Campylobacter in Human Cases and Retail Chicken in two Health Units in Ontario

by

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ABSTRACT

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Professor Scott McEwen

This thesis is an investigation of human campylobacteriosis and Campylobacter from retail chicken in two Ontario health units. The objectives were to investigate the symptoms, severity, antimicrobial use (AMU), antimicrobial resistance (AMR), and duration of illness (DI) in clinical campylobacteriosis, the prevalence and AMR of Campylobacter from contaminated retail chicken, and the relationship between clinical and chicken Campylobacter isolates based on Comparative Genomic Fingerprinting (CGF).

In the participating health units, campylobacteriosis case data were collected and retail chicken was sampled from randomly selected stores. Campylobacter isolates from clinical cases and chicken were antimicrobial susceptibility tested and CGF typed. A Cox proportional hazard model was used to investigate the DI in campylobacteriosis. Logistic regression models were used to explore the relationship between clinical and chicken CGF types.

Of 250 cases, 52% reported taking antimicrobials for their campylobacteriosis. In 124 cases with accompanying isolate and AMR information, 6 (4.8%) and 2 (1.6%) isolates were resistant to ciprofloxacin and erythromycin, respectively. In 749 chicken isolates, 14 (1.9%) and 25 (3.3%) isolates were resistant to ciprofloxacin and erythromycin, respectively. No isolates were
resistant to both antimicrobials. While the low prevalence of AMR to ciprofloxacin and
erythromycin was encouraging, the high proportion of cases treated with antimicrobials was
concerning and efforts should be made to reduce unnecessary treatment.

The Cox model identified that use of a macrolide for less than the recommended duration, use of
ciprofloxacin for the recommended duration, and use of other antimicrobials, were factors
associated with decreased DI. The impact of AMU was consistent regardless of when in the
course of illness it began.

The CGF results were available from 115 clinical and 718 chicken isolates. A *Campylobacter*
CGF reference database was used to identify CGF types that comprised at least 80% of isolates
from chicken, based on 90% fingerprint similarity (CA90). Isolates from urban cases were
significantly more likely than rural cases to be CA90. In Canada, the majority of
campylobacteriosis cases are urban dwellers. Therefore, the association between urban cases and
CA90 emphasizes the importance of *Campylobacter* from retail chicken on public health.
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# Table of Contents

Chapter 1: Introduction and Review of Literature................................................................. 1  

1. *Campylobacter* in human illness ...................................................................................... 2  
   1.1 Incidence ......................................................................................................................... 2  
   1.2 Severity and sequelae ...................................................................................................... 3  
   1.3 Clinical presentation ....................................................................................................... 4  
   1.4 Immunity ........................................................................................................................ 5  
   1.5 Indications for treatment of campylobacteriosis in humans ............................................. 6  
   1.6 Evaluation of antimicrobial treatment efficacy ............................................................... 7  
   1.7 Symptomatic treatment .................................................................................................. 9  
   1.8 Surveillance of campylobacteriosis in Canada ................................................................. 9  

2. Antimicrobial resistance in *Campylobacter* from human clinical cases ............................. 10  
   2.1 Ciprofloxacin resistance ................................................................................................. 10  
   2.2 Erythromycin resistance .............................................................................................. 12  
   2.3 Clindamycin and azithromycin resistance .................................................................... 12  
   2.4 Gentamicin resistance .................................................................................................. 13  
   2.5 Tetracycline resistance .................................................................................................. 13  
   2.6 Trimethoprim-sulfamethoxazole resistance .................................................................. 13  

3. Risk factors for human campylobacteriosis ...................................................................... 14  
   3.1 Chicken .......................................................................................................................... 14  
   3.2 Ruminants ....................................................................................................................... 16  
   3.3 Flies, rodents and wild birds .......................................................................................... 16  
   3.4 Direct contact ................................................................................................................ 17  
   3.5 Water .............................................................................................................................. 17  
   3.6 Other factors .................................................................................................................. 17  
   3.7 Urbanization .................................................................................................................. 18  

4. *Campylobacter* in retail chicken ..................................................................................... 19  
   4.1 Prevalence ...................................................................................................................... 19  
   4.2 Ciprofloxacin resistance ............................................................................................... 20
Chapter 2: Antimicrobial resistance and antimicrobial use associated with laboratory–confirmed cases of *Campylobacter* in two health units in Ontario

Abstract .................................................................................................................. 49

Introduction ............................................................................................................ 51

Methods .................................................................................................................. 52

Cases ....................................................................................................................... 53

Study area .............................................................................................................. 53

Isolation ................................................................................................................... 53

Minimum inhibitory concentrations ..................................................................... 54

Questionnaire data ................................................................................................. 54

Data analysis .......................................................................................................... 55

Results .................................................................................................................... 55

Discussion .............................................................................................................. 59

Conclusions ........................................................................................................... 65

Acknowledgements ............................................................................................... 66

References ............................................................................................................. 67

Chapter 3: Burden of illness and factors associated with duration of illness in clinical campylobacteriosis

Abstract .................................................................................................................. 79

Introduction ............................................................................................................ 80

Methods .................................................................................................................. 81

Questionnaire data ................................................................................................. 81

Survival analysis ..................................................................................................... 82

Results .................................................................................................................... 85
Chapter 6: Summary Discussion and Conclusions ................................................................. 157
References: ......................................................................................................................... 170

Chapter 5: Molecular epidemiology of Campylobacter jejuni human and chicken isolates from two health units in Ontario ................................................................. 137
Abstract: .............................................................................................................................. 137
Introduction .......................................................................................................................... 138
Methods ............................................................................................................................... 139
Statistical analysis ............................................................................................................... 140
Results ................................................................................................................................. 142
Exact logistic regression analysis ....................................................................................... 143
Discussion .......................................................................................................................... 144
Conclusions: ...................................................................................................................... 149
Acknowledgements: .......................................................................................................... 150
References: ......................................................................................................................... 153

Chapter 4: Prevalence and antimicrobial resistance in Campylobacter spp. isolated from retail chicken in two health units in Ontario ................................................................. 113
Abstract ............................................................................................................................. 113
Materials and Methods ...................................................................................................... 115
Sample collection ............................................................................................................... 115
Isolation ............................................................................................................................... 116
Minimum inhibitory concentrations .................................................................................. 117
Data analysis ....................................................................................................................... 118
Results and Discussion ...................................................................................................... 118
Acknowledgements ........................................................................................................... 125
References .......................................................................................................................... 127

Survival analysis .................................................................................................................. 88
Discussion ............................................................................................................................ 91
Acknowledgements ............................................................................................................ 96
References ............................................................................................................................ 98
Appendix A.1: Health unit recruitment and consent script for laboratory-confirmed cases of campylobacteriosis in the Perth District and Wellington-Dufferin-Guelph health units, 2002-2004 .. 174
Appendix A.2: Telephone survey administered to laboratory-confirmed cases of campylobacteriosis in the Wellington-Dufferin-Guelph and Perth District health units, 2002-2004. .......................................................... 175
Appendix A.4: Primary Isolation Laboratory Protocol for study of laboratory-confirmed cases of campylobacteriosis in the Perth District and Wellington-Dufferin-Guelph health units, 2002-2004. . 197
Appendix A.5: Flow Chart of roles and responsibilities for study of laboratory-confirmed cases of campylobacteriosis in the Perth District and Wellington-Dufferin-Guelph health units, 2002-2004. . 199
List of Tables

Table 2.1: Antimicrobial resistance in *Campylobacter* isolates from human cases in the Perth District and Wellington-Dufferin-Guelph health units, Ontario, Canada ................................................................. 77

Table 2.2: Antimicrobial resistance patterns in *Campylobacter* isolates from human cases in the Perth District and Wellington-Dufferin-Guelph health units, Ontario, Canada ............................................. 78

Table 3.1: Summary of reported symptoms by severity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units ............................................. 110

Table 3.2 Summary of reported days of missed work or school by severity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units......................... 111

Table 3.3: Potential covariates evaluated in univariable survival analysis in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units ................................. 112

Table 4.1: Prevalence of *Campylobacter* by sample type from fresh retail chicken in two health units in Ontario, Canada ........................................................................................................... 132

Table 4.2: Antimicrobial resistance in *Campylobacter* isolates from fresh retail chicken in two health units in Ontario, Canada ........................................................................................................... 133

Table 4.3: Antimicrobial resistance in *Campylobacter* associated with chicken* from selected literature ........................................................................................................................................ 134

Table 4.4: Multiple resistance in *Campylobacter* isolates from retail chicken in two health units in Ontario, Canada ........................................................................................................... 135

Table 4.5: Antimicrobial resistance patterns in *Campylobacter* isolates from fresh retail chicken in two health units in Ontario, Canada (n=749) ........................................................................................................... 136

Table 5.1: Potential covariates evaluated in univariable exact logistic regression analysis of “Chicken Associated” Comparative Genomic Fingerprint type in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units ................................................................. 151

Table 5.2: Exact logistic regression model for Chicken-associated CGF* types at the 90% fingerprint similarity level in laboratory-confirmed cases of *Campylobacter* in Perth District and Wellington-Dufferin-Guelph health units (n=89) ........................................................................................................................................ 152
List of Figures

Figure 2.1: Percentage of cases by age category over a ten year period in the Wellington-Dufferin-Guelph health unit and in laboratory-confirmed cases of Campylobacter in the Perth District and Wellington-Dufferin-Guelph health units during the study period. ................................................................. 74

Figure 2.2: Antimicrobial use in cases during their illness, by severity in laboratory-confirmed cases of Campylobacter in the Perth District and Wellington-Dufferin-Guelph health units (n=244). ........................................ 75

Figure 2.3: Frequency and timing of antimicrobial use relative to availability of fecal culture results in laboratory-confirmed cases of Campylobacter in the Perth District and Wellington-Dufferin-Guelph health units (n=138). ......................................................................................................................................................... 76

Figure 3.1: Duration of illness in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units (n=249). ............................................................................................................................................... 104

Figure 3.2: Self-reported severity of illness according to a defined severity scale and mean number of days of limited activity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units. ........................................................................................................................................ 105

Figure 3.3: Reported symptoms by severity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units ........................................................................................................................................ 106

Figure 3.4: Cox proportional hazard model for duration of illness in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units (n=227): Hazard ratios* and 95% Confidence intervals ........................................................................................................................................ 107

