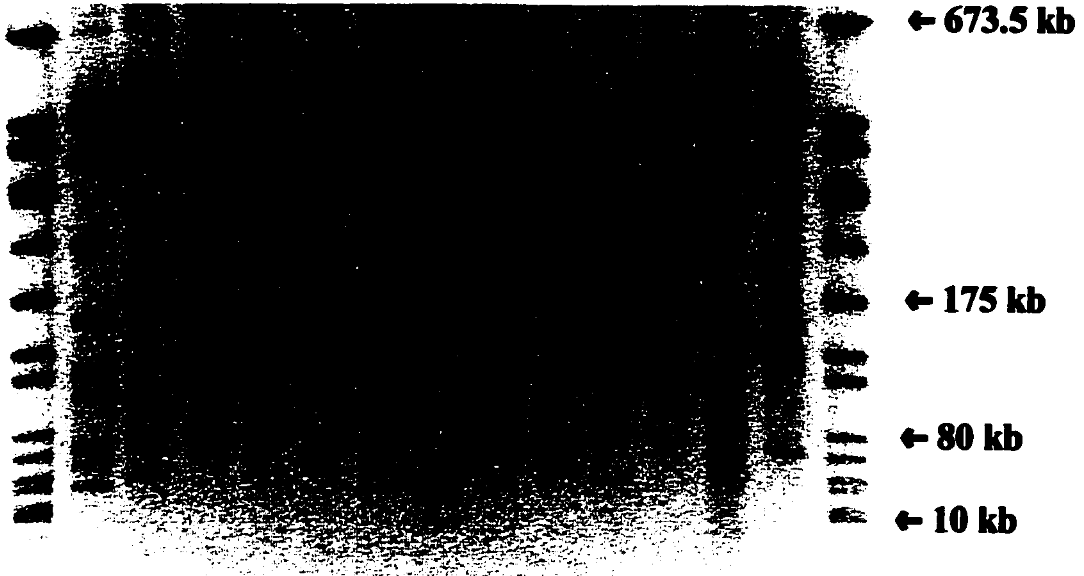
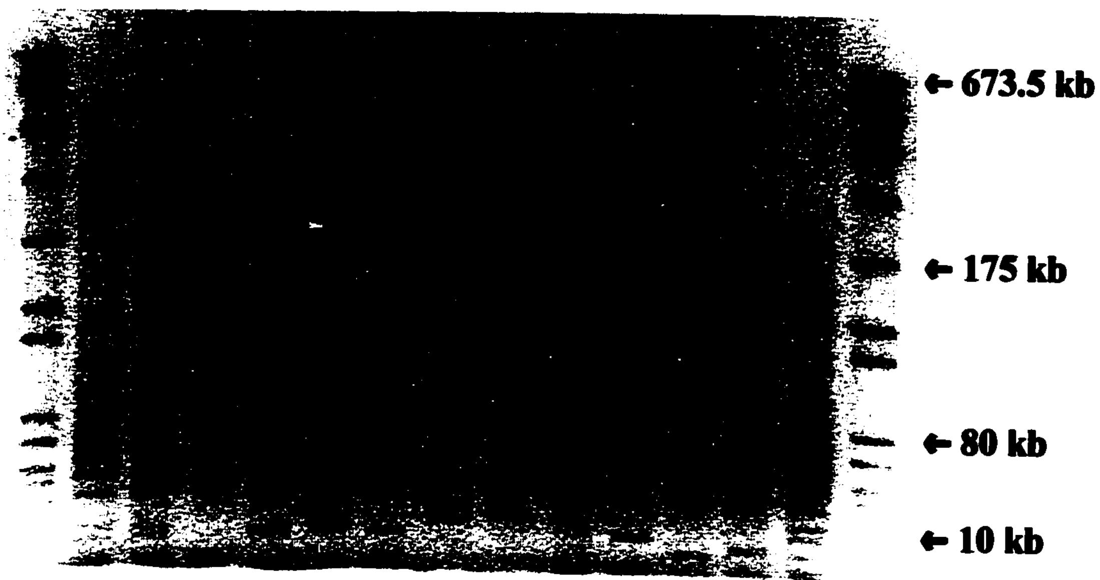

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
← 673.5 kb
← 175 kb
← 80 kb
← 10 kb

Figure 9. *KpnI* PFGE banding patterns. Lanes: 1, *Staphylococcus aureus* NCTC 8325 (reference/standard strain); 2, strain no. C140; 3, strain no. C254; 4, strain no. C118; 5, strain no. C200; 6, strain no. C207; 7, strain no. C203; 8, *Staphylococcus aureus* NCTC 8325; 9, strain no. C288; 10, strain no. F13; 11, strain no. C242; 12, strain no. C201; 13, strain no. F41; 14, strain no. C50; 15, *Staphylococcus aureus* NCTC 8325.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
← 673.5 kb
← 175 kb
← 80 kb
← 10 kb

Suzuki et al., 1993; On et al., 1998). In addition to the increased complexity of *Kpn*I-derived PFGE profiles, pulsotyping of isolates with *Kpn*I proved to be more discriminatory, i.e., the discriminatory power of *Kpn*I, as determined by Simpson's Index of Diversity, was higher than that of *Sma*I (Table 13). This correlates well with the findings of Michaud et al. (2001a) and others (Gibson et al., 1995; On et al., 1998), who have shown that *Sma*I-defined types can be further distinguished by *Kpn*I. Although PFGE typing with *Kpn*I proved more discriminatory than typing with *Sma*I, both enzymes possessed an index of diversity of greater than 0.90. Thus, according to Hunter and Gaston (1998), the results of PFGE typing using either of these enzymes can be interpreted with confidence.

4.2.2 PFGE profiles/Pulsotypes

The dendrogram obtained from numerical analysis of the *Sma*I PFGE profiles showed that strains were grouped into 124 unique profiles, and 47 shared profiles (or clusters) from a total of 278 strains, where the majority (26; 55%) of clusters consisted of two strains (Figure 10; Figure 1C and Table 1C, Appendix C). Eight (17%) of these clusters involved three isolates, four (8%) involved four isolates, two (4%) consisted of five strains, three (6%) included 6 strains, two (4%) included 7 strains, and the remaining two clusters consisted of 9 and 11 strains (Figure 10; Figure 1C and Table 1C, Appendix C). Analysis of the *Kpn*I dendrogram revealed the presence of far fewer clusters (i.e., 23) than observed in the *Sma*I dendrogram (Figure 11; Figure 2C and Table 1C, Appendix C). Like the *Sma*I clusters, these clusters consisted primarily of two strains; in fact, 19 (83%) of the *Kpn*I clusters involved two strains. However, unlike the *Sma*I clusters, the largest *Kpn*I cluster consisted of five strains (Figure 11; Figure 2C and Table 1C, Appendix C).

The largest *Sma*I cluster, 11 strains of pulsotype 68, consisted primarily of clinical

Figure 10. *Sma*I PFGE profiles and clusters. Profile numbers are shown in parentheses on the top, left-hand corner of the cells. Clusters are represented by the shaded cells. C and F numbers each represent a *Campylobacter* spp. isolate (see Appendix A, Table 1A and 2A).

(1) C144	(2) C152	(3) F41, F42, C202	(4) C309	(5) C276	(6) C8	(7) C214	(8) C5	(9) C268	(10) C271
(11) C291	(12) C285	(13) C205	(14) C124	(15) C130	(16) C153	(17) C156	(18) F33	(19) C7	(20) C262
(21) C174	(22) C188	(23) C255	(24) C185	(25) C138	(26) C239, F36, F29, F37	(27) C61, F47	(28) C45	(29) C112	(30) F48
(31) C230	(32) C213	(33) C292, C162	(34) C120, C79	(35) C154	(36) C2	(37) C207	(38) C49	(39) C44	(40) C168
(41) C257	(42) C172	(43) F35	(44) C90, C235	(45) F1	(46) C133	(47) C150, F13, C28	(48) C114	(49) C4	(50) C175
(51) F15	(52) C123	(53) C136	(54) C227, C254, C170, C197, C109, C178	(55) C140	(56) C165	(57) C20	(58) C203	(59) C110, F30	(60) F31
(61) C208, C209	(62) C308	(63) C47, C307, C69, C304, C316, C297, C267, C195, C189	(64) C132, C89, F43, C228, C279, C73, F8	(65) C3	(66) C105, C243, C211	(67) C103, C56	(68) C104, C108, C78, C64, C82, C218, C55, C216, C194, C167, C200	(69) C147	(70) C135
(71) C183	(72) C241	(73) C93, C60, C286, C287, C317, C329	(74) C198	(75) C283	(76) C1, C84	(77) C141	(78) C151, C157	(79) C159	(80) C219
(81) C70	(82) C258	(83) C91	(84) C15	(85) C77	(86) C313	(87) F11, F18	(88) C319	(89) C53	(90) C225, C290
(91) C246	(92) C131, C236, C266	(93) C81	(94) C48	(95) C193	(96) C321, C270	(97) C217	(98) F7	(99) C173	(100) C107
(101) C196	(102) C312	(103) C220	(104) C164	(105) C248	(106) C50, C19	(107) C98, C256	(108) C139	(109) F5, C88	(110) C118

(111) C275	(112) C251	(113) C127, C119	(114) C57	(115) C116, C245, C95, C74, C106	(116) C121, C41, F4	(117) C117	(118) CFA	(119) C12	(120) C261
(121) C300, C171, C163, C161	(122) C92, F2	(123) C240	(124) C80, F27	(125) C13	(126) C128, C269	(127) C186	(128) F44, F45	(129) C6, C16, C296	(130) F46
(131) C191	(132) C192	(133) C87	(134) C232	(135) C264	(136) C234, C182	(137) C26	(138) C238	(139) C252	(140) C201, C212
(141) C71	(142) C59, C126	(143) C101	(144) C85	(145) F28	(146) C115, C134, F25	(147) F38	(148) C11	(149) C9	(150) C42
(151) C242	(152) F10	(153) C72	(154) C155	(155) C233	(156) C46	(157) C206	(158) C102, C226	(159) C282	(160) C68, C293, C263, F26, F34, C224
(161) C52, C295, C169, C294	(162) C75, C76	(163) C122, C99, C314, F3, C187	(164) C83	(165) C289, C315	(166) C58, C210, F39, F14	(167) F32	(168) C51, C280, C310, F9, F12, C222, C288	(169) C25	(170) F6, F16, F17
(171) C96									

Figure 11. *Kpn*I PFGE profiles and clusters. Profile numbers are shown in parentheses on the top, left-hand corner of the cells. Clusters are represented by the shaded cells. C and F numbers each represent a *Campylobacter* spp. isolate (see Appendix A, Table 1A and 2A).