Figure 3.5: Predicted survival curves of significant antimicrobial use variables from Cox proportional hazard model for duration of illness in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units (n=227) with adjustment for prior use of non-antimicrobials ........................................................................................................................................ 108
Chapter 1: Introduction and Review of Literature

Although various species of Campylobacter have been recognized as veterinary pathogens for many years, it is only in the last 40 years that the role of Campylobacter as the most common cause of bacterial gastroenteritis in humans was recognized (1, 2). This is likely due to the adoption of laboratory tests that recognized some of the organisms’ growth requirements that include: a micro-aerophilic environment, increased carbon dioxide, and higher temperatures than most gastrointestinal pathogens (3). Campylobacter is a Gram negative spiral, motile bacillus that belongs to the family Campylobacteriaceae (4). There are approximately 15 species and 6 subspecies of Campylobacter. Campylobacter jejuni and to a lesser extent Campylobacter coli are the most commonly associated with human illness (5-10). Campylobacter is most frequently associated with sporadic disease in humans and symptoms of campylobacteriosis include severe, bloody diarrhea, fever, and abdominal pain (1, 3). The natural course of disease is typically less than two weeks in duration (11-13). Although most infections resolve without antimicrobial therapy, treatment with macrolides or fluoroquinolones may be required in severe cases or in immuno-compromised patients. Resistance to these antimicrobials in Campylobacter presents treatment difficulties and may be associated with an increased severity of disease. Human illness has frequently been associated with the handling and consumption of chicken in case-control studies (2). Poultry including chicken are asymptomatic carriers of Campylobacter and the prevalence of C. jejuni on retail chicken quite high (50-90%) (144-146,151-158). Therefore, the transmission of both susceptible and resistant Campylobacter through the food supply is of concern. Investigation of the relationship between human cases and potential sources including
chicken has been impeded by the lack of molecular sub-typing methods with sufficient discriminatory ability that are feasible to conduct on large numbers of isolates. 

The objectives of this chapter are to review the literature pertaining to the epidemiology of antimicrobial resistant *Campylobacter* infections in humans, and to identify the research objectives of this thesis. The literature review summarizes published research on:

1) Campylobacteriosis in humans including the burden of illness, duration of illness, and antimicrobial use

2) Antimicrobial resistance of *Campylobacter* from human clinical isolates

3) Risk factors for human campylobacteriosis

4) The prevalence and antimicrobial resistance of *Campylobacter* on retail chicken

5) The use of Comparative Genomic Fingerprinting (CGF) for examining the relationship between *Campylobacter* isolates from sporadic cases of illness in humans and *Campylobacter* from retail chicken

1. *Campylobacter* in human illness

1.1 Incidence

The incidence of campylobacteriosis varies widely in developed countries. In 2010, the FoodNet Surveillance system in the United States reported a rate of 13.5 per 100,000 people across their sites (14). European incidence rates ranged from less than 1 case per 100,000 in Italy to 201 per 100,000 in the Czech Republic in 2010 (15). New Zealand has historically had the highest incidence rate of any developed country. In 2003 the incidence rate was 396 per 100,000,
although after aggressive risk control strategies were implemented, that rate dropped to 157 per 100,000 in 2008 (2, 16). In 2004 the National Notifiable Disease Program in Canada reported 31.8 isolations of *Campylobacter* per 100,000 people in the province of Ontario and 30.2 per 100,000 nationally, which is well within the range reported by other developed countries (17). Regional differences in incidence rate have been reported, for example in the Netherlands, Scotland, and Denmark (18-20). In Canada recent studies have shown that rates in urban areas significantly differed from those in rural areas (21).

Gastrointestinal illness is typically under-reported in surveillance systems, therefore the true incidence rate is likely substantially higher than described (22-25). In Canada, it has been estimated that for every officially reported case there are actually 23 to 49 cases of campylobacteriosis that occur in the population (26). Taking into account this degree of under-reporting, the estimated number of cases of campylobacteriosis in Canada ranged from 221,899 to 472,741 cases in 2004 (27).

### 1.2 Severity and sequelae

The reported percentage of campylobacteriosis cases that were hospitalized has ranged from 10% to 14% in a variety of countries (11, 24, 28, 29) however in Canada, the reported percentage was lower, 1.9 to 6.9% (30-32). Duration of hospitalization is quite variable, for example while the reported mean duration of hospital stay in two studies was relatively short (3 and 5 days) (11, 32), the range was quite large (1 to 147) (32).

The reported case fatality rate for *Campylobacter* was 1 to 2 per thousand cases in the United States and the European Union (2, 15, 33). It has been estimated that 9-14% of cases develop
post-infectious irritable bowel syndrome (34, 35) and approximately 1 in one thousand cases progress to Guillain-Barré syndrome (36). Reactive arthritis is another potential sequel that occurs in 1-5% of campylobacteriosis cases (4, 37).

1.3 Clinical presentation

Symptoms commonly reported by campylobacteriosis patients include diarrhea, fever, stomach pain, abdominal pain, and nausea (11, 12, 37-39). Less commonly reported symptoms include headache, vomiting, rash, and joint pain (11, 12, 38, 40, 41). Bloody diarrhea is a commonly mentioned symptom of campylobacteriosis, but was reported in only 10-43% of cases (11, 12, 30, 37, 38). The true proportion of cases with bloody diarrhea may be even lower since blood in the stool has been cited as significantly associated with seeking health care (42). Overall, symptoms of campylobacteriosis are similar to other causes of gastroenteritis, thus a diagnosis based on symptoms is not possible (1, 37).

Duration of diarrhea is commonly reported in both observational studies and clinical trials, however the overall duration of illness is less frequently reported (13, 43-46). Since campylobacteriosis often involves a variety of symptoms other than diarrhea, the overall duration of illness may not be equivalent to the duration of diarrhea (11). Overall duration is a more appropriate measure of the impact of campylobacteriosis on both the individual and society (11, 44, 47).

The mean duration of illness has been reported to be 10 to 15 days with a range of 2 to 67 days (11, 13, 48). The number of days cases were unable to perform normal activities and/or attend...
work or school ranged from a median of 1 to 3 days with a range of 0 to 30 days in previous research (11, 12).

Plots of the age distribution of campylobacteriosis cases frequently show a substantial peak in children under the age of five (11, 24, 32, 49, 50). It has been suggested that this peak may be due to an increased sampling frequency in young children with gastrointestinal illness in comparison to older children and adults (49). It may also be due to risk factors that are more prevalent in young children, such as direct exposure to animals and drinking untreated water (51-55).

The monthly distribution of *Campylobacter* cases in temperate climates shows a seasonal pattern with a peak in the summer and early fall, as well as a substantial decline in cases over the winter months (5, 11, 24, 49, 56-61). The strength of this pattern has been shown to increase with increasing latitude (56).

**1.4 Immunity**

Prior exposure to *Campylobacter* may result in at least partial protective immunity (62-65). Since immunity may be strain specific, time-limited, and/or inadequate in the presence of large challenge doses (63, 64), repeated or chronic exposure to a variety of *Campylobacter* strains may be required to produce protective immunity (63, 64). In an experiment with volunteers, re-exposure with a homologous strain resulted in colonization in some cases but not illness (64). In an outbreak associated with unpasteurized milk, those with previous regular exposure to unpasteurized milk did not become ill, whereas 19 of 26 people without previous exposure developed campylobacteriosis (66). Another study in rural Wisconsin identified increased C.
jejuni seropositivity with age among rural children (67). Occupational exposure in poultry processing plants has also been associated with the development of immunity to Campylobacter (65, 68). As well, in developing countries infection and illness are quite common in young children but in older children and adults Campylobacter colonization is common but typically asymptomatic (62, 63).

1.5 Indications for treatment of campylobacteriosis in humans

Campylobacteriosis is usually a self-limiting disease and antimicrobial treatment is not recommended for uncomplicated cases (37). However, when antimicrobial treatment is indicated, macrolides and fluoroquinolones are the antimicrobials of choice (1, 37, 69-72). Antimicrobials may also be used in daycare or other group settings in order to decrease the duration of excretion and minimize person-to-person transmission, however adequate hygiene and infection control practices are the preferred control measures (44, 45, 69, 73-76).

The percentage of campylobacteriosis cases treated with antimicrobials varied widely in published studies: 16% in Norway, 33% in Denmark, 41% in Australia, 74% in Finland and 81%-83% in the United States (7, 11, 12, 38, 77, 78). However, it seems unlikely that antimicrobial treatment was indicated in many of these cases. The criteria used by physicians to determine the need for antimicrobial therapy, and which antimicrobial to prescribe, have not been reported. The most commonly used antimicrobials for treatment of campylobacteriosis are ciprofloxacin, erythromycin and to a lesser extent azithromycin and clindamycin (7, 11, 37). Erythromycin is commonly used in both adults and children and ciprofloxacin is exclusively used in adult cases (37). This is due to the association between fluoroquinolone use and
chondrotoxicity in children and adolescents (4). Historically, tetracycline was used to treat *Campylobacter* infections, however high levels of resistance have decreased its usefulness (79). For severe systemic cases, intravenous aminoglycosides are considered (37).

### 1.6 Evaluation of antimicrobial treatment efficacy

Clinical studies of the effectiveness of treatment options have often focused on mean duration of diarrhea or illness post-treatment versus placebo in study groups with approximately equal durations of illness pre-treatment (46). A meta-analysis examined 11 clinical trials that evaluated the effects of antimicrobial treatment of campylobacteriosis on duration of diarrhea and found that results from the individual studies varied, but overall, antimicrobial treatment appeared to decrease the mean duration of diarrhea by approximately 3 days when compared to a placebo using a random effects model (46). Some of the variability in effect that has been seen in these studies may be due to the use of mean duration of diarrhea as an outcome. Due to the natural short course of disease in campylobacteriosis, the reported distribution of illness has been typically right-skewed (11, 38, 73). Censored observations have also been reported (11). Therefore the assumption of normal distribution, upon which is based the comparison of mean values, has been violated. Variability in these results may also be due to co-infection with other organisms, use of hospitalized populations, insufficient sample size, and poor compliance with antimicrobial treatment protocols (43-45, 47, 80).