(1) C275	(2) C150	(3) C263	(4) C59	(5) C101	(6) C232	(7) C57	(8) C9	(9) C219	(10) C28
(11) C169	(12) C289	(13) C315	(14) C26	(15) C4	(16) F13	(17) C292	(18) C121, F4	(19) C106	(20) C41, C74, C95 C116
(21) C117	(22) C51	(23) CFA	(24) F2	(25) C13	(26) C88	(27) C80	(28) C238	(29) C191	(30) C119, C127
(31) C87	(32) C12	(33) C276	(34) C164	(35) F27	(36) C161, C163	(37) C171	(38) C6, C16	(39) C192	(40) C209
(41) C2	(42) C220	(43) C258	(44) C77	(45) C68	(46) F28	(47) C85	(48) C139	(49) C207	(50) C196
(51) C197	(52) C131	(53) C141	(54) C312	(55) C136	(56) C243	(57) C246	(58) C225	(59) C98	(60) C283
(61) C242	(62) C103	(63) F15	(64) C151, C157	(65) C168	(66) C135	(67) C126	(68) C217	(69) F41	(70) F42
(71) C202	(72) C155	(73) C206	(74) F33	(75) C153	(76) C156	(77) C255	(78) F10	(79) C42	(80) F38
(81) F44	(82) F46	(83) F45	(84) F26	(85) C144	(86) C152	(87) C115, 134	(88) F25	(89) C109	(90) F11
(91) F18	(92) C107	(93) C72	(94) F5	(95) C248	(96) C81	(97) C234	(98) C165	(99) F31	(100) C140
(101) C89, C256	(102) C147	(103) C290	(104) C236, C266	(105) C132, C319	(106) C270, C321	(107) C55, C194	(108) C200	(109) C167	(110) C78, C218
(111) C48	(112) C82, C216	(113) C114	(114) C110	(115) C203	(116) F30	(117) C105	(118) C108	(119) C56	(120) C211
(121) C170	(122) C20	(123) C104	(124) C70, C189, C267	(125) C227	(126) C47, C187	(127) C195, C297, C304 C307, C316	(128) C296	(129) C178	(130) C73, F8
(131) F43	(132) C254	(133) C228, C279	(134) C193	(135) C186	(136) C92	(137) C162	(138) C50	(139) C300	(140) C49

(141) C245	(142) C128	(143) F7	(144) C252	(145) C269	(146) C182	(147) C313	(148) C8	(149) C233	(150) C90
(151) C235	(152) F1	(153) C257	(154) C46	(155) C118	(156) C3	(157) C15	(158) C251	(159) C261	(160) C198
(161) C287	(162) C241	(163) C183	(164) C201, C212	(165) C93	(166) C264	(167) C286, C329	(168) C317	(169) C308	(170) C61
(171) C19	(172) C71	(173) C175	(174) C208	(175) C84	(176) C1	(177) C285	(178) C188	(179) C174	(180) C262
(181) C11	(182) C185	(183) C53	(184) C47	(185) C268	(186) C5	(187) C138	(188) C205	(189) C123	(190) C213
(191) C309	(192) C124	(193) C130	(194) C214	(195) C91	(196) F48	(197) F35	(198) C159	(199) C173	(200) F36, F37
(201) F29	(202) C45	(203) C172	(204) C64	(205) C112	(206) C239	(207) C222	(208) C210	(209) F39	(210) F12
(211) C293	(212) C291	(213) C310	(214) C58	(215) F32	(216) F14	(217) C69	(218) F34	(219) C25	(220) C44
(221) C52, C294, C295	(222) C7	(223) C282	(224) C76	(225) C75	(226) C314	(227) C83	(228) F3	(229) C99	(230) F17
(231) C60	(232) C96	(233) C280	(234) C288	(235) C224	(236) C122	(237) C226	(238) C102	(239) C240	(240) F9
(241) F6	(242) F16	(243) C154	(244) C133	(245) C120	(246) C79	(247) C230	(248) C271		

isolates that originated from campylobacteriosis cases during the months of July through September in 1998 and 1999. In fact, cases shared a close temporal relationship, with the first faecal sample in 1998 being submitted for analysis on July 7, and the final faecal sample being analysed on September 15. This pattern was also observed in 1999, where the first faecal sample was submitted for analysis on July 7, and the final faecal sample was tested on September 9. These observations suggest that particular *Campylobacter* strains may predominate during the summer months, and is in agreement with the observations of other groups, including Hänninen et al. (1998) and Nielsen et al. (2000), who found that certain pulsotypes predominated in June through August. Analysis of this *Sma*I cluster also revealed that the majority of cases did not share a close geographical relationship, with cases residing in the former cities of Ottawa, Nepean, and Orleans, as well as the cities of Oakville, Appleton and Blondeau (Table 1A, Appendix A). Further analysis revealed that there was a fairly even gender and age distribution among the cases in this cluster. Interestingly, this *Sma*I cluster was further subdivided by *Kpn*I into 8 different profiles, including pulsotypes 104, 108, 109, 110, 112, 118, 123 and 204, attesting to the improved discriminatory power of PFGE typing using *Kpn*I. This lack of concordance between *Sma*I and *Kpn*I profiles suggests that this was not a true cluster and, as a result, may not represent an outbreak(s). There is also the possibility that *Kpn*I may be too discriminatory.

The second most common *Sma*I cluster involved 9 isolates of pulsotype 63, which were obtained from campylobacteriosis cases in 1998, 1999 and 2000. Three of the isolates in this cluster shared a very interesting commonality; strains C297, C304 and C307 were obtained from individuals residing in the same household. It is not uncommon for members of the same

household to develop campylobacteriosis and, in fact, there are a number of ways in which *Campylobacter* can be transmitted within a home (Oberhelman and Taylor, 2000). The organism may have been present in a contaminated food item consumed by the household members, or on a kitchen surface where it could have been in contact with other foods, leading to cross-contamination. Another possibility is that one of the individuals in this household was excreting *Campylobacter* in their faeces, and that other household members became infected via the faecal-oral route. It is also possible that a household pet, such as a dog or cat, was excreting *Campylobacter* in their faeces, and that the household members became infected via the faecal-oral route. Contact with animals has previously been cited as a risk factor for development of campylobacteriosis (Blaser et al., 1978; Ponka et al., 1984) as have the other modes of transmission described above (Blaser et al., 1981; Oberhelman and Taylor, 2000).

Analysis of *Kpn*I profiles also revealed an association between strains C297, C304 and C307, along with two of the other isolates in the afore-mentioned *Sma*I cluster (i.e., strains C195 and C316). The fact that both *Sma*I and *Kpn*I dendrograms grouped these strains into one cluster, suggests that there was a small, household-related outbreak. Hänninen et al. (1998) reported over five small family outbreaks with identical PFGE patterns among the 176 *C. jejuni* isolates examined in Denmark.

Unlike the *Sma*I clusters described above, cluster isolates of pulsotype 64 were of human and food origin, suggesting a possible source of infection. The food (raw chicken isolates F8 and F43) and clinical (C73, C89 and C132) strains in this cluster were not temporally related, either longitudinally or seasonally, nor did they share any other commonalities. *Kpn*I profile analysis supported the clustering of strain C73 with F8 (raw chicken isolate), suggesting that these

isolates were somehow related, even though strain F8 was isolated 6 months after C73. Fourteen other *Sma*I clusters consisted of both food and clinical isolates, and included strains belonging to pulsotypes 3, 26, 27, 47, 59, 109, 116, 122, 124, 146, 160, 163, 166, and 168. Only one of these associations was supported by the *Kpn*I data. *Kpn*I pulsotype 18 included C121 and F4 (raw chicken isolate), as did *Sma*I pulsotype 116. These isolates shared a close temporal relationship (i.e., isolated in September and December of 1998, respectively), suggesting a possible source of infection. These data are suggestive of chicken being a risk factor for the development of campylobacteriosis, and correlate well with the findings of a case-control study conducted by Kapperud et al. (1992), in which handling and eating of poultry were determined to be major risk factors in sporadic cases of campylobacteriosis. Others have also cited the handling and/or consumption of chicken as a major risk factor for development of *Campylobacter* infection (Brieseman et al., 1990; Ikram et al., 1994; Adak et al., 1995; Eberhart-Phillips et al., 1997). The presence of a common PFGE profile for C121 and F4 may also be explained by chance, by a predominant clone in circulation, or by a common-source infection for both humans and chicken.

The next most common *Sma*I profiles were pulsotypes 54, 73 and 160, which each represented 6 strains. Cluster isolates belonging to *Sma*I pulsotype 54 were further subdivided by *Kpn*I into 6 different profiles, as were cluster isolates belonging to *Sma*I pulsotype 160. Cluster isolates belonging to *Sma*I pulsotype 73 were subdivided further by *Kpn*I into four different profiles. The association of strains C286 and C329 in this former *Sma*I cluster was supported by the *Kpn*I data.

Of the remaining *Sma*I clusters, 17 did not correlate with the *Kpn*I data. PFGE typing using *Kpn*I further subdivided these clusters. Conversely, 11 other *Sma*I clusters were

represented among the *KpnI* clusters, and included the following isolate pairs: C151 and C157, C236 and C266, C260 and C321, C119 and C127, C161 and C163, C6 and C16, C115 and C134, C201 and C212, as well as groups C52, C294 and C295, and C116, C74 and C95. A closer examination of these concordant pairs revealed that the majority of clusters represented strains obtained from the same person. For example, strains C74 and C95 were both obtained from the clinical sample of a 67-year old woman from Kanata. Similarly, strains C6 and C16 were obtained from the same individual, as were strains C161 and C163, C115 and C134, and strains C201 and C212. This phenomenon may be explained in a number of ways. It is possible that the patients may have experienced a worsening of symptoms and returned to their physician. This appears to be the case for three of these individuals, since a second faecal sample was received within a few days or weeks of the original one (e.g., strains C201 and C212, C74 and C95, as well as C161 and C163). Laboratory reports for the remaining individuals revealed that a second faecal sample was retrieved from these patients over a month after the first, suggesting that these persons may have become re-infected with *C. jejuni*. Strain C6 was obtained from a faecal sample received on February, 6 of 1998, while C16 was obtained from a faecal sample received on March, 2 of 1998. Similarly, strains C115 and C134 were obtained from faecal samples retrieved within just over a month of each other (i.e., September, 25 of 1998 and October 29 of 1998, respectively). These individuals may have become re-infected through exposure to the same sources, for example, contact with pets or consumption of contaminated food and water. They may also have re-developed the infection from within (i.e., a state where resistant *Campylobacter* cells may reside in a protective area of the body). Re-infection from within or carrier states have been reported for other foodborne pathogens, including *Salmonella* spp. and

Listeria monocytogenes (Neill et al., 1991; Yew et al., 1991; Nathwani et al., 1992; Sauders et al., 2001). It is also possible that these individuals did not follow the prescribed antibiotic treatment, or, that it was followed, but did not effectively clear the organism (i.e., resistant isolate). However, antimicrobial susceptibility testing revealed that all of these strains were susceptible to fluoroquinolones (i.e., ciprofloxacin, norfloxacin), tetracycline, erythromicin, and ampicillin, antibiotics commonly used in the treatment of campylobacteriosis (Farber, unpublished data). Thus, resistance to antibiotic treatment, cannot account for the phenomenon described above.

Both the *Sma*I and *Kpn*I dendrograms showed 100% identity between isolates C119 and C127. Complete similarity between isolates C151 and C157 was also noted, as well as for isolates C236 and C266, C270 and C321, C55 and C194, C78 and C218, C82 and C216, C189 and C267, suggesting that these were true associations. Most of the isolates in these clusters did not share a close temporal relationship (i.e., isolated about one year apart from each other). For example, strains C82 and C216 were isolated in August of 1998 and September of 1999, respectively. This was also seen for strains C78 and C218 and strains C55 and C194, suggesting that particular *Campylobacter* strains may predominate during the summer months. This is in agreement with our earlier observations, as well as the observations of others (Hänninen et al., 1998; Nielsen et al., 2000). A few of the remaining clusters consisted of isolates obtained in close proximity of one another, for example, strains C119 and C127 were isolated in September and October of 1998, respectively. Strains C151 and C157 were also isolated within a month of one another.

Among the 154 isolates assigned to clusters by *Sma*I, only 42% gave concordant results

with *Kpn*I. In contrast, among the 53 isolates assigned to 23 clusters by *Kpn*I, 87% gave concordant results with *Sma*I. Five of the 20 concordant clusters represented isolates obtained from the same person (i.e., isolate pairs C74 and C95, C161 and C163, C6 and C16, C115 and C134, C201 and C212), whereas another cluster represented isolates obtained from individuals residing in the same household (i.e., strains C297, C304 and C307).