It has been frequently stated that antimicrobial treatment is most effective when given early in the course of campylobacteriosis (37, 46, 81). However, some of the research upon which this recommendation is based evaluated the usefulness of empirical treatment of cases of
undifferentiated diarrhea, only a proportion of which were subsequently determined to be due to *Campylobacter* (82-84). Importantly, these studies compared antimicrobial treatment to placebo when given early in the course of disease and no direct comparisons between early and late treatment were investigated (45, 82-85).

Treatment of culture-confirmed campylobacteriosis early in the course of disease is problematic however, due to the relatively long period of time from initiation of symptoms to the availability of culture results. Cases have been reported to take a median of 4 days (mean 7 days) to seek medical attention after symptoms begin, and a median of 6 days (mean 9 days) elapsed from the time symptoms began until a fecal sample was taken (11). Once fecal samples are received by the laboratory, culture of *Campylobacter* may take an additional 5 days (3, 11, 79). These factors, combined with the relatively short natural course of *Campylobacter* illness, limit the usefulness of culture and susceptibility information in guiding treatment decisions. Nevertheless, fecal culture results are essential from a surveillance perspective in order to estimate the incidence rate in the population as well as the importance of various risk factors in the epidemiology of this disease (69).

Treatment of undifferentiated gastroenteritis with antimicrobials is only recommended for international travellers (71, 72, 86). Although children are often considered a vulnerable group, antimicrobial treatment is not recommended for acute gastroenteritis in children due to the high proportion of cases of viral etiology and the potential negative consequences of antimicrobial treatment on enterohemorrhagic *E. coli* and *Salmonella* infections (69).
1.7 Symptomatic treatment

Symptomatic treatment of campylobacteriosis with rehydration solutions is recommended in affected children but is of questionable benefit in otherwise healthy adults with adequate fluid intake (81, 86). In spite of this recommendation, there is no evidence that rehydration fluids decrease the duration of illness (69, 87). Antidiarrheal drugs are recommended in cases over the age of two years that do not have bloody diarrhea (69, 86). Antidiarrheal drugs have been reported in some studies to decrease the duration of diarrhea (87-89), but in one large American study a significant increase in the duration of diarrhea was observed among cases taking an antidiarrheal (12). Persistence of symptoms other than diarrhea may prevent these medications from having an impact on the overall duration of illness. Other symptomatic medications include analgesics, which may result in a shorter duration of fever and headache but would not affect other clinical symptoms of Campylobacter infections.

1.8 Surveillance of campylobacteriosis in Canada

Although Campylobacter is the most commonly reported cause of gastroenteritis in Canada, the actual number of cases is estimated to be substantially higher due to under-reporting (26). Currently when Campylobacter is isolated from a clinical sample the laboratory is required to notify the respective health unit which reports to the provincial ministry of health (5,22,31). However, the collection and reporting of risk factor data from cases of campylobacteriosis varies substantially between health units which impacts the completeness of risk factor data at the provincial level (5,31). With the exception of Saskatchewan the isolating laboratory is not required to forward Campylobacter isolates to their provincial laboratory. As a result, the Campylobacter isolates received by provincial laboratories are primarily those requiring
additional laboratory testing to confirm genus or species (17). Therefore, although there are national data on the incidence of laboratory-confirmed campylobacteriosis, there are no comprehensive national risk factor data and a representative national collection of *Campylobacter* isolates from human cases does not exist.

2. Antimicrobial resistance in *Campylobacter* from human clinical cases

Differences in the panel of antimicrobials tested makes comparison of multi-drug resistance between studies difficult. Similarly, differences in isolation methodologies, including use of antimicrobials in selective media, pose challenges to the comparison of individual resistance results. In addition, the lack of internationally standardized breakpoints for all antimicrobials of interest for *Campylobacter*, makes comparisons problematic unless minimum inhibitory concentration (MIC) data are reported to allow the direct calculation of the proportion of resistant isolates (90-92). Standardization of all aspects of laboratory methods for *Campylobacter* would facilitate a better understanding of the global epidemiology of antimicrobial resistance in this organism.

2.1 Ciprofloxacin resistance

Ciprofloxacin is frequently recommended for the treatment of campylobacteriosis when antimicrobials are indicated. Of concern is an increase in ciprofloxacin resistance in *Campylobacter* isolated from clinical infection that has been demonstrated in many countries (37, 70, 93-95) although comparisons between countries and over time are problematic due to differences in the breakpoint used (4, 96, 97). Over a ten year period from 1992 to 2001, in a hospital-based study in Montréal, the prevalence of resistance to ciprofloxacin increased from
3.5% of isolates to 47% (98, 99). A 2001 American study found that 18% of \textit{C. jejuni} isolates were resistant to ciprofloxacin and the adjusted odds for a \textit{Campylobacter} isolate to be resistant were 2.5 times higher in 2001 than in 1997 (7). In Ontario, a study of clinical cases found no resistance to nalidixic acid in 1981; resistance to ciprofloxacin was not investigated (100). The type of population studied is likely to have affected the prevalence of resistance reported, for example, many studies only included isolates from hospital or reference laboratories (6, 90, 91, 98, 99, 101, 102). Hospitalized patients may be more likely to have been treated with antimicrobials and isolates from referral laboratories may not be representative. Regional differences in specific risk factors (e.g. antimicrobial prescribing practices, antimicrobial access, travel, water supply and prevalence of ciprofloxacin resistance in \textit{Campylobacter} on retail poultry) have been shown to impact the proportion of clinical \textit{Campylobacter} isolates resistant to ciprofloxacin, and therefore the utility of this antimicrobial for treatment (78, 91, 101). For example, in Australia where fluoroquinolones have never been approved for use in food animals, the prevalence of ciprofloxacin resistance among clinical isolates is low (2%) (77). As well, patients from developed countries who acquire campylobacteriosis internationally are more likely to have a ciprofloxacin resistant isolate (4, 13, 18, 77, 78, 94, 101, 103). It has also been demonstrated that the prevalence of ciprofloxacin resistant infections increases in the winter months, which may be related to international travel (18). However, isolates from urban cases have been associated with an increased prevalence of ciprofloxacin resistance even after accounting for foreign travel (18).

Cases with ciprofloxacin resistant \textit{Campylobacter} isolates have been reported to have longer durations of illness in several studies, but not in others (13, 77, 78, 104). Further research
including molecular sub-typing, is required to determine the factors responsible for these differing results. Ciprofloxacin resistance has also been associated with an increased risk of invasive illness or death (105).

2.2 Erythromycin resistance
In 2005, the breakpoint established by the Clinical Laboratory Standards Institute (CLSI) for erythromycin was changed from \( \geq 8 \mu/ml \) to \( \geq 32 \mu/ml \) (96, 101). Therefore, many studies that describe levels of resistance to erythromycin are based on the lower breakpoint and cannot be directly compared to more recent results. Unlike resistance to ciprofloxacin, where variation in prevalence appears to relate to antimicrobial access, this does not appear to be the case for erythromycin (90, 91, 99, 101). The prevalence of resistance to erythromycin at the current CLSI breakpoint (\( \geq 32 \mu/ml \)) was consistently low in previous studies from a variety of countries, between 0% and 5.1% (90, 91, 99, 100, 105-107). Even at the previous lower breakpoint (\( \geq 8 \mu/ml \)) the reported prevalence of resistance was less than 20% in both developed and developing countries (37). This may explain why international travel has not been identified as a risk factor for becoming ill with an erythromycin resistant *Campylobacter* isolate (13, 105). It has also been shown that cases with an erythromycin resistant isolate have a higher risk of invasive disease or death than cases with an erythromycin sensitive isolate (105).

2.3 Clindamycin and azithromycin resistance
The reported prevalence of resistance to clindamycin is usually low (0.6%-3.6%) (8, 100, 106), with the exception of a study by Guévremont et al. in Québec where 10.3% of isolates were resistant (108). Typically isolates resistant to clindamycin exhibit cross-resistance to
erythromycin (100, 109). In the limited number of reports available, the prevalence of resistance to azithromycin also appears to be quite low (0%-2%) (90, 110).

2.4 Gentamicin resistance

There was no resistance to gentamicin detected in several studies from North America and Europe (7, 10, 106). There was a low prevalence found in Finland and Sweden although the isolates from that research were primarily acquired internationally (8, 90). A low percentage of resistance to gentamicin (0.9%) was also found in domestically acquired infections in France (111).

2.5 Tetracycline resistance

Studies from several countries found that resistance to tetracycline ranged from 32.6% to 61% (7, 10, 90, 91, 98, 102, 103). This validates the removal of tetracycline from the list of the recommended antimicrobials for the treatment of Campylobacter infections.

2.6 Trimethoprim-sulfamethoxazole resistance

Trimethoprim-sulfamethoxazole has been used for the clinical treatment of laboratory-confirmed campylobacteriosis, however there has been a demonstrated lack of clinical effect (10, 83, 100, 112, 113). Campylobacter has been reported to possess intrinsic resistance to trimethoprim (100, 109). A bacterial species is intrinsically resistant to an antimicrobial when every isolate demonstrates resistance (109). Although Campylobacter may be intrinsically resistant to trimethoprim, the reported levels of resistance to trimethoprim-sulfamethoxazole do not meet the technical definition of intrinsic resistance (10, 100). In a German study where both human and chicken isolates were investigated, 47.7% of human clinical C. jejuni isolates were resistant to trimethoprim-sulfamethoxazole and 54.6% of retail chicken C. jejuni isolates were resistant (10).
However, in a hospital-based study, 96% of *Campylobacter* isolates from human cases were resistant to trimethoprim-sulfamethoxazole (106). These differences may be due to differences in methodologies since trimethoprim is often included in isolation protocols for *Campylobacter*, making the assessment of resistance levels to trimethoprim-sulfamethoxazole problematic.

3. Risk factors for human campylobacteriosis

Campylobacteriosis typically presents as sporadic infections and outbreaks are uncommon, which makes the identification of risk factors more difficult (1, 2). A large number of zoonotic reservoirs have been identified for human campylobacteriosis including poultry, ruminants, companion animals and wild birds (2, 53, 114). Exposure routes include direct contact, food, contaminated milk, and contaminated water (2, 53, 114, 115). Human to human transmission may occur, particularly in institutional or daycare settings, but does not play a significant role in the overall epidemiology of campylobacteriosis (2, 3, 75, 76). International travel is also a very commonly identified risk factor (2, 53, 116) and is likely due to increased exposure to novel strains through contaminated water or the food chain. Although studies have investigated the risk based on destination, very little work has been done to determine the specific risk factors for campylobacteriosis acquired while in a foreign country (57, 117).