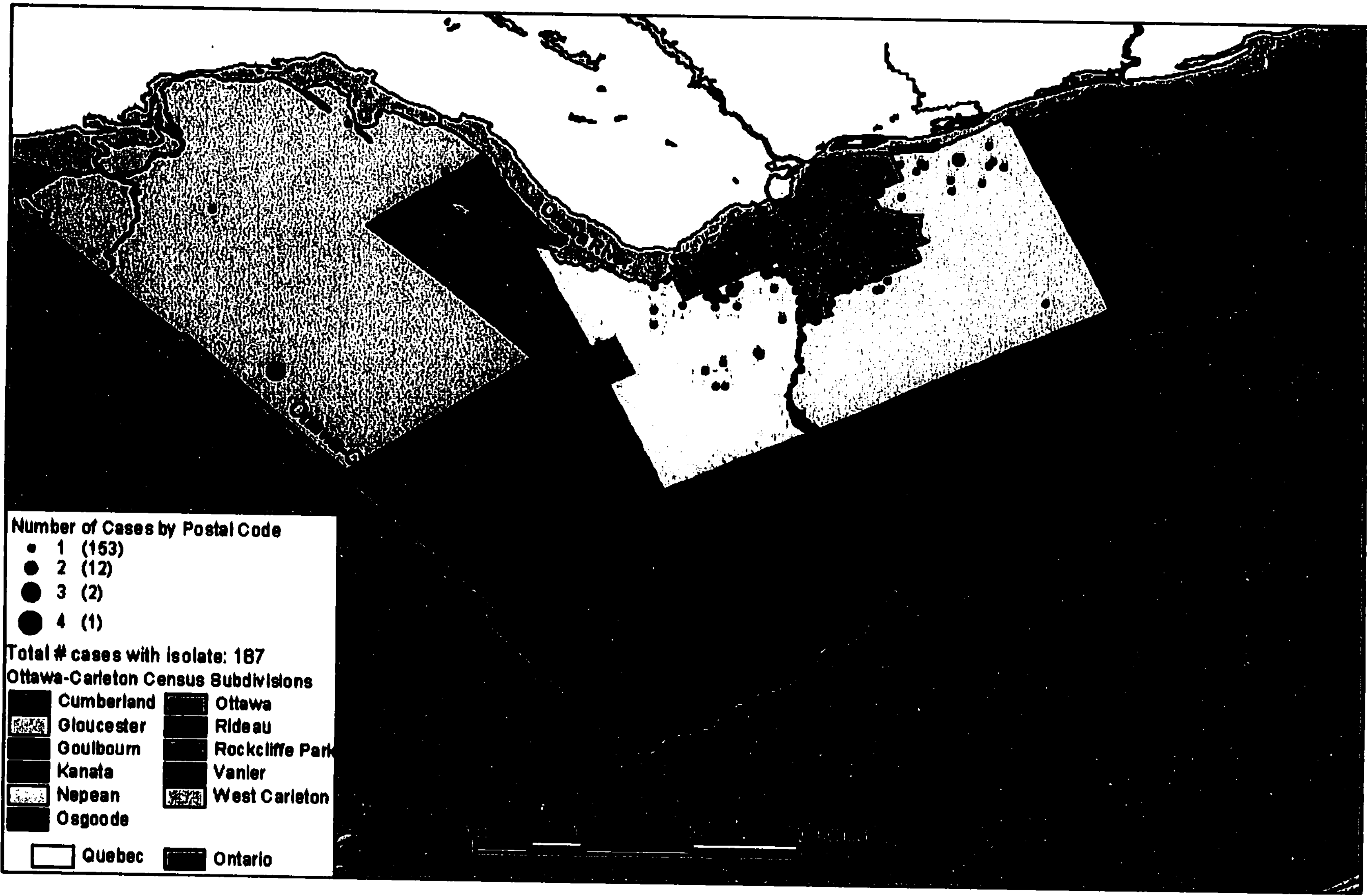
4.3 Campylobacteriosis cases

4.3.1 Spot map

Campylobacteriosis cases were further analysed by preparing spot maps. Spot map analysis was designed to explore the geographical distribution of these cases. Visual inspection of the Ottawa-Carleton spot map revealed a significant clustering of cases in the former city of Ottawa, and proximal cities (Figure 12). Very few cases were seen in outlying areas such as West Carleton, Rideau, Osgoode and Cumberland. Although numerous campylobacteriosis cases were observed in the former city of Ottawa, few belonged to the same postal code region. In fact, the majority of cases did not share a postal code. In contrast, cases in more remote regions tended to be clustered into a particular postal code. For example, of the 6 cases observed in the city of Cumberland, four belonged to the same postal code. Similarly, three of the 6 cases in West Carleton belonged to the same postal code. This pattern was also observed in Goulbourn, where three of the eight campylobacteriosis cases belonged to the same postal code. These data suggest the presence of local/locally-confined outbreaks in outlying regions, possibly related to a common exposure source. However, this pattern may also be related to the fact that rural postal codes cover a larger area that urban postal codes.

Spot map analysis also revealed that the majority of cases in the former cities of Kanata

Figure 12. Spot map of campylobacteriosis cases in the Ottawa-Carleton region, 1998-2000.



Number of Cases by Postal Code
1 (153)
2 (12)
3 (2)
4 (1)
Total # cases with isolate: 187
Ottawa-Carleton Census Subdivisions
Cumberland
Gloucester
Goulbourn
Kanata
Nepean
Osgoode
Ottawa
Rideau
Rockcliffe Park
Vanier
West Carleton
Quebec
Ontario

and Nepean were clustered around the Northeastern limits of these cities, whereas the cases in the former city of Gloucester were clustered around its Northwestern limits. This may be related to the presence of a common exposure, or it may simply reflect a higher population density along these borders. These clusters were not confirmed by PFGE.

5. CONCLUSIONS AND FUTURE WORK

One of the major aims of the present study was to identify foods that harbor *Campylobacter* spp. and thus, could possibly cause illness. Three hundred raw and ready-to-eat foods were analyzed for the presence of this organism. Of these food items, only four proved positive for *Campylobacter*, and included both raw chicken and raw beef liver. These data suggest that *Campylobacter* spp. were either not present in the majority of the foods tested, were present in very small numbers (i.e., below the detection limit of the method used), or were in a form that could not be detected (e.g., VBNC). The use of an alternative *Campylobacter* isolation method, such as that of the USDA or FDA, may provide some insight into whether the method used here was sensitive enough to detect small numbers of campylobacters. The use of a molecular detection method (e.g., PCR) would also be very informative, since it would detect both viable and non-viable cells.

Ultimately, the best way to determine which foods harbor *Campylobacter* spp. would be to identify foods responsible for sporadic campylobacteriosis cases. This can only be achieved through an active surveillance program. Such a program would involve prompt questioning of cases about their exposures, and retrieval of food samples; and would require strong collaboration between physicians, private and government laboratories and regional health units.

Continued surveillance of foods at the retail level is also essential in determining which foods harbor *Campylobacter* spp. The testing of ready-to-eat foods is especially important since these foods are becoming more and more available to the consumer, and are generally not processed (e.g., washed or cooked) in any way before consumption. Unpasteurized fruit juices and nuts represent some of the ready-to-eat foods that need to be examined for the presence of

Campylobacter spp., since they have been implicated in *E. coli* and *Salmonella*-related outbreaks (FSNET, 2001). Tap water may also harbor *Campylobacter* spp. In fact, there is some evidence that campylobacters can survive within water-borne protozoa, making them significantly more resistant to chlorine (Snelling, 2001).

Another goal of this study was to identify environments that harbor *Campylobacter* spp. thereby, identifying possible niches for this organism. The prevalence of *Campylobacter* spp. in a retail foodservice operation was determined. *Campylobacter* spp. were not detected in any of the 125 environmental samples analysed, suggesting that this organism was not present on the surfaces tested, or that it simply could not be detected. Since the detection limit of the method used was 10^2 to 10^3 cells/ml, it is quite possible that there were small numbers of campylobacters on some of these restaurant surfaces which fell below this detection limit and thus, could not be detected. Improving the detection limit of this method, or the application of an alternative environmental sampling method may lead to improved recovery of *Campylobacter* spp. from these types of surfaces. An increased sampling period and sampling pool (i.e., more restaurants) would also help in determining which surfaces harbor *Campylobacter* spp., and if there is an effect of seasonality (i.e., more *Campylobacter* spp. present on surfaces in the summer months).

In addition to determining which foods and environments harbor *Campylobacter* spp., another goal of this study was to characterize *Campylobacter* clinical and food isolates using PFGE, in order to determine whether there were common pulsotypes associated with the development of campylobacteriosis. An underlying objective was also to compare the discriminatory power and reproducibility of PFGE typing using the restriction enzymes *Sma*I and *Kpn*I. PFGE using both *Sma*I and *Kpn*I yielded highly discriminatory and reproducible

profiles, with *Kpn*I being the most discriminatory enzyme (i.e., 225 unique profiles versus 124 unique profiles for *Sma*I). PFGE analysis did not identify any clear, common pulsotypes instead, it clustered certain small groups of strains. For example, PFGE analysis using *Sma*I resulted in 47 clusters, the largest of which consisted of 11 strains. PFGE analysis using *Kpn*I was even more discriminatory, with only 23 clusters identified, the largest consisting of five strains. These data suggest that *Campylobacter* strains associated with illness are extremely diverse. It also suggests that *Kpn*I may be too discriminatory thus, the use of an additional restriction enzyme may be required. The use of an additional typing method may also be advisable, since analysis of PFGE profiles can be quite difficult. Difficulties may arise due to the subjective nature of certain software analysis tools, and in interpreting band differences. Moreover, genetic instability, a single or series of events that lead to a change in the genetic organization of a given bacterial strain, also confounds the interpretation process. Genetic instability can lead to minor or major changes in PFGE profiles, thereby making it difficult to elucidate the true relationship between strains (Tenover et al., 1995; On et al., 1998; Wassenaar et al., 1998). This does not imply that PFGE analysis is not a valid typing method; in fact, others have found that strains of *Campylobacter* remain genetically stable over long periods of time, and in completely different environments (Hanninen et al., 1999; Manning et al., 2001). However, the use of an additional typing method (e.g., ribotyping, AFLP) may aid in further understanding the relationship between *Campylobacter* clinical and food isolates.

This study was also designed to examine the geographical distribution of campylobacteriosis cases in the Ottawa-Carleton region, by producing spot maps. Analysis of these spot maps revealed that the majority of cases were within the boundaries of the former city

of Ottawa. However, it also revealed that few of these cases were clustered (i.e., within the same postal code region). Furthermore, there did not appear to be a correlation between PFGE profiles and the geographical location of cases.

Overall, this study identified raw poultry and beef as sources of *Campylobacter* spp., which is in accordance with the findings of others. This study has also reported the findings of an environmental sampling effort that took place in a major chicken foodservice operation, which few groups have reported. It has also made evident the diversity of *Campylobacter* strains responsible for causing illness, as determined by PFGE. Moreover, the findings of the present study have added to our understanding of the epidemiology of campylobacteriosis in Ontario, while also highlighting the need for an active *Campylobacter* surveillance network, designed to promptly follow-up on sporadic cases of campylobacteriosis.

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APPENDIX A

***Campylobacter* clinical and food isolate characteristics**

Table 1A. Description of *Campylobacter* clinical isolates used for PFGE analysis.

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
1	1	jejuni	98/02/01	M/18	Nepean
2	2	jejuni	98/02/02	M/28	Nepean
3	3	jejuni	98/02/02	M/7	Limoges
4	4	jejuni	98/02/04	M/45	Hawkesbury
5	5	coli	98/02/04	M/62	Ottawa
6	6	jejuni	98/02/06	F/25	Nepean
7	7	jejuni	98/02/10	M/60	Gloucester
8	8	jejuni	98/02/11	M/50	Gloucester
9	9	jejuni	98/02/10	M/33	Eganville
10	11	lari	98/02/23	F/59	Ottawa
11	12	jejuni	98/02/25	F/53	Ottawa
12	13	jejuni	98/03/02	M/39	Kanata
13	15	jejuni	98/03/09	F/20	Maberly
14	16	jejuni	98/03/13	F/25	Nepean
15	19	lari	98/03/16	M/25	Orleans
16	20	jejuni	98/03/13	F/38	Nepean
17	25	jejuni	98/04/17	M/27	Gatineau
18	26	lari	98/04/20	M/47	Greely
19	28	jejuni	98/05/12	F/47	-[b]
20	41	jejuni	98/06/10	F/62	Kanata
21	42	jejuni	98/06/12	F/19	Carp
22	44	jejuni	98/06/17	F/29	Gloucester
23	45	jejuni	98/06/17	M/47	Ottawa

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
24	46	jejuni	98/06/22	F/34	Merrickville
25	47	jejuni	98/06/23	F/26	Gatineau
26	48	jejuni	98/06/29	M/30	Ottawa
27	49	jejuni	98/06/30	M/47	Kanata
28	50	jejuni	98/06/29	M/56	Ottawa
29	51	coli	98/06/26	F/11	Vankleek Hill
30	52	coli	98/06/30	M/30	Nepean
31	53	jejuni	98/07/03	M/40	St-Albert
32	55	jejuni	98/07/07	M/39	Appleton
33	56	jejuni	98/07/10	F/10	Manotick
34	57	jejuni	98/07/10	F/31	Ottawa
35	58	jejuni	98/07/08	F/43	Stittsville
36	59	jejuni	98/07/15	F/10	Ottawa
37	60	jejuni	98/07/17	M/4	-
38	61	jejuni	98/07/17	M/5	Etobicoke
39	64	jejuni	98/07/23	F/24	Blondeau
40	68	jejuni	98/07/27	F/10	Gloucester
41	69	jejuni	98/07/28	F/7	Manotick
42	70	jejuni	98/07/27	M/33	Vankleek Hill
43	71	jejuni	98/07/29	M/4	Oxford Mills
44	72	coli	98/07/26	F/25	Lefaivre
45	73	jejuni	98/07/29	F/4	Ottawa
46	74	jejuni	98/07/30	F/67	Kanata

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
47	75	coli	98/07/29	M/54	Nepean
48	76	coli	-	-	-
49	77	jejuni	98/08/12	F/81	Vanier
50	78	jejuni	98/08/12	F/3	Ottawa
51	79	jejuni	98/08/04	F/53	Nepean
52	80	jejuni	98/08/05	M/14	Rockliffe
53	81	jejuni	98/08/05	F/4	Ottawa
54	82	jejuni	98/08/05	F/48	Orleans
55	83	jejuni	98/07/31	M/51	Munster
56	84	jejuni	98/07/31	F/18	Kanata
57	85	jejuni	98/08/24	M/30	Hawkesburry
58	87	jejuni	98/08/24	F/36	Carlton Place
59	88	jejuni	98/08/20	F/31	Ottawa
60	89	jejuni	98/08/26	F/44	Nepean
61	90	jejuni	98/08/18	F/57	Walkerton
62	91	jejuni	98/08/14	F/32	Hull
63	92	jejuni	98/08/19	M/19	Ottawa
64	93	jejuni	-	-	-
65	95	jejuni	98/08/14	F/67	Kanata
66	96	jejuni	98/08/14	M/47	Nepean
67	98	jejuni	98/08/20	M/38	Ottawa
68	99	jejuni	98/08/15	F/11	Stittsville
69	101	jejuni	98/08/25	F/40	Nepean

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
70	102	jejuni	98/09/10	M/37	Arnprior
71	103	jejuni	98/09/10	F/47	Nepean
72	104	coli	-	-	-
73	105	jejuni	98/09/17	M/74	Greely
74	106	jejuni	98/09/11	F/49	-
75	107	jejuni	98/09/11	M/34	Gloucester
76	108	coli	98/09/15	M/56	Ottawa
77	109	jejuni	98/09/10	M/47	Ottawa
78	110	jejuni	98/09/09	M/30	Ottawa
79	112	jejuni	98/09/19	M/39	North Gower
80	114	coli	98/09/23	M/51	Osgoode
81	115	jejuni	98/09/25	F/57	Ottawa
82	116	jejuni	98/09/24	F/38	Ottawa
83	117	jejuni	-	-	-
84	118	jejuni	98/09/25	F/77	Constance Bay
85	119	jejuni	98/09/25	M/46	Ottawa
86	120	jejuni	-	-	-
87	121	jejuni	-	-	-
88	122	jejuni	98/09/25	F/46	Ottawa
89	123	jejuni	98/09/24	F/45	Gloucester
90	124	lari	98/10/14	M/27	Ottawa
91	126	jejuni	98/10/16	F/27	Carlton Place
92	127	jejuni	-	-	-

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
93	128	jejuni	98/10/15	M/51	Ottawa
94	130	coli	98/10/20	F/9months	Pembroke
95	131	jejuni	98/10/15	F/37	Ottawa
96	132	jejuni	98/10/16	F/40	Ottawa
97	133	coli	98/11/11	M/4	Ottawa
98	134	jejuni	98/10/29	F/57	Ottawa
99	135	jejuni	-	-	-
100	136	jejuni	98/11/10	F/54	Ottawa
101	138	coli	98/10/30	M/48	Ottawa
102	139	jejuni	-	-	-
103	140	jejuni	-	-	-
104	141	jejuni	-	-	-
105	144	jejuni	-	-	-
106	147	jejuni	98/12/18	M/42	Rockland
107	150	jejuni	98/11/16	M/39	Ottawa
108	151	jejuni	99/01/07	M/19	Ottawa
109	152	jejuni	99/02/08	M/45	Ottawa
110	153	jejuni	99/02/09	F/24	Ottawa
111	154	jejuni	99/02/01	M/45	Kanata
112	155	jejuni	99/01/22	F/27	Ottawa
113	156	coli	99/01/28	M/35	Ottawa
114	157	jejuni	99/02/11	F/1	Ottawa
115	159	jejuni	99/02/19	M/58	Ottawa

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
116	161	jejuni	99/02/23	M/36	Ottawa
117	162	jejuni	99/02/24	F/6	Ottawa
118	163	jejuni	99/03/02	M/36	Ottawa
119	164	jejuni	99/03/01	F/25	Hull
120	165	jejuni	99/03/04	M/2	Gloucester
121	167	jejuni	99/03/13	F/42	Nepean
122	168	jejuni	99/03/13	F/34	Nepean
123	169	jejuni	99/03/21	M/31	Gloucester
124	170	jejuni	99/03/23	F/78	Ottawa
125	171	jejuni	99/03/25	M/34	Ottawa
126	172	jejuni	99/04/24	M/25	Ottawa
127	173	jejuni	99/04/09	F/54	Carp
128	174	coli	99/04/27	M/2	Nepean
129	175	jejuni	99/04/26	F/43	Orleans
130	178	jejuni	99/05/06	M/51	Hull
131	182	jejuni	99/05/20	M/2	Nepean
132	183	jejuni	99/05/11	M/74	Kanata
133	185	lari	99/05/07	M/44	Ottawa
134	186	jejuni	99/06/08	M/72	Ottawa
135	187	jejuni	99/06/08	F/53	Metcalfe
136	188	coli	99/06/15	F/65	Nepean
137	189	jejuni	99/06/08	F/38	Rockland
138	191	jejuni	99/06/30	M/33	Ottawa

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
139	192	jejuni	99/06/15	F/25	Ottawa
140	193	jejuni	99/06/30	M/32	Ottawa
141	194	jejuni	99/06/17	M/52	Ottawa
142	195	jejuni	99/07/03	M/27	Gloucester
143	196	jejuni	99/07/07	M/35	Ottawa
144	197	coli	99/07/07	F/30	Ottawa
145	198	jejuni	99/07/07	F/27	Ottawa
146	200	jejuni	-	-	-
147	201	jejuni	99/07/30	F/50	Nepean
148	202	jejuni	99/07/30	M/65	-
149	203	jejuni	99/07/29	F/18	Gloucester
150	205	lari	99/07/20	M/55	Gloucester
151	206	jejuni	99/07/22	F/17	Kanata
152	207	jejuni	99/07/20	M/39	Ottawa
153	208	jejuni	99/08/20	F/24	Ottawa
154	209	jejuni	99/08/20	M/64	Ottawa
155	210	jejuni	99/08/19	M/48	Nepean
156	211	jejuni	99/08/11	F/31	Ottawa
157	212	jejuni	99/08/03	F/50	Nepean
158	213	lari	99/08/30	M/3	Kanata
159	214	coli	99/08/30	F/1	Nepean
160	216	jejuni	99/09/09	M/12	Oakville
161	217	jejuni	99/08/26	M/58	Manotick

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
162	218	jejuni	99/09/03	F/38	Ottawa
163	219	jejuni	99/09/14	F/3	North Gower
164	220	jejuni	99/09/24	M/41	Ottawa
165	222	jejuni	99/09/28	F/72	Nepean
166	224	jejuni	99/09/30	M/1	Embrun
167	225	jejuni	99/10/01	F/30	Lazo, B.C.
168	226	jejuni	99/10/07	F/63	Vars
169	227	jejuni	99/10/18	M/31	Nepean
170	228	jejuni	99/10/15	F/22	Ottawa
171	230	jejuni	99/10/14	M/1	Embrun
172	232	jejuni	99/10/14	M/28	Ottawa
173	233	jejuni	99/10/15	F/44	Vernon
174	234	jejuni	99/10/21	M/60	Nepean
175	235	jejuni	99/10/15	M/16	Ottawa
176	236	jejuni	99/10/15	F/23	Richmond
177	238	jejuni	99/10/04	F/62	Osgoode
178	239	jejuni	99/10/28	M/17	Orleans
179	240	jejuni	99/10/26	F/51	North Augusta
180	241	jejuni	-	M/-	-
181	242	jejuni	99/11/23	F/9	Ottawa
182	243	jejuni	99/12/14	M/28	Gloucester
183	245	jejuni	99/12/09	M/25	Ottawa
184	246	jejuni	99/12/15	F/41	Ottawa

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
185	248	jejuni	99/11/17	M/84	Ottawa
186	251	jejuni	99/11/17	F/72	Nepean
187	252	jejuni	99/11/22	F/1	Nepean
188	254	jejuni	00/01/27	M/38	Ottawa
189	255	jejuni	00/01/14	M/22	Ottawa
190	256	jejuni	00/01/13	M/28	Ottawa
191	257	jejuni	00/01/08	M/33	Ottawa
192	258	jejuni	00/02/03	F/58	Kanata
193	261	jejuni	-	F	-
194	262	lari	00/02/08	F/37	Kanata
195	263	jejuni	-	-	-
196	264	jejuni	-	-	-
197	266	jejuni	00/02/19	M/30	Dunrobin
198	267	jejuni	00/03/06	M/21	Kanata
199	268	coli	00/03/06	M/16	Clarence Creek
200	269	jejuni	00/03/10	F/25	Ottawa
201	270	jejuni	00/03/07	M/19	Stittsville
202	271	jejuni	00/04/20	F/73	Kanata
203	275	jejuni	00/05/04	F/60	Ottawa
204	276	jejuni	00/04/27	M/44	Cantley
205	279	jejuni	00/05/02	M/32	Gloucester
206	280	coli	00/04/15	F/25	Kanata
207	282	jejuni	00/05/05	F/48	Richmond

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
208	283	jejuni	00/06/02	M/30	Ottawa
209	285	lari	00/05/17	F/52	Routhier
210	286	jejuni	00/05/29	F/36	Ottawa
211	287	jejuni	00/05/13	F/46	Kanata
212	288	jejuni	00/05/17	M/5	Nepean
213	289	jejuni	00/05/31	F/2	Ottawa
214	290	jejuni	00/05/31	F/25	Ottawa
215	291	jejuni	00/07/14	M/5	St-Bernadite
216	292	jejuni	00/07/14	F/37	Ottawa
217	293	jejuni	-	M/-	-
218	294	jejuni	00/07/12	F/47	Ottawa
219	295	jejuni	00/07/12	M/4	St. Isidore
220	296	jejuni	00/07/11	M/55	Orleans
221	297	jejuni	00/07/11	F/23	Orleans
222	300	jejuni	00/06/20	M/26	Ottawa
223	304	jejuni	00/07/07	F/7	Orleans
224	307	jejuni	00/07/07	M/24	Orleans
225	308	jejuni	00/07/21	F/52	Langley, B.C.
226	309	jejuni	00/07/16	M/22	Carlsbad Springs
227	310	jejuni	00/07/18	M/30	Richmond
228	FA	jejuni	-	-	-
229	312	jejuni	00/08/21	M/45	Manotick
230	313	jejuni	00/08/22	F/36	Kanata

Table 1A (cont.)

Isolate	HPFB[a] no.	Species	Service date - case report (Gamma-Dynacare)	Gender/Age	City
231	314	jejuni	00/08/23	M/8	Stittsville
233	316	jejuni	00/08/21	M/44	Ottawa
234	317	jejuni	00/08/10	F/23	Gloucester
235	319	jejuni	00/08/23	F/36	Ottawa
236	321	jejuni	00/08/15	M/25	Ottawa
237	329	jejuni	01/01/22	M/26	Ottawa

[a] HPFB, Health Products and Food Branch.
[b] -, Data not available.

Table 2A. Description of *Campylobacter* food isolates used for PFGE analysis.

Isolate	HPFB[a] no.	Species	Source	Date of isolation
1	1	jejuni	cooked chicken - confirmed campylobacteriosis case	98/09/14
2	2	jejuni	raw chicken	98/12/11
3	3	jejuni	raw chicken	98/12/11
4	4	jejuni	raw chicken	98/12/14
5	5	jejuni	raw chicken	98/12/14
6	6	jejuni	raw chicken	99/01/28
7	7	jejuni	raw chicken	99/01/28
8	8	jejuni	raw chicken	99/02/05
9	9	jejuni	raw chicken	99/02/05
10	10	jejuni	raw chicken	99/02/12
11	11	jejuni	raw chicken	99/02/12
12	12	jejuni	raw chicken	99/02/26
13	13	jejuni	raw chicken	99/02/26
14	14	jejuni	raw chicken	99/02/26
15	15	jejuni	raw chicken	99/03/05
16	16	coli	raw chicken	99/03/05
17	17	jejuni	raw chicken	99/03/20
18	18	jejuni	raw chicken	99/03/20
19	25	jejuni	raw chicken	99/04/26
20	26	jejuni	raw chicken	99/04/26
21	27	jejuni	raw chicken	99/05/03
22	28	jejuni	raw chicken	99/05/03
23	29	jejuni	raw chicken	99/05/10
24	30	jejuni	raw chicken	99/05/10

Table 2A (cont.)