3.1 Chicken

Estimates of the proportion of human campylobacteriosis cases that can be attributed to the consumption of chicken range from 20 to 80 percent (114, 118, 119). Case-control studies have consistently identified chicken handling and consumption as major risk factors for campylobacteriosis (2, 31, 48, 53, 116, 120-123). A recent meta-analysis of thirty-eight case-
control studies identified consumption of chicken prepared outside of the home and consumption of undercooked chicken as important risk factors (53). Other chicken-related risk factors that have been associated with campylobacteriosis include involvement in poultry husbandry and contact with/ preparation of raw chicken meat (2, 51, 115, 122, 124, 125). Lower risk has been associated with consumption of frozen chicken versus fresh chicken (124). *Campylobacter* does not tolerate cold temperatures well and frozen chicken has been shown to have significantly less *Campylobacter* contamination than fresh chicken (124). Unfortunately, most case-control studies did not differentiate between the consumption of previously frozen or fresh chicken. The variety of risk factors associated with chicken combined with the overall high rates of chicken handling and consumption in the population, make it difficult to assess the importance of specific risk factors for campylobacteriosis from chicken through the food pathway (48, 122). However overall, exposure to chicken through the food chain appears to be more important than direct exposure to live birds (53).

The importance of chicken handling and consumption in the epidemiology of human campylobacteriosis was demonstrated in 1999 when chicken and eggs were removed from the market in Belgium due to dioxin-contaminated feed. A mathematical model based on national surveillance data collected in prior years showed that the withdrawal of these products resulted in 40% fewer cases of campylobacteriosis than expected. The number of cases returned to the expected level when chicken sales resumed (126).
3.2 Ruminants

Previous research has shown that ruminants may also be a significant source of *Campylobacter* in human infection, particularly for young, rural children (20, 119, 127). Direct exposure and/or exposure through fecal-contaminated water are likely pathways (19, 20, 116, 127, 128). Cattle are commonly asymptomatic carriers of *C. jejuni* and some individuals can shed large numbers of the organism (129). This can result in contamination of the local environment as well as contamination of surface water through pasture run-off (128, 130). *Campylobacter* has also been found to survive for extended periods in manure storage (129). Unpasteurised milk is another route for human infection from ruminant sources. Although often associated with outbreaks of campylobacteriosis, unpasteurized milk consumption is also a frequent risk factor for sporadic infections (2, 129, 131). There are very few reports of beef consumption as a risk factor which is likely due to the low prevalence of *Campylobacter* on retail beef (111, 114, 132).

3.3 Flies, rodents and wild birds

Flies may play a role in the epidemiology of *Campylobacter* in broiler flocks and dairy herds and have been suggested as a mechanical vector for human infection, similar to their role in the transmission of shigellosis (133-135). Further investigation of the role flies play in human campylobacteriosis is warranted but difficult to conduct. Rodents and wild birds have been shown to be asymptomatic carriers of *Campylobacter* and have been suggested as potential reservoirs for dairy cattle and broiler chickens through fecal contamination of water and/or feed (134, 136).
3.4 Direct contact

Direct contact with animals including cats and dogs, in particular kittens and puppies, as well as cattle, chickens and pigs is a frequently identified risk factor for campylobacteriosis (2, 51, 52, 54, 116, 120, 121). Considering that asymptomatic carriage is very common in these animal species, risk through direct exposure is not surprising.

3.5 Water

Untreated water has been identified as an important source of *Campylobacter* infections in humans (2, 53, 128). The presence of *Campylobacter* in surface water and shallow wells is likely the result of contamination by wild bird feces, manure run-off from dairy or poultry farms, or human sewage (137, 138).

3.6 Other factors

Workers in poultry slaughter and processing plants are at higher risk for campylobacteriosis, particularly in the first few weeks of employment (68). This is likely due to the aerosolisation of *Campylobacter* from the chickens during processing, with inhalation by exposed workers and subsequent ingestion (65). Although this is a significant risk factor within poultry workers, due to the small percentage of the population with this occupation, the impact on the overall population is quite small.

Less commonly reported risk factors for campylobacteriosis include: eating at a restaurant, consumption of various foods (raw seafood, milk from bird-pecked bottles, pork, barbequed meat, organ meats), antimicrobial use in the 28 days prior to illness, and taking antacid drugs (2, 54, 116, 120, 121). Additional risk factors specifically associated with children include:
consumption of fruit or vegetables at home and a person in the household with gastroenteritis (51, 139).

Some variation among infecting *Campylobacter* species in risk factors has been observed, with non-poultry risk factors predominating in cases of infection with *C. coli* (54).

### 3.7 Urbanization

Studies in Scotland, Denmark, the Netherlands and Canada have identified differences in the incidence rate of campylobacteriosis in urban versus rural populations (19, 20, 54, 124, 127, 140). In the Netherlands there was a significantly higher incidence rate of campylobacteriosis in urban populations whereas in Denmark and Canada there was a higher incidence rate in rural populations (19, 54, 124, 140). The Scottish study found a significantly higher incidence rate in rural populations but only for children (20). The reasons for these differences require further investigation. Several studies have shown that urban cases are more likely to have travelled internationally than rural cases (54). However, other differences among these groups with respect to exposure to specific risk factors may also play a role. For example, it would be expected that rural cases would have more likelihood of exposure through unpasteurized milk, untreated water, and direct farm animal contact, particularly ruminants. It has also been demonstrated that young children comprise a greater proportion of cases in rural populations (19, 20, 140) and may have different risk factors for campylobacteriosis (51, 52, 139).

Several studies have used multilocus sequence typing (MLST) to demonstrate that strains of *Campylobacter* isolated from urban cases are more closely related to chicken strains than ruminant strains, whereas rural cases are more closely related to ruminant strains (16, 20, 127).
In addition, New Zealand implemented a Code of Practice for poultry processors, as well as broiler carcass performance targets and found that these interventions had a larger impact on incidence rates of campylobacteriosis in urban areas than in rural ones (16).

4. Campylobacter in retail chicken

4.1 Prevalence

In poultry, Campylobacter is a commensal organism, seldom causing disease (33) and Campylobacter jejuni is the most common species isolated from poultry (108, 141-150). The reported prevalence of Campylobacter on retail chicken in recent studies ranged from 49.5% to 93.2% (144-146, 151-158). This large range may reflect true differences in prevalence among regions or countries, but may also be due to differences in isolation methodologies, the use of selective media, season of sampling, geographical scope of project, type and size of meat sample, fresh versus previously frozen chicken sampled, and utilization of a sampling frame vs. convenience or other biased sampling. Similar factors may also play a role in the proportion of each Campylobacter species that is recovered (101, 108, 141, 144, 147-152, 159-161).

Antimicrobial resistance profiles can differ substantially between the various Campylobacter species, therefore the proportion of each species in the pool of isolates tested can impact the overall results (162, 163). Consequently, standardization of methodologies for sampling, isolation, and antimicrobial susceptibility testing would facilitate valid comparisons of results and a better understanding of the epidemiology of Campylobacter on retail chicken.
4.2 Ciprofloxacin resistance

Resistance to fluoroquinolones occurs rapidly in *Campylobacter* in the presence of selection pressure (4, 92) where, in contrast to some other foodborne pathogens, a single mutation in the *gyrA* gene can result in a high level of resistance (4, 92). Since fluoroquinolones are utilized in the treatment of severe campylobacteriosis in adults, the emergence of fluoroquinolone resistance in isolates from retail chicken is of concern for public health (2). Cross-resistance between nalidixic acid and ciprofloxacin is a common finding in *Campylobacter*. The proportion of isolates resistant to nalidixic acid and ciprofloxacin can vary widely between countries and regions with less than 25% reported in Europe and the United States and 50-92% reported in Asia (101, 132, 146, 147, 153, 154, 158, 160, 163). In Canadian studies, less than 6% of *C. jejuni* isolates from retail chicken were resistant to ciprofloxacin (101, 132). The observed differences between countries may be due in part to differences in antimicrobial usage within their respective broiler chicken industries. In developing countries, antimicrobial usage in the agriculture sector is largely unregulated (164). In Australia, fluoroquinolones are prohibited in all food-producing animals and no resistance to ciprofloxacin has been found in *Campylobacter* isolated from chicken (165). Fluoroquinolones were approved for use in broiler chickens in the United States in 1995 and that approval was withdrawn in 2005 due to concerns regarding antimicrobial resistance and public health (166). Fluoroquinolones have not been approved for use in broiler chickens in Canada, although they may be used in an extra-label manner (96).

4.3 Erythromycin resistance

As with isolates from human infection, many older studies that investigated erythromycin resistance in retail chicken cannot be compared to more recent results due to the change in the
breakpoint established by the CLSI for erythromycin in 2005. Recent studies in Europe and North America report less than 4% of *Campylobacter* isolates from retail chicken were resistant to erythromycin (96, 146, 153, 158, 160). Macrolides were withdrawn for use as growth promoters in Europe in 2000 but are still widely used in North America (167). This difference in use practices does not yet appear to be reflected in the prevalence of resistance to erythromycin. A much higher prevalence of resistance to erythromycin (19.4%) was reported in Korea (153). However, the proportion of *C. coli* isolated in this study was quite high (42.8%) and *C. coli* is reported to have a higher prevalence of resistance to erythromycin than *C. jejuni* (37, 96, 153, 158, 163).