Isolate	HPFB[a] no.	Species	Source	Date of isolation
25	31	jejuni	raw chicken	99/05/10
26	32	jejuni	raw chicken	99/05/10
27	33	jejuni	raw chicken	99/06/21
28	34	jejuni	raw chicken	99/06/21
29	35	jejuni	raw chicken	99/07/05
30	36	jejuni	raw chicken	99/07/05
31	37	jejuni	raw chicken	99/07/12
32	38	jejuni	raw chicken	99/07/12
33	39	jejuni	raw chicken	99/07/12
34	41	jejuni	raw chicken	99/07/29
35	42	jejuni	raw chicken	99/07/29
36	43	jejuni	raw chicken	99/09/01
37	44	jejuni	raw chicken	99/09/01
38	45	jejuni	raw chicken	00/07/10
39	46	jejuni	raw chicken	00/08/14
40	47	jejuni	raw chicken	00/08/16
41	48	jejuni	beef liver	01/02/19

[a] HPFB, Health Products and Food Branch.

APPENDIX B

Stainless steel chip experiments
(environmental sampling method)

Table 1B. The effect of inoculum concentration and size on the recovery of *Campylobacter* spp. from the surface of stainless steel chips. [a]

Inoculum concentration[b] (cells/ml)	Inoculum size (μl)	Recovery[c]
10^2	10	-[d]
	100	-
10^3	10	-
	100	+/-
10^4	10	+/-
	100	+
10^5	10	+
	100	+
10^6	10	+
	100	+
10^8	10	+
	100	+

[a] A modification of the method of Humphrey et al. (1995) was used. Five test strains of *Campylobacter* (C72, C127, C134, F26, F34) were used as the inoculum.
[b] The inoculum was prepared in peptone water.
[c] Chips were left to dry at room temperature for 5 min.
[d] -, no recovery; +, complete recovery; +/-, complete recovery of all strains except F26.

Table 2B. The effect of inoculum concentration and drying time on the recovery of *Campylobacter* spp. from stainless steel chips.[a]

Inoculum concentration[b] (cells/ml)	Drying time[c]	Recovery[d]
10^2	15 min	-
	30 min	-
	45 min	-
	1h	-
	2h	-
10^3	15 min	+
	30 min	+
	45 min	+/-
	1h	-
	2h	-
10^4	15 min	+
	30 min	+
	45 min	+/-
	1h	-
	2h	-
10^5	15 min	+
	30 min	+
	45 min	+/-
	1h	-
	2h	-
10^6	15 min	+
	30 min	+
	45 min	+/-
	1h	-
	2h	-
10^8	15 min	+
	30 min	+
	45 min	+/-
	1h	-
	2h	-

[a] A modification of the method of Humphrey et al. (1995) was used. Five test strains of *Campylobacter* (C72, C127, C134, F26, F34) were used as the inoculum.
[b] The inoculum was prepared in peptone water, and an inoculum size of 100 μl was used.
[c] Chips were left to dry at room temperature and at 4°C, with no observed difference in recovery.
[d] -, no recovery; +, complete recovery; +/-, complete recovery of all strains except F26.

APPENDIX C

PFGE profiles and dendrograms

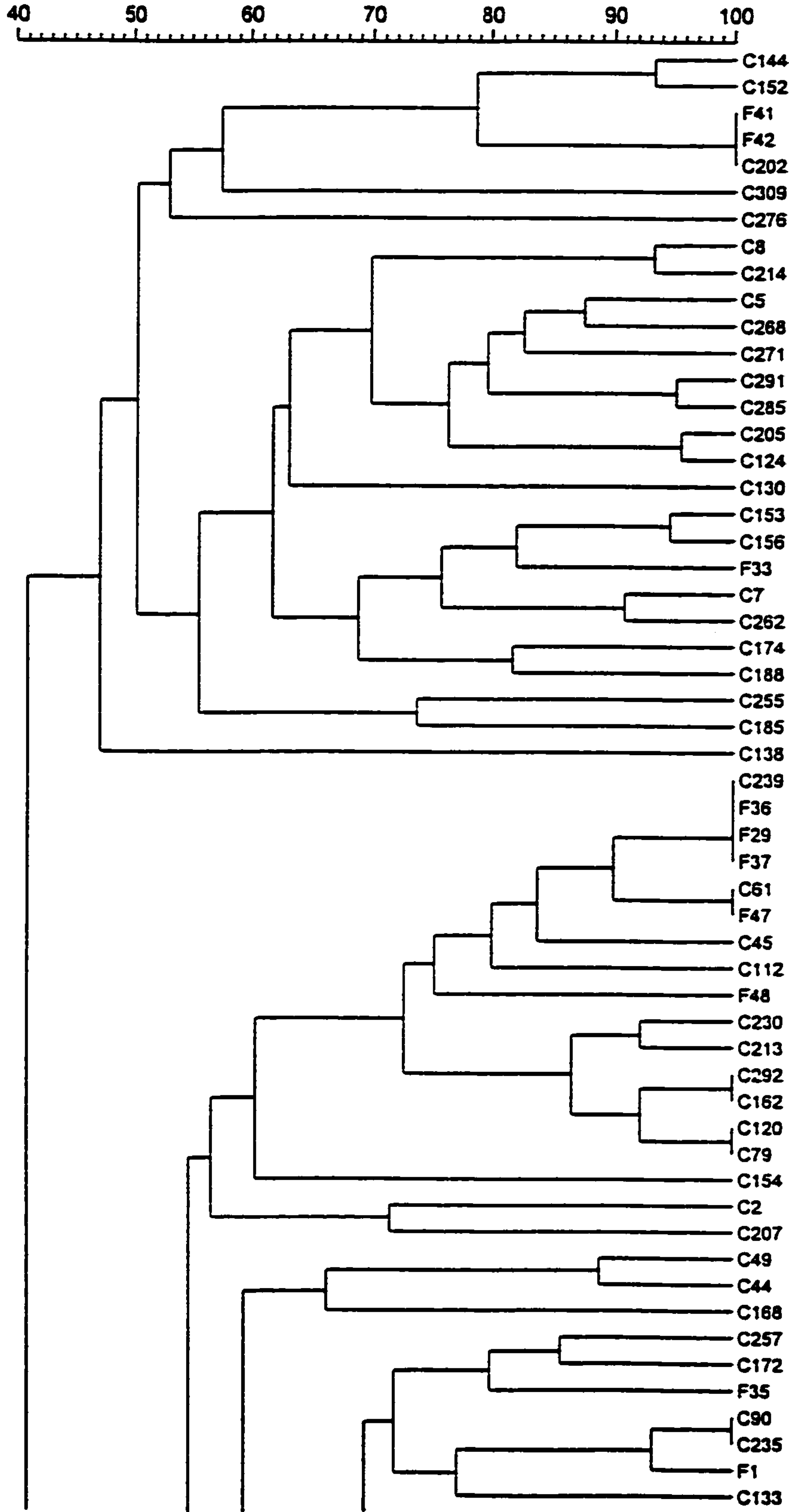
Figure 1C. *SmaI* **dendrogram of** ***Campylobacter*** **isolates obtained using Molecular Analyst®.**

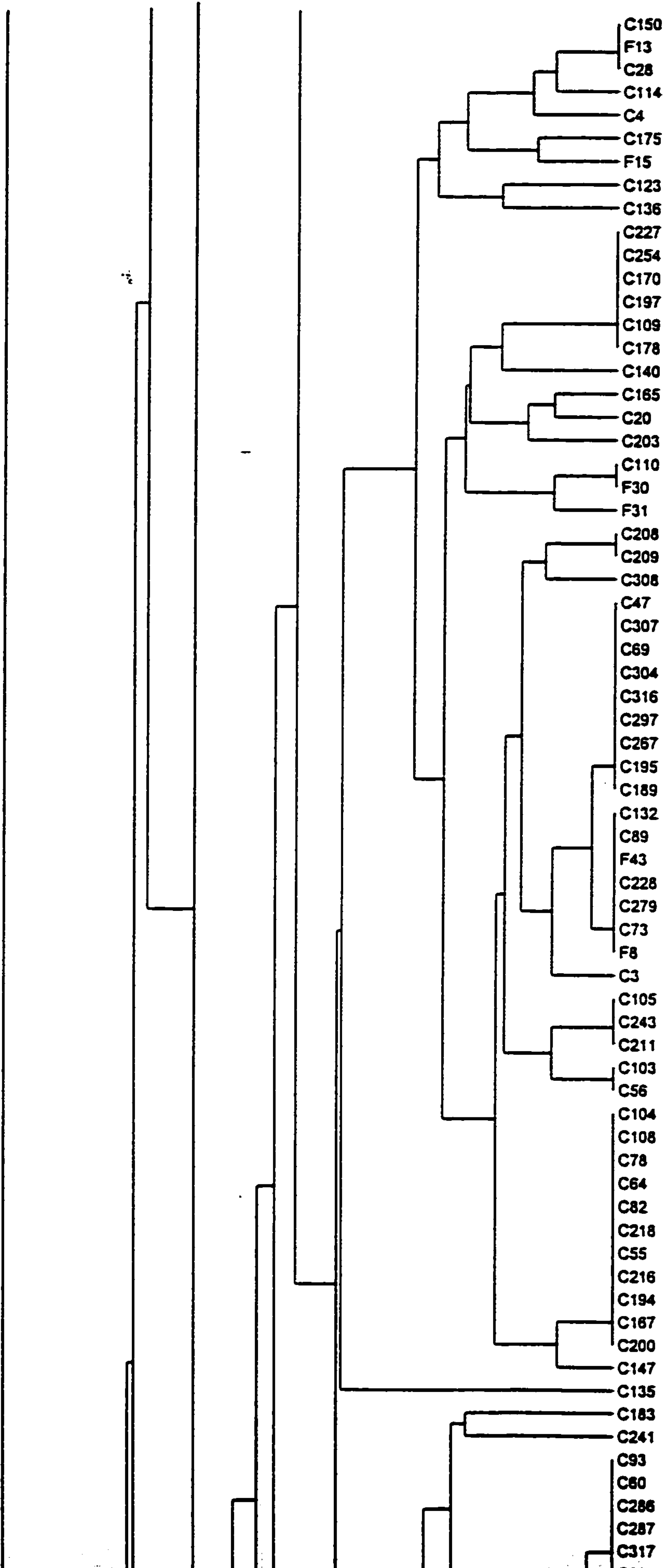
The horizontal bar is a measure of similarity (i.e., percent similarity).

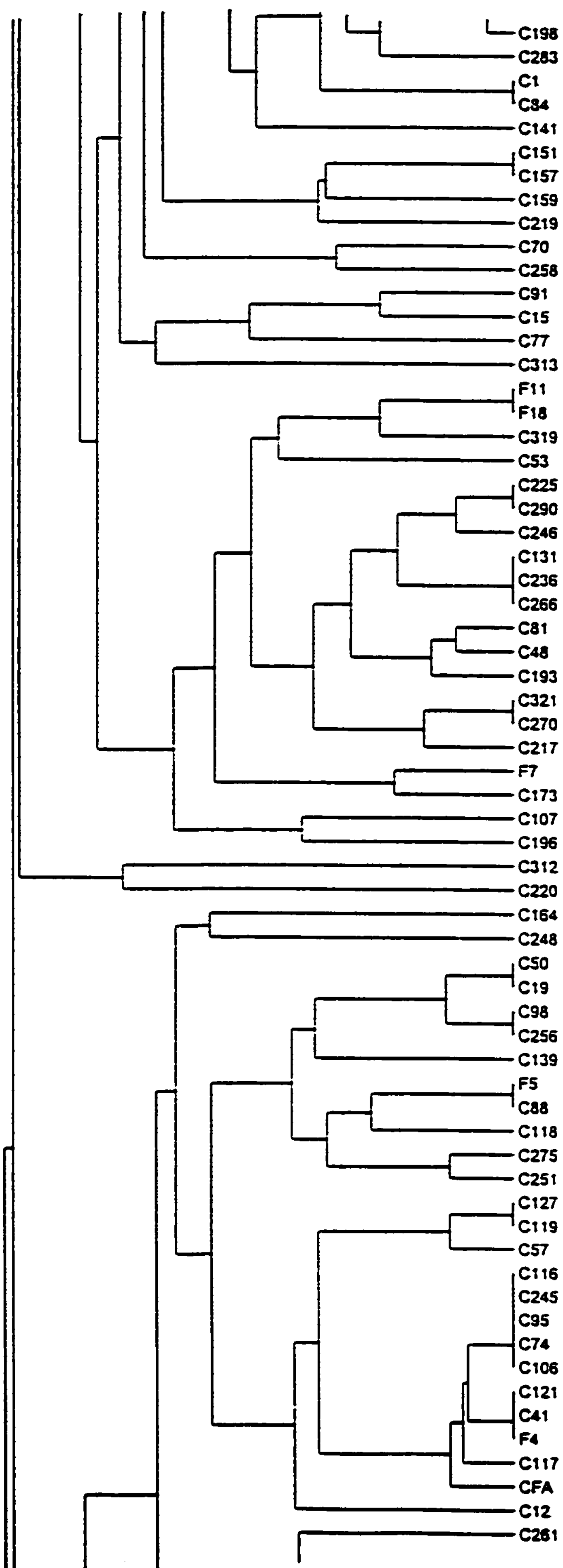
Molecular Analyst

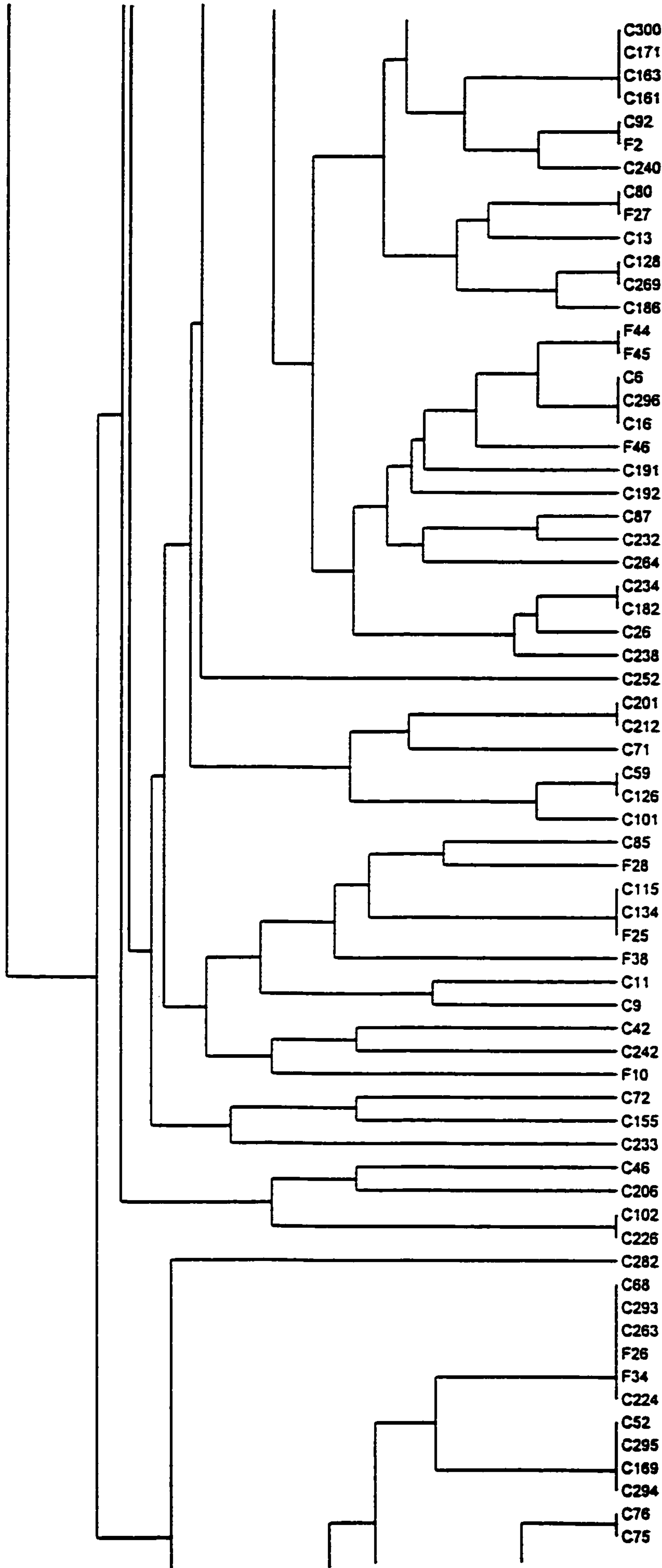
MA-F

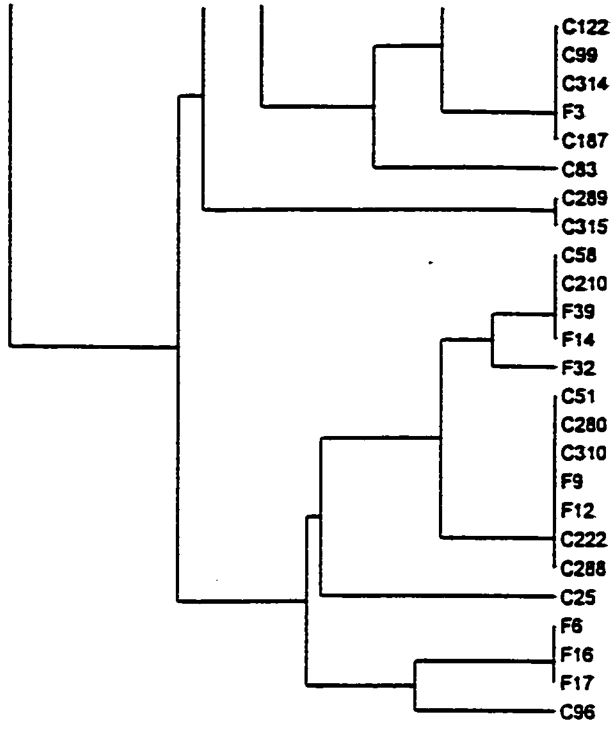
List: SMAF
Entries: 278
Correlation: Bands, Dice
Zones: [1-400]
Clustering: UPGMA




C150
F13
C28
C114
C4
C175
F15
C123
C136
C227
C254
C170
C197
C109
C178
C140
C165
C20
C203
C110
F30
F31
C208
C209
C308
C47
C307
C69
C304
C316
C297
C267
C195
C189
C132
C89
F43
C228
C279
C73
F8
C3
C105
C243
C211
C103
C56
C104
C108
C78
C64
C82
C218
C55
C216
C194
C167
C200
C147
C135
C183
C241
C93
C60
C286
C287
C317


C198
C283
C1
C34
C141
C151
C157
C159
C219
C70
C258
C91
C15
C77
C313
F11
F18
C319
C53
C225
C290
C246
C131
C236
C266
C81
C48
C193
C321
C270
C217
F7
C173
C107
C196
C312
C220
C164
C248
C50
C19
C98
C256
C139
F5
C88
C118
C275
C251
C127
C119
C57
C116
C245
C95
C74
C106
C121
C41
F4
C117
CFA
C12
C261


C300
C171
C163
C161
C92
F2
C240
C80
F27
C13
C128
C269
C186
F44
F45
C6
C296
C16
F46
C191
C192
C87
C232
C264
C234
C182
C26
C238
C252
C201
C212
C71
C59
C126
C101
C85
F28
C115
C134
F25
F38
C11
C9
C42
C242
F10
C72
C155
C233
C46
C206
C102
C226
C282
C68
C293
C263
F26
F34
C224
C52
C295
C169
C294
C76
C75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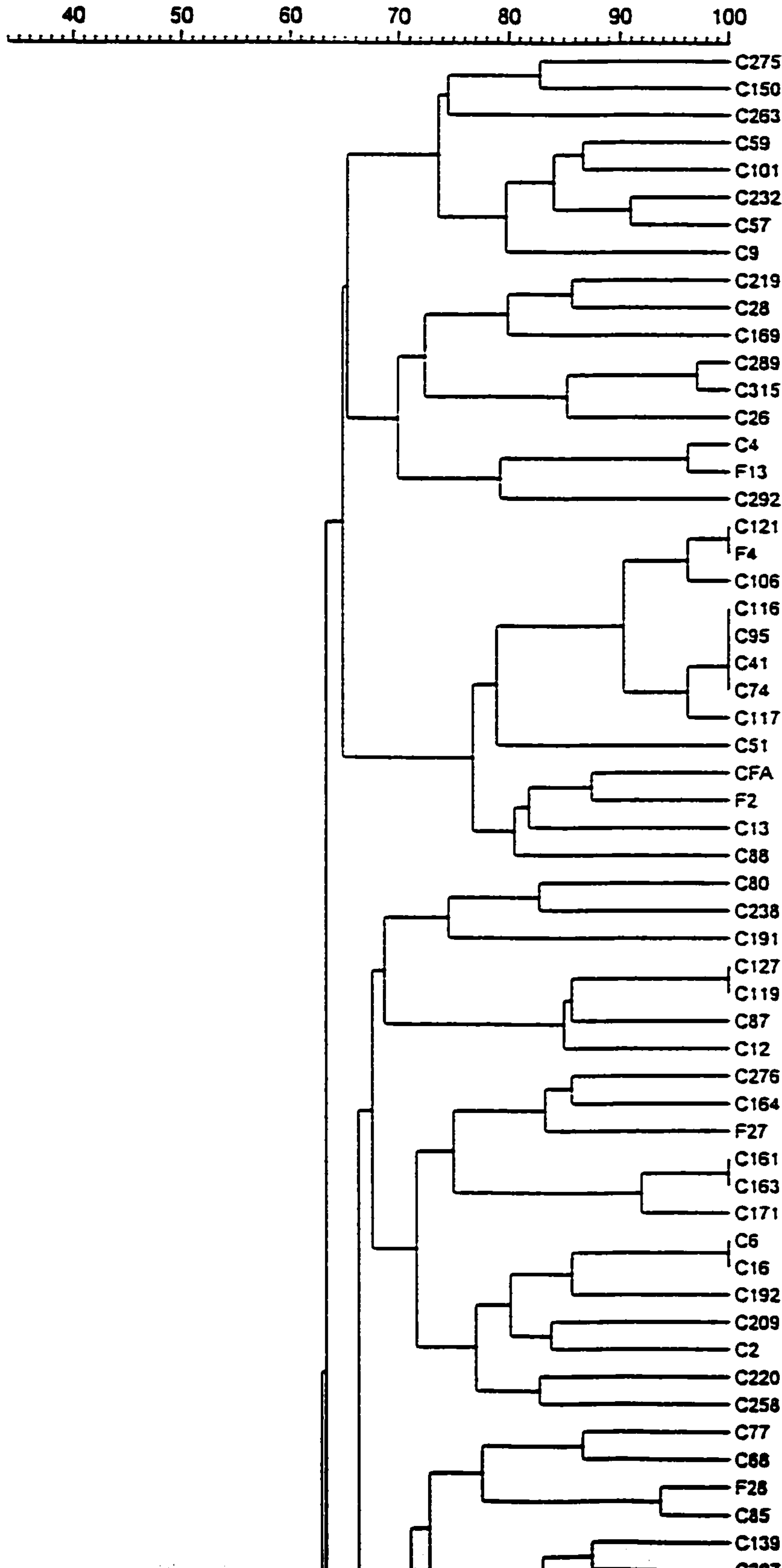
C99
C314
F3
C187
C83
C289
C315
C58
C210
F39
F14
F32
C51
C280
C310
F9
F12
C222
C288
C25
F6
F16
F17
C96

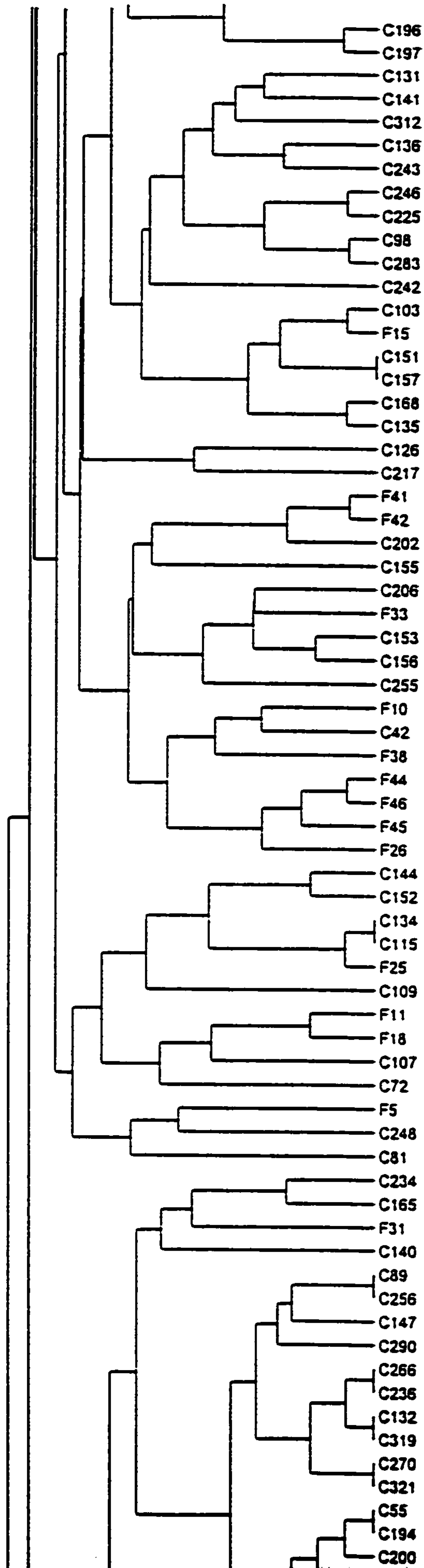
Figure 2C. *Kpn*I dendrogram of *Campylobacter* isolates obtained using Molecular Analyst®.