**4.4 Tetracycline resistance**

Prevalence of resistance to tetracycline also varies widely, for example only 7.6% of isolates were resistant in Denmark, in contrast to 47.2% in the UK, and 87.2% in Korea (146, 153, 160). Canadian and American studies found approximately half of *Campylobacter* isolates from chicken were resistant to tetracycline (96, 101, 148, 158). The wild type distributions reported by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) showed no resistance to tetracycline for either *C. jejuni* or *C. coli* (162). Resistance to tetracycline is commonly seen in both pathogenic and commensal bacteria isolated from food animals and their products (96, 158). In North America, this is likely due at least in part to the extensive history of tetracycline use in food animal production.
4.5 Multi-resistance

Comparisons between studies for multi-resistance frequencies are problematic due to differences in the antimicrobial resistance panels utilized. In four studies reporting multi-resistance data for *Campylobacter* from broiler chickens, the number of antimicrobials on the panels ranged from 3 to 10 (101, 146, 148, 153), and ciprofloxacin, erythromycin, and tetracycline were found on all panels. From a public health perspective, multi-resistance to ciprofloxacin and erythromycin is of the most interest because these drugs are commonly used for therapy of campylobacteriosis. Andersen and collaborators in Denmark found that 0.4% (2/767) of isolates of *C. jejuni* were resistant to both ciprofloxacin and erythromycin (160). Levesque and co-workers in Québec, Canada found 1.8% (1/56) of *C. jejuni* isolates from chicken were resistant to these two antimicrobials (101). Similarly, the Canadian Integrated Program for Antimicrobial Resistance Surveillance program (CIPARS) found 1 isolate resistant to both ciprofloxacin and erythromycin in each of 2003, 2004, and 2008 from 179, 316, and 281 isolates, respectively (96), but none was found in 2005-2007 (96). These low frequencies of resistance to ciprofloxacin and erythromycin are encouraging but ongoing monitoring is necessary to detect changes over time.

5. Molecular sub-typing

Molecular sub-typing methods may be useful in elucidating the role of animals and other potential reservoirs as sources of *Campylobacter* infection of humans. However, the development of useful molecular sub-typing techniques for *Campylobacter* has been complicated by its high genetic diversity and the strong tendency of *Campylobacter* towards recombination and mutation (168-170). The most commonly used methods include PFGE, ribotyping, amplified fragment length polymorphism (AFLP), multilocus sequence typing (MLST), and
those based on the flagellin genes: *fla* typing, *fla*-restriction length polymorphism (*fla*-RFLP), and *fla*-short variable region sequencing (*fla*-SVR). These methods have provided useful information, but to date have been applied in only a limited way to large isolate collections due to cost, the level of expertise required, complexity, low throughput, and/or lack of discriminatory ability (78, 169, 171). Standardization of methodology within a sub-typing method is essential for the comparison of results between different laboratories and research studies but has not been achieved for most methods (171). MLST methodology has been standardized and is conducive to data sharing and data comparisons. This has led, in part, to the adoption of MLST as the standard for molecular sub-typing of *Campylobacter* (172). A study in Québec used MLST to demonstrate that *Campylobacter* isolates from humans and chicken, raw milk and water samples were genetically linked (173). Among a collection of isolates (primarily from human and chicken sources), approximately 25% were contained within 5 clonal complexes (173). In the United Kingdom, Wilson and colleagues used MLST to model the probable source of human clinical isolates, and estimated that more than half of them had sequence types attributable to chicken while approximately 35% were attributed to cattle (138). Similarly, a study in New Zealand using MLST estimated that 80% of human cases were associated with chicken genotypes (114). The limitations of MLST are its cost and labour-intensiveness as well as the tendency for a large proportion of isolates to be grouped into a small number of genotypes (168-170, 173).

The recently developed and validated Comparative Genomic Fingerprinting (CGF40) method is rapid, low cost and highly discriminatory (168). This assay is based on the presence/absence of 40 marker genes that are assessed with eight multiplex PCRs that target five loci each. The PCR
results are converted to binary values. These are used to generate a CGF40 fingerprint based on the simple matching distance coefficient and unweighted-pair group method using average linkages (UPGMA) of clustering (168). The CGF40 types are then identified at the 90%, 95%, and 100% fingerprint similarity levels. When isolates were tested with both methods, CGF40 had a higher discriminatory ability than MLST (168). When duplicate isolates were tested, 1152/1160 loci matched, resulting in a reproducibility estimate of 99.3% (Steven Mutschall – personal communication, 2012). Unlike other methods (173) there is a concordance between CGF40 and MLST in the clusters identified (168), which is an advantage as it allows comparisons with previous and ongoing work. These qualities suggest that CGF40 may be increasingly adopted, particularly for source attribution studies and surveillance programs.

Research Objectives

Although campylobacteriosis is the most common cause of bacterial gastroenteritis in developed countries including Canada, there is still a lack of information regarding key aspects of the epidemiology of this disease. Components of the burden of illness in human cases including symptoms, severity of illness and duration of illness are not well understood. Various factors including antimicrobial use in cases and antimicrobial resistance in isolates may impact the duration of illness, however very little is reported on these factors outside of clinical trials. This is due in part to a lack of data from observational studies. Currently, culture-confirmed campylobacteriosis is reportable in Canada but there is no requirement for isolates to be provided to government laboratories at the federal or provincial level with the exception of Saskatchewan. Therefore, antimicrobial resistance information from representative human cases is scant. Previous investigations in other countries have evaluated the factors affecting the duration of
illness with the mean duration of diarrhea/illness as the outcome (46). The right-skewed distribution of duration of illness in campylobacteriosis and the issue of censored observations may impact these results. Survival analysis is a non-parametric method better suited to investigating factors associated with duration of illness and has not previously been used in this context.

Although several risk factors for campylobacteriosis have been identified including; consumption of contaminated water, contact with ruminants, and international travel, the transmission of *Campylobacter* from chicken through the food chain appears to be a primary risk factor in sporadic infections. However the role of retail chicken in human cases of susceptible and resistant campylobacteriosis in Canada is unknown.

In order to better understand campylobacteriosis in the Canadian population, as well as investigate the relationship between human illness and retail chicken, a population-based study was conducted in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario. The objectives of this research were to investigate:

1. the clinical signs, symptoms and severity of clinical cases of campylobacteriosis
2. antimicrobial use and antimicrobial resistance associated with clinical cases of campylobacteriosis
3. the factors associated with duration of illness in clinical cases of campylobacteriosis using survival analysis
4. the prevalence of *Campylobacter* contaminated retail chicken sold in these regions
5. antimicrobial resistance among *Campylobacter* isolates from retail chicken in these health units

6. the association between isolates from human sporadic cases of campylobacteriosis and *Campylobacter* isolates from retail chicken based on CGF molecular sub-typing.
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Chapter 2: Antimicrobial resistance and antimicrobial use associated with laboratory–confirmed cases of Campylobacter in two health units in Ontario

Abstract

A population based study was conducted over two years in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario, to document antimicrobial resistance and antimicrobial use associated with clinical cases of laboratory-confirmed campylobacteriosis. The Etest® was used to determine the minimum inhibitory concentration (MIC) of amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), and tetracycline (TCY) in Campylobacter isolates from human cases. Data on antimicrobial use were collected from 250 cases.

One hundred and sixty-five cases (65.74%) reported staying home or being hospitalized due to their campylobacteriosis. Fifty-four percent of cases (135/249) including 62% (26/42) of children in this study reported taking antimicrobials for their campylobacteriosis. In 115 cases (51.1%), fecal culture results were not utilized for treatment decisions because they were not available prior to the initiation of antimicrobial treatment and/or they were not available prior to the cessation of symptoms. There were 124/250 cases (49.6%) with available Campylobacter isolates, and sixty-six isolates (53.2%) were resistant to at least one of the antimicrobials tested. No resistance to AMC, CHL, or GEN was found in these isolates. Six isolates (4.8%) were resistant to CIP. Two isolates (1.6%) were resistant to ERY, however no isolates were resistant to CIP and ERY.
Since antimicrobials are not indicated for undifferentiated gastroenteritis or campylobacteriosis in most cases including children, the high proportion of cases in this study that were treated with antimicrobials for their illness is concerning. Prudent use practices should be promoted among physicians in order to reduce the use of antimicrobials in the treatment of gastroenteritis in general and Campylobacter in particular, as well as to minimize the future development of resistance to these antimicrobials in Campylobacter.

Key Words: Campylobacter, Ontario, Canada, antimicrobial use, antimicrobial resistance
Introduction

*Campylobacter* is an important enteric pathogen of humans which can cause severe, bloody diarrhea, fever, and abdominal pain (1, 2). In Canada, the National Notifiable Disease Program reported 31.8 isolations of *Campylobacter* per 100,000 people in the province of Ontario and 30.2 per 100,000 nationally in 2004, which is the most recent year for which results are available (3). There were 3,945 campylobacteriosis cases reported in Ontario and 9,345 in Canada that year (3). In 2010, the FoodNet Surveillance system in the United States reported 6,033 cases of campylobacteriosis and a rate of 13.5 per 100,000 people across their sites (4).

Since gastrointestinal illness is significantly under-reported, the number of cases is likely substantially higher (5, 6). In Canada, it was estimated that for every reported case of campylobacteriosis there are 23 to 49 cases that occur in the population resulting in an estimate of 8.9 to 18.8 cases per 100,000 people in 2001 (7).

The case fatality rate for *Campylobacter* was reported as one in one thousand in the United States in 1999 (7). It has been estimated that 9-14% of cases develop post-infectious irritable bowel syndrome (8, 9) and approximately one in one thousand cases progress to Guillain-Barré syndrome (10).

Campylobacteriosis is usually a self-limiting infection and treatment is recommended only in vulnerable populations with severe or invasive disease (13, 14). However observational studies have shown that a substantial percentage of patients take antimicrobials for their campylobacteriosis (11-14). There is currently no information on the types and prevalence of
antimicrobial treatment for campylobacteriosis in Canada. Erythromycin is commonly used in both adults and children and fluoroquinolones, such as ciprofloxacin, are used in adult cases (15). There is also evidence that antimicrobial resistance in _Campylobacter_ may be associated with increased virulence and adverse outcomes in human cases (16) as well as prolonged duration of diarrhea (13, 17, 18). Therefore, resistance in _Campylobacter_ organisms to antimicrobials in general, and these clinically important antimicrobials in particular, is of concern.

A population based study was conducted over a two year period in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario to document antimicrobial resistance and antimicrobial use associated with clinical cases of campylobacteriosis.

Methods

This research project was approved by the University of Guelph Research Ethics Board. Agreements with hospital and private laboratories were established so that isolates obtained from clients living in these health unit areas were forwarded for further characterization to the Central Public Health Laboratory (CPHL), Ontario Ministry of Health and Long-term Care (now Public Health Ontario Laboratory). These agreements ensured the confidentiality of patient information. Case physicians were not provided with the results of the further characterization conducted for this research. The testing delays inherent in a research project precluded the results from being available in a clinically relevant time-frame.
Cases

Laboratory-confirmed cases of *Campylobacter* occurring between February 2002 and February 2004 and residing in the PD and WDG health unit areas were eligible for inclusion in the study. Cases were excluded if they could not speak English or did not have a home telephone. Cases were informed of the purpose of the study and provided informed consent for participation at the time of questionnaire administration. Questionnaires were administered by health unit personnel via telephone.

Study area

The average population of the PD and WDG health units during the time period of the study was 77,188 and 252,844, respectively (Provincial Health Planning Database, Ontario Ministry of Health and Long Term Care). Both health unit areas have a combination of urban and rural populations.

Isolation

*Campylobacter* strains were confirmed and speciated at the Central Public Health Laboratory by: Gram stain, oxidase test, hippurate hydrolysis, susceptibility to nalidixic acid (30 ug) and cephalothin (30 ug), microaerophilic growth at 25, 36, and 42 degrees Celcius, aerobic growth at 25 degrees Celcius, catalase production, and indoxyl acetate hydrolysis. Supplementary tests included: urea hydrolysis, nitrate reduction, H$_2$S production, and growth on 1% glycine, and on MacConkey agar.
**Minimum inhibitory concentrations**

The Etest® was used for the determination of the minimum inhibitory concentration (MIC) of amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), and tetracycline (TCY) in the isolates. Sub-cultures were grown on a Mueller-Hinton blood agar plate for 24 h under micro-aerobic conditions at 42ºC prior to determining the MICs using Mueller-Hinton agar with 5% laked horse blood and micro-aerobic conditions with incubation for 48 h at 37ºC. Control strains were *C. jejuni* ATCCC 33560, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 29213. Breakpoints from Clinical Laboratory Standards Institute (CLSI) and CIPARS were used in this study (19). The breakpoints utilized were AMC, ≥ 32 μg/ml; AMP, ≥ 32 μg/ml; CHL, ≥ 32 μg/ml; CIP, ≥ 4 μg/ml; CLI, ≥ 4 μg/ml; ERY, ≥ 32 μg/ml; GEN, ≥ 16 μg/ml; NAL, ≥ 32 μg/ml; and TCY, ≥ 16 μg/ml. Isolates were considered resistant when the MIC was greater than or equal to the breakpoint. The concentration of each antimicrobial that inhibited 50% (MIC50) and 90% (MIC90) of isolates was calculated.

**Questionnaire data**

The dates of fecal collection and availability of culture results to the physician were reported to the health unit by the primary laboratory. Information on the use of antimicrobials during the course of *Campylobacter* infection was collected as part of a comprehensive case questionnaire that also elicited information on gender, age, and severity of illness. Severity of illness was reported by cases based on the following definitions: Quite mild - feeling slightly unwell but able to do all normal activities; Fairly mild - feeling quite unwell but able to do most normal
activities; Moderate - having to stay at home but able to get out of bed for limited activities; Fairly severe - confined to bed at home and unable to do any normal activities; Quite severe - hospitalized. Age was categorized based on the age distribution of *Campylobacter* cases in the WDG health unit from 1991-2000 into < 5 years, 5 years to less than 17 years, 17 years to less than 41 years, 41 years to less than 61 years, and greater than or equal to 61 years (Figure 2.1).

**Data analysis**

Test results and antimicrobial use information were analyzed using Stata Intercooled version 11 (Stata Corporation, College Station, Texas, USA). Dichotomous variables were analyzed using the Chi-square and Fisher’s exact tests as appropriate at p<0.05. Differences between groups of continuous variables were evaluated with the two-tailed Student’s t-test at p<0.05.

**Results**

There were 317 laboratory-confirmed cases during the study period with 49.4 cases/100,000 in 2002 and 46.7 cases/100,000 in 2003. There was a marked difference in this rate between the two health units with 84.5 and 63.3 cases/100,000 in PD in 2002 and 2003 respectively in comparison to 38.6 cases/100,000 and 41.7 cases/100,000 in WDG (p<0.05). Seventy-nine percent of cases (n=252) were successfully contacted and agreed to participate. Two cases were excluded: one due to an inability to communicate in English and one that did not reside in the study area. Therefore, questionnaire data were collected from 250 cases, 51 from PD and 199 from WDG.
The ages of cases ranged from 4 months to 85 years with a median of 27.4 years. The age of cases was not significantly different between health units (p>0.05). There were 140 (56.2%) male cases and 109 (43.8%) female cases, resulting in a male:female ratio of 1.3:1. In both 2002 and 2003, more than half of all cases reported that their symptoms began from June to September (2002: 72/119 [61%], 2003: 68/122 [56%]. The months with the highest number of cases in both years were July (2002: 27, 2003: 18) and September (2002: 21, 2003: 18). Forty-eight per cent of cases (114/237) reported blood in the stool but the proportion of cases with blood in the stool was significantly higher for those under five years of age (p=0.001). Fever was reported in seventy-three percent (180/246) of cases. Eighty-four cases (34%) reported Quite Mild or Fairly Mild symptoms. One hundred and sixty-five cases (66%) reported staying home or being hospitalized due to their campylobacteriosis. The overall mean number of days that cases reported having symptoms was 10 (median 8, sd=8.6).

Fifty-two percent of cases (130/249) reported taking antimicrobials for their campylobacteriosis. Cases treated with antimicrobials did not differ from those that were not treated with antimicrobials with regards to age category, severity of illness, chronic medical condition or history of recent international travel (p> 0.05) (Figure 2.2). Cases reporting blood in the stool were not more likely to be treated with antimicrobials (p=0.30) but cases reporting fever were more likely to be treated (p=0.004). The mean number of days from the beginning of symptoms to the collection of the fecal sample was 6.01 (median=4, sd=9.00), to the availability of the results of the fecal culture was 12.26 (median=10, sd=10.35), and to the beginning of antimicrobial treatment was 9.81 (median=8, sd=10.08). The mean number of days from
collection of the fecal sample until the results were available was 6.33 days (median=5, sd=4.35).

Of the 227 cases for whom there were known dates when symptoms ended and fecal culture results were received at the health unit, 43 had culture results available on the day symptoms ended (18.9%) and 136 (59.9%) did not have culture results until after symptoms had ended. Therefore, in 179 cases (78.9%) fecal culture results were not available for use in making treatment decisions. Thirty-two cases (24.4%) began taking antimicrobials after their symptoms had ended.

Of the 96 cases for whom the date of stool collection was available, and who took antimicrobials prior to their symptoms ending, 11 (11/96, 11.5%) began antimicrobial treatment prior to the collection of the fecal sample and twenty (20/96, 20.8%) began antimicrobial treatment on the same day that the fecal sample was collected. Overall, seventy-seven cases (77/99, 77.8%) began antimicrobial treatment prior to the results of the fecal sample becoming available.

Antimicrobials taken for campylobacteriosis by 138 cases included: amoxicillin (2), cephalexin (1), ciprofloxacin (45), azithromycin (32), clarithromycin (11), erythromycin (32), metronidazole (7), sulfonamides (5), doxycycline (1), and tetracycline (2) (Figure 2.3). Eight cases reported treatment with more than one antimicrobial. Of cases which began antimicrobial treatment after fecal culture results were available, 7 were treated with ciprofloxacin, 3 with erythromycin, 1 with trimethoprim-sulfamethoxazole, 1 with azithromycin and 1 with clarithromycin.

*Campylobacter* isolates from 124/250 cases (49.6%) were received by the CPHL and therefore had antimicrobial susceptibility information. Of these, 121 (97.6%) were *C. jejuni* and 3 (2.4%)...
C. coli. Isolates from the remaining cases were not available from the primary laboratory or were unable to be matched with case information. There were no significant differences in age category, gender or severity of illness between cases with and without accompanying isolates (p>0.05). There were also no significant differences between health units in the proportion of cases with accompanying isolates (p>0.05). Among cases with isolate susceptibility results that were treated with ciprofloxacin, erythromycin, clarithromycin, and/or azithromycin, only 5.3% (3/57) were resistant to the antimicrobial class utilized.

Sixty-six isolates (53.2%) were resistant to at least one of the nine antimicrobials tested, including all 3 C. coli. Six isolates (4.8%, 5 C. jejuni, 1 C.coli) were resistant to CIP (Table 2.1) and all isolates resistant to NAL were also resistant to CIP. Two isolates (1.6%, 1 C. jejuni, 1 C.coli) were resistant to ERY, however no isolates were resistant to CIP and ERY (Table 2.2). The MIC50 and MIC90 for both CIP and ERY was well below the breakpoint (Table 2.1)

The three cases with a Campylobacter coli isolate did not appear to be substantially different than the cases with a Campylobacter jejuni isolate with respect to the parameters described in this research. All three cases were adult males, 1 from WDG and 2 from PD. Two of these cases had blood in the stool and 1 reported fever. One of these cases had travelled internationally. Severity was evenly distributed among the Fairly Mild, Moderate, and Fairly Severe categories. Two of the cases used ciprofloxacin for their illness and the use began prior to the availability of fecal culture results. The median duration of illness was 4 days (range 4-7) for these three cases.
Discussion

Campylobacteriosis is usually a self-limiting disease and antimicrobial treatment is not recommended in uncomplicated cases, however macrolides and fluoroquinolones are the primary antimicrobials utilized for the treatment of campylobacteriosis in vulnerable populations (13, 14, 23-25). Antimicrobials may also be used in conjunction with infection control practices in daycare or other group settings in order to decrease the duration of excretion and minimize person-to-person transmission, however adequate hygiene and infection control practices are the preferred control measures (20-25).

Although there are limited data available, the percentage of campylobacteriosis cases treated with antimicrobials varied in published studies from 16% in Norway, 52% in this study, 74% in Finland and 81%-83% in the United States (15-18).

The number of days in this study from the initiation of symptoms to antimicrobial treatment (mean 9.8, median 8) were slightly shorter than in a Norwegian study (mean 11.3, median 10) (11). As well, the number of days from the beginning of symptoms to the submission of a fecal sample (mean 6.0, median 4) were somewhat lower than in previous work (mean 9.0, median 6) (11) but similar to the time period between the beginning of symptoms and seeking medical attention in other studies (21-23).