The horizontal bar is a measure of similarity (i.e., percent similarity).

MA-F

List: KPNDENT
Entries: 278
Correlation: Bands, Dice
Zones: [1-400]
Clustering: UPGMA

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C197
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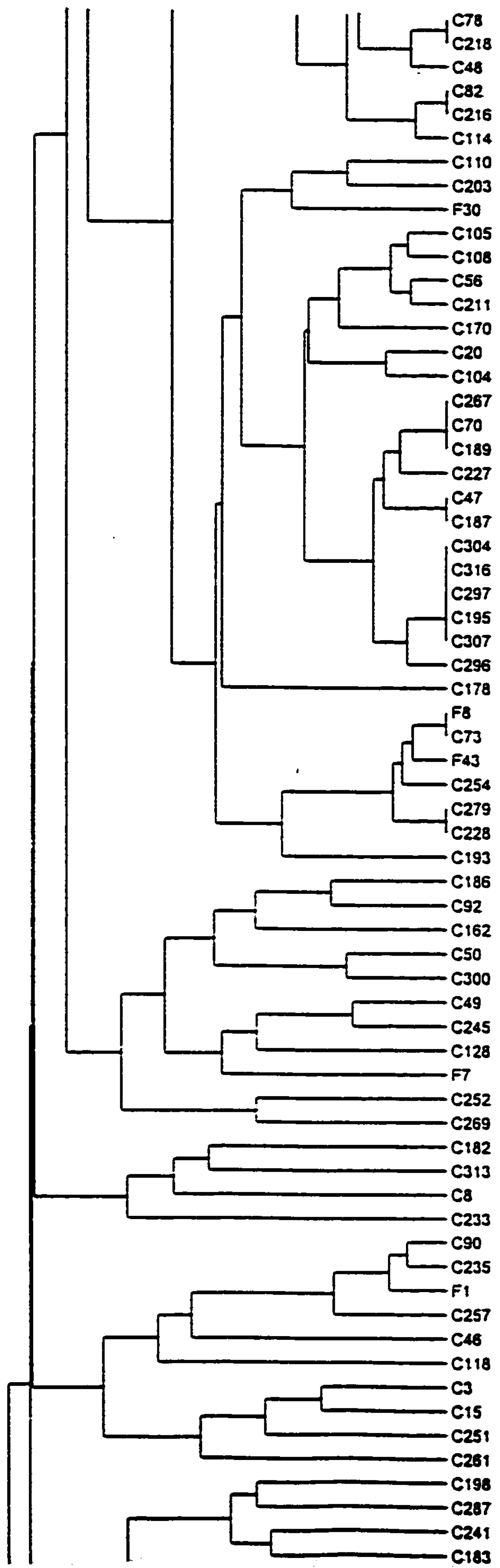
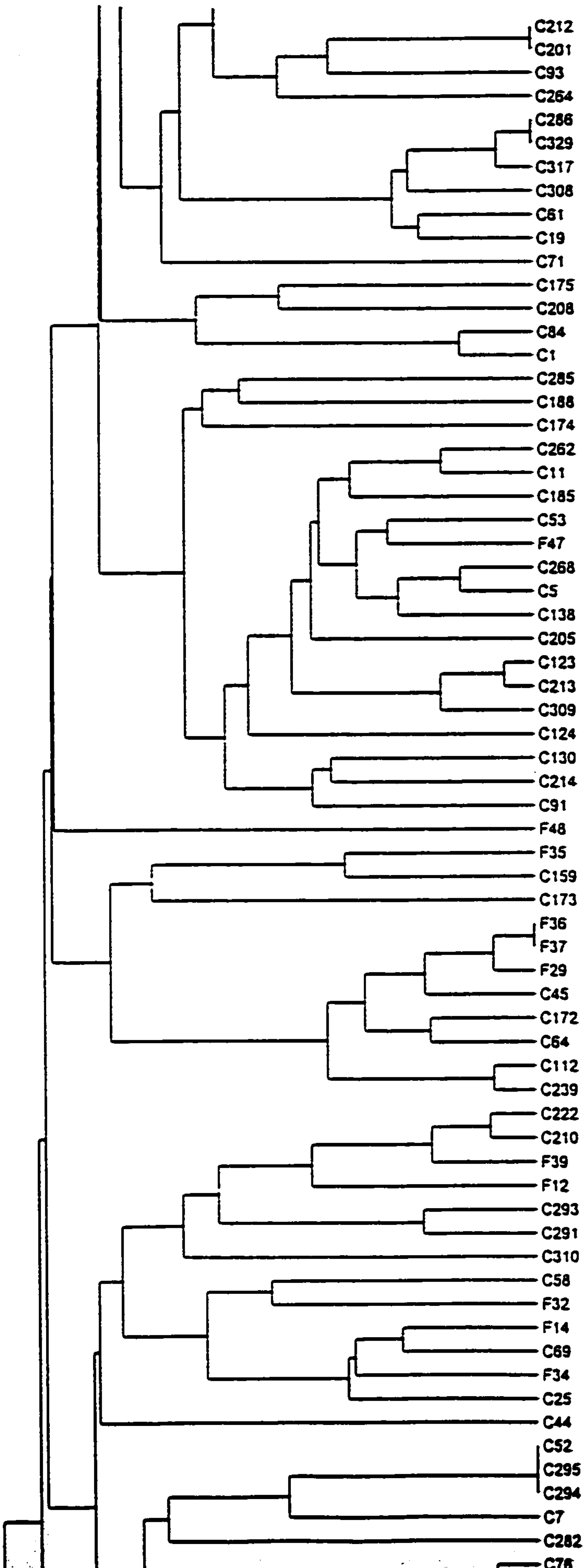
C141
C312
C136
C243
C246
C225
C98
C283
C242
C103
F15
C151
C157
C168
C135
C126
C217
F41
F42
C202
C155
C206
F33
C153
C156
C255
F10
C42
F38
F44
F46
F45
F26
C144
C152
C134
C115
F25
C109
F11
F18
C107
C72
F5
C248
C81
C234
C165
F31
C140
C89
C256
C147
C290
C266
C236
C132
C319
C270
C321
C55
C194
C200


C78
C218
C48
C82
C216
C114
C110
C203
F30
C105
C108
C56
C211
C170
C20
C104
C267
C70
C189
C227
C47
C187
C304
C316
C297
C195
C307
C296
C178
F8
C73
F43
C254
C279
C228
C193
C186
C92
C162
C50
C300
C49
C245
C128
F7
C252
C269
C182
C313
C8
C233
C90
C235
F1
C257
C46
C118
C3
C15
C251
C261
C198
C287
C241
C183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C19
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C175
C208
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C285
C188
C174
C262
C11
C185
C53
F47
C268
C5
C138
C205
C123
C213
C309
C124
C130
C214
C91
F48
F35
C159
C173
F36
F37
F29
C45
C172
C64
C112
C239
C222
C210
F39
F12
C293
C291
C310
C58
F32
F14
C69
F34
C25
C44
C52
C295
C294
C7
C282
C76

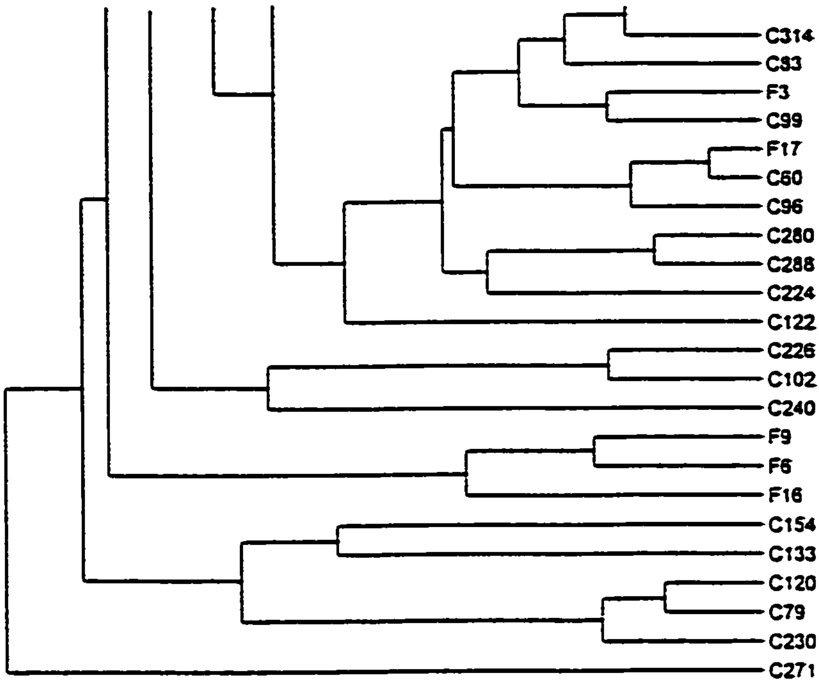

C314
C83
F3
C99
F17
C60
C96
C280
C288
C224
C122
C226
C102
C240
F9
F6
F16
C154
C133
C120
C79
C230
C271

Table 1C. PFGE profiles assigned to *Campylobacter* clinical and food isolates.

HPFB[a] no.	PFGE	
i) Clinical	*Sma* I	*Kpn* I
1	76	176
2	36	41
3	65	156
4	49	15
5	8	186
6	129	38
7	19	222
8	6	148
9	149	8
11	148	181
12	119	32
13	125	25
15	84	157
16	129	38
19	106	171
20	57	122
25	169	219
26	137	14
28	47	10
41	116	20
42	150	79
44	39	220
45	28	202

Table 1C (cont.)

HPFB[a] no.	PFGE	
i) Clinical	*Sma* I	*Kpn* I
46	156	154
47	63	126
48	94	111
49	38	140
50	106	138
51	168	22
52	161	221
53	89	183
55	68	107
56	67	119
57	114	7
58	166	214
59	142	4
60	73	231
61	27	170
64	68	204
68	160	45
69	63	217
70	81	124
71	141	172
72	153	93
73	64	130
74	115	20

Table 1C (cont.)

HPFB[a] no.	**PFGE**	
i) Clinical	*Sma* I	*Kpn* I
75	162	225
76	162	224
77	85	44
78	68	110
79	34	246
80	124	27
81	93	96
82	68	112
83	164	227
84	76	175
85	144	47
87	133	31
88	109	26
89	64	101
90	44	150
91	83	195
92	122	136
93	73	165
95	115	20
96	171	232
98	107	59
99	163	229
101	143	5

Table 1C (cont.)

HPFB[a] no.	PFGE	
i) Clinical	*Sma* I	*Kpn* I
102	158	238
103	67	62
104	68	123
105	66	117
106	115	19
107	100	92
108	68	118
109	54	89
110	59	114
112	29	205
114	48	113
115	146	87
116	115	20
117	117	21
118	110	155
119	113	30
120	34	245
121	116	18
122	163	236
123	52	189
124	14	192
126	142	67
127	113	30

Table 1C (cont.)