In the majority of cases, fecal culture results were not utilized for treatment decisions because they were not available prior to the initiation of antimicrobial treatment and/or they were not
available prior to the cessation of symptoms. For seventy-eight percent of cases (78.9%) fecal culture results were not available while they were symptomatic. This is due in part to the delay in seeking medical treatment, the relatively short duration of symptoms in the typical patient (mean: 10.0 days) and the fastidious nature of *Campylobacter* which can require up to 5 days to culture (2, 11, 26). Therefore, the primary value of fecal culture in clinical campylobacteriosis may be to properly manage complicated or prolonged cases by adjusting therapy based on susceptibility results as well as to meet public health needs (27). Information on the number, demographics, and risk factors for cases is essential in ongoing public health activities (27).

Ontario has a universal health care system, therefore access to physicians and submission of stool samples should not be influenced by willingness or ability to pay for these services. However the cost of antimicrobials is not universally covered and therefore the utilization of antimicrobials may be affected by cost. Since antimicrobials for oral therapy of humans are not available over-the-counter in Canada and all cases in this study accessed the healthcare system, it is assumed that the antimicrobials taken were prescribed for this illness by a physician. Further research is required to determine the factors utilized by physicians when determining whether to prescribe antimicrobials for acute onset diarrhea.

Only 22.2% of cases receiving antimicrobials in this study began treatment after fecal results were available. Although ten different antimicrobials were utilized in this study, those used after fecal results were available were primarily CIP, and ERY, which is similar to previous studies (15, 16). It should be noted that antimicrobials are not indicated for acute undifferentiated diarrhea in children due to the high proportion of cases with a viral etiology and the self-limiting
nature of bacterial gastroenteritis in children (28). However, in this study, 26/42 (61.9%) cases less than 5 years old were treated with antimicrobials. The 3 cases in this age group with reported daycare exposure were all treated with antimicrobials. With regards to severity of illness, none of the cases under the age of 5 were hospitalized or in the Fairly Severe category, however 46% were in the Quite Mild category. This is similar to the results from cases overall where those treated with antimicrobials did not differ from those that were not treated with antimicrobials with regards to severity of illness. However, cases in this dataset may be biased towards an increased level of severity due to the requirement for submission of a fecal sample. Empiric treatment of gastroenteritis in adults is only recommended in specific populations including international travellers (29, 30), yet there was no significant difference in the proportion treated in cases with and without histories of international travel (p>0.05). In the case of fluoroquinolones, this is of concern since their use is a risk factor for fluoroquinolone resistant Clostridium difficile associated diarrhea (CDAD) (31). Clostridium difficile associated diarrhea is an increasing public health concern in both hospital and community settings increasing the importance of the judicious use of fluoroquinolones (34). Amoxicillin, cephalexin, and metronidazole were utilized for treatment in 10 cases in this study although these drugs are not indicated for undifferentiated gastroenteritis (29, 30). Although metronidazole is recommended for the treatment of Giardia, one of the distinguishing clinical features of Giardia is persistent diarrhea (>7 days) however 6/7 patients treated with metronidazole initiated treatment at ≤7 days. The use of trimethoprim-sulfamethoxazole (SXT) for the clinical treatment of laboratory-confirmed campylobacteriosis was unexpected due to the high proportion of Campylobacter isolates with resistance to SXT and the demonstrated lack of clinical effect (35-38).
Trimethoprim-sulfamethoxazole is recommended for the treatment of other enteric pathogens but not *Campylobacter* or undifferentiated diarrhea in international travellers (14, 24, 35, 39).

The prevalence of ciprofloxacin and nalidixic acid resistance in the isolates from this study was less than 5%, which is consistent with older research but lower than other more recent Canadian and international data (16, 21, 32, 38, 40-44). In a 1992-1993 hospital-based Montreal study there were only 3.5% of isolates resistant to ciprofloxacin but the prevalence of resistance had increased to 12.7% by 1997 (32). An American study found that in 2001 18% of *C. jejuni* isolates were resistant and the adjusted odds for a *Campylobacter* isolate to be resistant to ciprofloxacin were 2.5 times higher than in 1997 (12). Similar increases in *Campylobacter* resistance to fluoroquinolones have been seen in many other countries (33, 34). The only previous study utilizing clinical cases from Ontario did not evaluate resistance to ciprofloxacin but found no resistance to nalidixic acid almost 20 years prior to the current study (35). The relatively low prevalence of resistance to ciprofloxacin in this study may be due to regional differences in specific risk factors including; antimicrobial prescribing practices, antimicrobial access, travel, water supply and prevalence of ciprofloxacin resistance in *Campylobacter* on retail poultry (21, 40, 42). Case-control studies have consistently identified chicken consumption in particular as a major risk factor for campylobacteriosis (33, 36-40). Additional research has estimated that the proportion of human campylobacteriosis cases that can be attributed to the consumption of chicken ranges from 20 to 80 percent (41-43). Therefore ciprofloxacin resistance in *Campylobacter* isolated from retail chicken may play an important role in ciprofloxacin resistance in human campylobacteriosis. Ciprofloxacin resistance in retail chicken sampled as an additional component to the current study was also quite low at 1.9%.
Antimicrobial use practices in poultry production in Canada are unknown but may impact antimicrobial resistance in *Campylobacter* from retail chicken (45). These results suggest that fluoroquinolone use in domestic poultry production during the time period of this study may have been uncommon.

Differences in the population studied may also play a role in the observed disparity in resistance since many studies only included isolates from hospital or referral laboratories (40-44, 46). Hospitalized patients are more likely to have been treated with antimicrobials including fluoroquinolones and isolates from referral laboratories may not be representative.

The breakpoint established by the CLSI for erythromycin was changed from $\geq 8 \, \mu g/ml$ to $\geq 32 \, \mu g/ml$ in 2005. Although the data collection and laboratory analysis for this study were conducted prior to 2005, the data was analyzed using the current breakpoint. Comparisons with many relevant studies are not possible since the prevalence of resistance to erythromycin was based on the lower breakpoint. In this study, there were 2 isolates (1.6%) that were resistant to erythromycin and both had an MIC of $> 256$. Therefore these isolates would be categorized as resistant at both the $\geq 8 \, \mu g/ml$ and $\geq 32 \, \mu g/ml$ breakpoints. Although differences in antimicrobial access may play a role in the high level of resistance to ciprofloxacin in human isolates from some countries, this does not appear to result in high levels of resistance to erythromycin (40-42, 46). The prevalence of resistance to erythromycin at the current breakpoint was between 0% and 2.9% in other studies (37, 40-42, 46-48). The prevalence of resistance to clindamycin is also low, both in this study and in other work with the exception of the Guévremont study in Québec where 10.3% of isolates were resistant to clindamycin (12, 35,
The two isolates in this study that were resistant to erythromycin with an MIC of >256 were also resistant to clindamycin, which is consistent with previous Canadian work (35).

Differences in the panel of antimicrobials tested makes comparisons of multi-drug resistance difficult between studies and differences in isolation methodologies make comparisons of individual resistance results difficult as well. In addition, the lack of standardized breakpoints utilized for *Campylobacter* makes comparisons problematic unless MIC data are provided in a way that allows the re-calculation of the proportion of resistant isolates (40, 46).

The incidence rate of campylobacteriosis per 100,000 in WDG was similar to that found in neighbouring Waterloo region (49.69/ 100,000) (49), in the province of Ontario (42.3/100,000 1997-2001) (50) and nationally (34.9 cases/ 100,000 2001-2004) (51). The incidence rate in the PD health unit was substantially higher than in WDG which was expected due to a historically high incidence rate in this health unit. This may be related to differences in exposure to risk factors. This may include the presence of a chicken abattoir as a major employer in this region as *Campylobacter* has been associated with occupational exposure to chicken processing (52). As well, there may be an increased proportion of rural properties with chickens and exposure to raw milk due to the presence of a farm-based religious community in the PD health unit. The PD and WDG health units are also in different watersheds which may result in a different level of exposure through water. Differences in physician awareness of zoonoses and subsequent differences in levels of stool testing may also play a role.

The age distribution of cases in this study was consistent with previously observed age distributions in these health units and with other literature although the substantial peak in
children under the age of five that is reported in some studies was not observed (Figure 2.1) (15, 53, 56, 57, 61). It has been suggested that this peak may be due to an increased sampling frequency in young children with gastrointestinal illness in comparison to older children and adults (11, 53, 54). It is possible that this increased sampling frequency in young children does not occur in this region, possibly due to the length of time required to obtain fecal culture results and the shorter duration of clinical signs that has been found in children under the age of 10 (23). Alternatively, the true incidence of Campylobacter infection in young children in this region may be lower than reported elsewhere.

Data from this study were collected between 2002 and 2004. Antimicrobial use and antimicrobial resistance in these two health units may have changed since that time.

Conclusions

Since antimicrobials are not indicated for undifferentiated gastroenteritis or campylobacteriosis in most cases including children, the high proportion of cases in this study that were treated with antimicrobials (52% overall, 62% of children) for their illness is concerning. However, among cases who were treated with an antimicrobial considered efficacious against Campylobacter, approximately 95% of tested isolates were susceptible to that antimicrobial. This is not surprising considering the low levels of resistance found in this work to erythromycin and ciprofloxacin, although in the majority of cases the treatment decision was made prior to the availability of fecal culture results. Therefore, although the utilization of antimicrobials seems
Quite high, the majority of cases were treated appropriately from a microbiological perspective. However, the value of this treatment is questionable considering the minimal expected impact on illness, the potential increased risk for CDAD, and the increased opportunity for the development of antimicrobial resistance. The low levels of antimicrobial resistance to ciprofloxacin, erythromycin and nalidixic acid in Campylobacter from this region are encouraging. Prudent use practices should be promoted among physicians in family practice, walk-in clinics, and emergency rooms in order to reduce the inappropriate use of antimicrobials in the treatment of gastroenteritis in general and Campylobacter in particular, as well as to minimize the future development of resistance to these antimicrobials in Campylobacter.

Acknowledgements

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Figure 2.1: Percentage of cases by age category over a ten year period in the Wellington-Dufferin-Guelph health unit and in laboratory-confirmed cases of *Campylobacter* in the Perth District and Wellington-Dufferin-Guelph health units during the study period.