HPFB[a] no.	PFGE	
i) Clinical	*Sma* I	*Kpn* I
128	126	142
130	15	193
131	92	52
132	64	105
133	46	244
134	146	87
135	70	66
136	53	55
138	25	187
139	108	48
140	55	100
141	77	53
144	1	85
147	69	102
150	47	2
151	78	64
152	2	86
153	16	75
154	35	243
155	154	72
156	17	76
157	78	64
159	79	198

Table 1C (cont.)

HPFB[a] no.	**PFGE**	
i) Clinical	*Sma* I	*Kpn* I
161	121	36
162	33	137
163	121	36
164	104	34
165	56	98
167	68	109
168	40	65
169	161	11
170	54	121
171	121	37
172	42	203
173	99	199
174	21	179
175	50	173
178	54	129
182	136	146
183	71	163
185	24	182
186	127	135
187	163	126
188	22	178
189	63	124
191	131	29

Table 1C (cont.)

HPFB[a] no.	PFGE	
i) Clinical	*Sma* I	*Kpn* I
192	132	39
193	95	134
194	68	107
195	63	127
196	101	50
197	54	51
198	74	160
200	68	108
201	140	164
202	3	71
203	58	115
205	13	188
206	157	73
207	37	49
208	61	174
209	61	40
210	166	208
211	66	120
212	140	164
213	32	190
214	7	194
216	68	112
217	97	68

Table 1C (cont.)

HPFB[a] no.	**PFGE**	
i) Clinical	*Sma* I	*Kpn* I
218	68	110
219	80	9
220	103	42
222	168	207
224	160	235
225	90	58
226	158	237
227	54	125
228	64	133
230	31	247
232	134	6
233	155	149
234	136	97
235	44	151
236	92	104
238	138	28
239	26	206
240	123	239
241	72	162
242	151	61
243	66	56
245	115	141
246	91	57

Table 1C (cont.)

HPFB[a] no.	PFGE	
i) Clinical	*Sma* I	*Kpn* I
248	105	95
251	112	158
252	139	144
254	54	132
255	23	77
256	107	101
257	41	153
258	82	43
261	120	159
262	20	180
263	160	3
264	135	166
266	92	104
267	63	124
268	9	185
269	126	145
270	96	106
271	10	248
275	111	1
276	5	33
279	64	133
280	168	233
282	159	223

Table 1C (cont.)

HPFB[a] no.	PFGE	
i) Clinical	*Sma* I	*Kpn* I
283	75	60
285	12	177
286	73	167
287	73	161
288	168	234
289	165	12
290	90	103
291	11	212
292	33	17
293	160	211
294	161	221
295	161	221
296	129	128
297	63	127
300	121	139
304	63	127
307	63	127
308	62	169
309	4	191
310	168	213
FA	118	23
312	102	54
313	86	147

Table 1C (cont.)

HPFB[a] no.	**PFGE**	
i) Clinical	*Sma* I	*Kpn* I
314	163	226
315	165	13
316	63	127
317	73	168
319	88	105
321	96	106
329	73	167
ii) Food		
1	45	152
2	122	24
3	163	228
4	116	18
5	109	94
6	170	241
7	98	143
8	64	130
9	168	240
10	152	78
11	87	90
12	168	210
13	47	16
14	166	216
15	51	63

Table 1C (cont.)

HPFB[a] no.	PFGE	
ii) Food	*Sma* I	*Kpn* I
16	170	242
17	170	230
18	87	91
25	146	88
26	160	84
27	124	35
28	145	46
29	26	201
30	59	116
31	60	99
32	167	215
33	18	74
34	160	218
35	43	197
36	26	200
37	26	200
38	147	80
39	166	209
41	3	69
42	3	70
43	64	131
44	128	81
45	128	83

Table 1C (cont.)

HPFB[a] no.	PFGE	
ii) Food	*Sma* I	*Kpn* I
46	130	82
47	27	184
48	30	196

[a] HPFB, Health Products and Food Branch.

APPENDIX D

Spot map analysis

Title: **Campylobacteriosis Cases With Idendified Isolate in Ottawa-Carleton Municipality, 1998-2001**

Date Created: August 31, 2001

Map Number: Map7

Client Info:

Client Name and Organization: Diane Medeiros - Bureau of Microbial Hazards, Health Canada
E-mail: Diane_medeiros@hc-sc.gc.ca
Date of Request: July 2001

Produced by:

Kara Linttell
GIS Infrastructure Project
Centre for Surveillance Coordination
Population and Public Health Branch, Health Canada
(613) 946-4816
kara_linttell@hc-sc.gc.ca

Data Set and Source:

1) Campy cases: Health data in .xls table provided by client in Map and Data Exchange, modified to include campy isolate value column.
A) MaDEx/HC-Microbial Hazards/Cycle 1/Spot map of campy cases/All campy cases 2001 06 20.xls (original table)
B) C:/ArcView/Client's Data/Diane Medeiros/all campy cases w isolate.dbf

2) Provincial Boundaries: Area theme file retrieved from server with plug-in.
gltp://127.0.0.1:80/shp/unhs103/dmti/postal/ArcView/Nad83/Canada
(Date of file: July 2000)

3) Census Division: Area theme file retrieved from server with plug-in.
gltp://127.0.0.1:80/shp/unhs103/dmti/ArcView/Nad83/Census/1996/Can/CD/BDYS
(Date of file: 1996)

4) Census Subdivisions: Area theme file retrieved from server with plug-in.
gltp://127.0.0.1:80/shp/unhs103/dmti/ArcView/Nad83/Census/1996/Can/CSD/BDYS
(Date of file: 1996)

5) Waterbody: Area theme file retrieved from server with plug-in.
gltp://127.0.0.1:80/shp/unhs103/dmti/postal/ArcView/Nad83/Canada
(Date of file: July 2000)

6) Postal Codes: DMTI's Unique Enhanced Postal code point file retrieved from local drive. C:/ArcView/Data to be loaded on server/DMTI Unique Enhanced PC
(Date of file: April 4, 2001)

<u>Method:</u>

The client provided an Excel table composed of 3 columns: HPB number, street address and postal code of 487 campy cases. The case reports, from 1998 to June 2001, were obtained by Microbiology Research Division at Health Canada from the Regional Municipality of Ottawa-Carleton (RMOC) Health Department, to whom Gamma-Dynacare reports the cases. The client performed a data extraction from that information to produce a table suitable for mapping.

The client had highlighted the records in the table that had no identified Campylobacter isolate. Since the client requested that these postal codes be identifiable in the map by using a different color, a new column titled "Campy Isolate" was added to the table and each highlighted record was assigned the value "no" while the remaining records were assigned the value "yes". This step was required in order for the records with no campylobacter isolate to have a different value than the others. This table was saved as "all campy cases.dbf" and opened in ArcView GIS v3.1 (1A).

In ArcView, a new view was created and the provincial boundaries were downloaded from the CANprv file of the postal data set (2). This file was modified by deleting all provinces except Ontario and Quebec. The two provinces were given different colors in the legend.

The Census Divisions (CD) were downloaded (3) and modified by selecting only the Ottawa-Carleton Regional Municipality (CD id = 3506) and converting it into a shapefile. In the legend editor, an outline of a 2 point width was selected in order to present an obvious outline of the municipal boundary. The Census Subdivisions (CSD) were downloaded (4) and modified by selecting only the CSDs within the Ottawa-Carleton CD. The CSDs were given unique colors and named in the legend in order to help identify the geographical location of postal codes in such a large area. The water layer was added (5) and text was added to label the Ottawa River and the municipal boundary.

DMTI's Unique Enhanced Postal Code file was downloaded (6) and joined to the table "all campy cases.dbf". There were several records that did not join, therefore some preliminary investigation was required in order to maximize the number of mappable cases. Using Canada Post's postal code search website (www.canadapost.ca/tools/pcl/bin and www.canadapost.ca/tools/pcl/bin/rural-e.asp) an atlas and the CanMap street file, the postal code of each record that did not join successfully was searched. On 2 separate occasions, the results of the search and suggested solutions were provided to the client, who agreed with each one.

The "all campy cases.dbf" (1) was then edited in order to capture the fixed or found postal codes and an unjoined version of the table, named **"campy cases enhanced table.dbf"** was posted in the Map and Data Exchange for the client. The search and editing resulted in 475 of 487 cases (97.5%) being mappable.

A query was performed on the "All campy cases.dbf" table to identify those postal codes located within the Ottawa-Carleton Municipality boundary (CD id=3506), then were converted to a new shapefile and saved as "Ottawa-Carleton campy cases". Of the 475 mappable cases, 398 are geographically located within the Ottawa-Carleton Municipality boundary and were mapped. An unjoined version of the table of cases within the specified boundary was saved as **"Ottawa-Carleton campy cases table.dbf"** and was posted in the Map and Data Exchange for the client. A query was run on the "Ottawa-Carleton campy cases table.dbf" in order to get a count of cases with an identified isolate per postal code. In the view, a new event theme was created by using the campy cases postal code latitude and longitude produced from the join. The isolate cases per postal code were mapped as graduated symbols and their counts were noted in the legend of Map7.

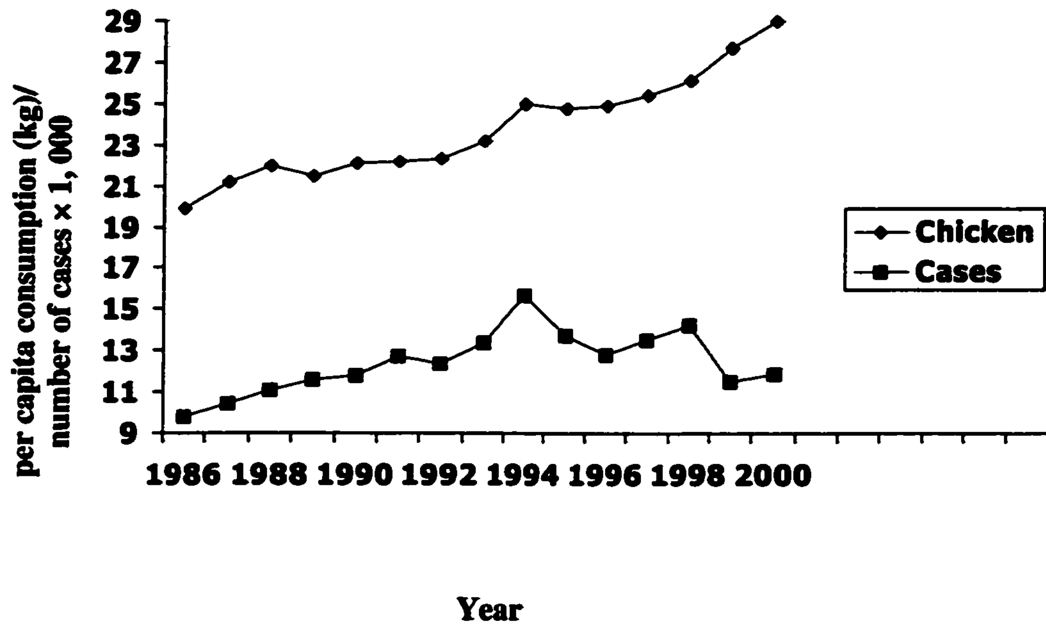
The source of the data was noted at the bottom of the map for reference. Forty-four percent of the cases are located within the Ottawa Census Subdivision with clusters in census subdivisions surrounding the Ottawa subdivision. The table below provides some preliminary statistics, from high to low number of cases:

Census Subdivision Name	**Count of Campy Cases**	**Percent of Ottawa-Carleton Campy cases**	**No Campy isolate**	**Campy isolate**
Ottawa	175	44.0%	96 (54.9%)	79 (45.1%)
Nepean	76	19.1%	43 (56.6%)	33 (43.4%)
Gloucester	49	12.3%	24 (49%)	25 (51%)
Kanata	34	8.5%	16 (47%)	18 (53%)
Cumberland	16	4.0%	10 (62.5%)	6 (37.5%)
West Carleton	14	3.5%	8 (57.1%)	6 (42.9%)
Goulbourn	10	2.5%	2 (20%)	8 (80%)
Osgoode	9	2.3%	4 (44.4%)	5 (55.5%)
Rideau	9	2.3%	4 (44.4%)	5 (55.5%)
Vanier	4	1.0%	3 (75%)	1 (25%)
Rockcliffe Park	2	0.5%	1 (50%)	1 (50%)
TOTAL:	**398**	**100%**	**211** (53%)	**187** (47%)

APPENDIX E

Poultry consumption trends

Figure 1E. Levels of poultry consumption and campylobacteriosis in Canada, 1986-2000 (Chicken Farmers of Canada, 2001).



Year