WDG  Wellington-Dufferin-Guelph
Figure 2.2: Antimicrobial use in cases during their illness, by severity in laboratory-confirmed cases of *Campylobacter* in the Perth District and Wellington-Dufferin-Guelph health units (n=244).
Figure 2.3: Frequency and timing of antimicrobial use relative to availability of fecal culture results in laboratory-confirmed cases of *Campylobacter* in the Perth District and Wellington-Dufferin-Guelph health units (n=138).
Table 2.1: Antimicrobial resistance in *Campylobacter* isolates from human cases in the Perth District and Wellington-Dufferin-Guelph health units, Ontario, Canada

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC50*</th>
<th>MIC90**</th>
<th># Resistant Isolates</th>
<th>% Resistant Isolates</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-Clavulanic acid</td>
<td>0.19</td>
<td>0.38</td>
<td>0</td>
<td>0</td>
<td>0.0 - 2.9</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>2.4</td>
<td>0.5 - 6.9</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.75</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>0.0 - 2.9</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.047</td>
<td>0.094</td>
<td>6</td>
<td>4.8</td>
<td>1.8 - 10.2</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.094</td>
<td>0.25</td>
<td>2</td>
<td>1.6</td>
<td>0.2 – 5.7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25</td>
<td>0.75</td>
<td>2</td>
<td>1.6</td>
<td>0.2 – 5.7</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.25</td>
<td>0.38</td>
<td>0</td>
<td>0</td>
<td>0.0 – 2.9</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1.5</td>
<td>3</td>
<td>6</td>
<td>4.8</td>
<td>1.8 - 10.2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.19</td>
<td>&gt;256</td>
<td>60</td>
<td>48.4</td>
<td>39.3 – 57.5</td>
</tr>
</tbody>
</table>

MIC50: concentration where 50% of isolates were inhibited

MIC90: concentration where 90% of isolates were inhibited
## Table 2.2: Antimicrobial resistance patterns in *Campylobacter* isolates from human cases in the Perth District and Wellington-Dufferin-Guelph health units, Ontario, Canada

<table>
<thead>
<tr>
<th>Resistance Profile</th>
<th># Isolates</th>
<th>% Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIP-NAL-TCY</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>CLI-ERY-TCY</td>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>CIP-NAL</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>AMP</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>TCY</td>
<td>55</td>
<td>44.4</td>
</tr>
<tr>
<td>Susceptible</td>
<td>58</td>
<td>46.8</td>
</tr>
</tbody>
</table>

AMP: ampicillin

CIP: ciprofloxacin

CLI: clindamycin

ERY: erythromycin

NAL: nalidixic acid

TCY: tetracycline
Chapter 3: Burden of illness and factors associated with duration of illness in clinical campylobacteriosis

Abstract

A population based study investigated the burden of illness, including the duration of illness associated with laboratory-confirmed cases of campylobacteriosis in two health unit areas. Questionnaire data were collected for 250 cases. The median duration of illness was 8 days and 66% of cases reported symptoms of moderate severity or greater. A Cox proportional hazard model identified antimicrobial use factors associated with a significantly increased rate of symptom resolution (shorter duration of illness): macrolide for less than the recommended number of days, ciprofloxacin for at least 3 days, and antimicrobials not recommended for campylobacteriosis. The impact of antimicrobial use was consistent regardless of when in the course of illness the antimicrobial use began. The effectiveness of ciprofloxacin in these results may be due to the low prevalence of resistance to ciprofloxacin in isolates from this study. The effect of antimicrobials not recommended for campylobacteriosis should be further investigated.
Introduction

Campylobacter is the one of the most commonly reported enteric bacterial pathogens in many countries, including Canada [1, 2]. The burden of illness of campylobacter cases on society results from lost time at work or school for either the case or a caregiver, and the costs of utilization of health care providers, hospitalization, laboratory testing and treatment. This impact is influenced by the duration, severity and scope of the patient’s symptoms. The case fatality rate for campylobacteriosis has been reported as 1 to 2 per one thousand cases [2-4]. Potential sequelae include post-infectious irritable bowel syndrome, Guillain-Barré syndrome, and reactive arthritis [5-9]. When all of these factors are considered, the overall cost of campylobacteriosis has been estimated at approximately $8000 USD per case [10].

Campylobacteriosis is usually a self-limiting infection and treatment with macrolides or fluoroquinolones is recommended only in vulnerable populations with severe or invasive disease [8, 11-13]. However observational studies have shown that a substantial percentage of patients take antimicrobials for their campylobacteriosis [11, 14]. The reported effect of antimicrobial treatment on duration of illness has been variable [15]. It has been suggested that the effect of antimicrobials on the duration of illness may vary with the period between initiation of symptoms and initiation of antimicrobial treatment [15, 16].

A population-based study was conducted over a two year period in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario, with an objective of documenting the burden of illness associated with clinical cases of campylobacteriosis. A second objective was to investigate the factors associated with the duration of illness in cases of campylobacter.
Methods

This research project was approved by the University of Guelph Research Ethics Board. Agreements were put in place with hospital and private laboratories to have isolates obtained from clients living in two health unit areas forwarded to the Public Health Ontario Laboratory – Toronto (PHL). These agreements ensured the confidentiality of patient information.

Data collection and laboratory methods for this project have been previously described [17]. In brief, laboratory-confirmed cases of campylobacteriosis during the study period were eligible for inclusion in the study. Cases provided consent for participation in the study at the time of questionnaire administration. Isolates were forwarded to the PHL for confirmation, speciation, and antimicrobial susceptibility testing. Minimum inhibitory concentrations were determined by the E-test® with the following breakpoints: amoxicillin/clavulanic acid (AMC; ≥ 32 μg/ml), ampicillin (AMP; ≥ 32 μg/ml), chloramphenicol (CHL; ≥ 32 μg/ml), ciprofloxacin (CIP; ≥ 4 μg/ml), clindamycin (CLI; ≥ 4 μg/ml), erythromycin (ERY; ≥ 32 μg/ml), gentamicin (GEN; ≥ 16 μg/ml), nalidixic acid (NAL; ≥ 32 μg/ml), and tetracycline (TCY; ≥ 16 μg/ml).

Questionnaire data

Data on the burden of illness associated with campylobacteriosis were collected as part of a comprehensive telephone questionnaire. The telephone interview was conducted by personnel from the local health unit when a laboratory-confirmed case of campylobacter was identified.
Diarrhea was defined as two or more loose stools or bowel movements in a 24 hour period and was self-reported. Burden of illness indicators included the reported type, duration, and severity of clinical signs, activity limitations, and health care utilization. Severity of illness was reported by cases based on the following definitions: Quite mild - feeling slightly unwell but able to do all normal activities; Fairly mild - feeling quite unwell but able to do most normal activities; Moderate - having to stay at home but able to get out of bed for limited activities; Fairly severe - confined to bed at home and unable to do any normal activities; Quite severe - hospitalized.

Antimicrobial use data captured through the questionnaire included: antimicrobial(s) used in the thirty days prior to illness, antimicrobial(s) used during illness, and the start and end dates of use during illness. Linear regression was used to investigate the relationship between severity of illness and the number of days cases were unable to perform their usual activities.

**Survival analysis**

Stata Intercooled version 11 (Stata Corporation, College Station, Texas, USA) was used for the analysis. A Cox proportional hazard model was developed to identify factors associated with duration of illness. The date of failure was the date all symptoms ceased. The date of censor was the date of last contact when symptoms were ongoing. Covariates included in the univariable analysis are listed in Table 3.2. Age was analyzed as both continuous and categorical variables; category breakpoints were determined based on historical age distribution data from the participating health units as previously described (5,17,41, and 61 years) [17]. Season was categorized as winter (December-February), spring (March-May), summer (June-
August) and fall (September-November). Individual variables were created for the most common medications taken during the four weeks prior to illness including gastroesophageal reflux, pain, high blood pressure, immunosuppression, and thyroid medications. A general variable was also created which included any non-antimicrobial medications taken during the four weeks prior to illness.

In order to account for time from initiation of symptoms to initiation of antimicrobial therapy, a categorical time varying variable was created. This variable was coded according to the antimicrobial utilized and if/when the antimicrobial was taken for the recommended duration. The antimicrobial categories evaluated were ciprofloxacin, macrolide or “other” antimicrobial. These categories were selected based on previous research and clinical guidelines for the treatment of campylobacteriosis [8, 12, 13, 16, 18-20]. For classification of completeness of therapy duration, the following recommended durations were used: 3 days for azithromycin [18]; 3 days for ciprofloxacin [18]; 5 days for erythromycin [12, 18]; and 7 days for clarithromycin [13]. Antimicrobials in the “other” category were not expected to be clinically effective and a recommended duration was not considered [8, 12, 13, 18, 20]. Therefore, antimicrobial use during illness (AMU) was categorized as no antimicrobial use, incomplete macrolide, complete macrolide, incomplete ciprofloxacin, complete ciprofloxacin, or “other” treatment. Cases were categorized as no use prior to antimicrobials being taken, as incomplete use once antimicrobials were initiated, and as complete use if/when the recommended duration was achieved. Although some cases continued to have symptoms after antimicrobial use had ended, the antimicrobial use variable was not returned to the value for “no use” due to the expected impact of antimicrobials on the gastrointestinal flora and the relatively short overall
duration of symptoms. In these instances, the antimicrobial use variable remained at its value when antimicrobial use ceased i.e. incomplete or complete. Antimicrobial use that continued after the date of failure (symptom resolution) was not included in the analysis.

The AMU variable was assessed for a time-varying effect by first: testing the proportional hazards assumption on the basis of the Schoenfeld residuals on the log and time scales and second: adding interaction terms between AMU and analysis time to the univariable Cox proportional hazard model. Aalen’s linear hazard model was used to parameterize the potential time-varying effect of the AMU variable [21].

Univariable analysis was performed with potential covariates utilizing Log rank and Wilcoxon tests of equality as well as univariable Cox proportional hazards models. Variables significant at p≤0.2 for any of these tests were considered for inclusion into the multivariable analysis. Variables with a prevalence of less than two per cent in cases were omitted from further analysis. Variables were examined for collinearity and when the correlation coefficient for two predictors was significant at p<0.05 after bonferroni adjustment, one was selected for inclusion into the multivariable analysis based on level of significance, number of missing observations, and reliability.

The preliminary main effects Cox proportional hazard model was developed utilizing manual backward selection. Variables were retained if the likelihood ratio test was significant (p≤0.05) or if the coefficient(s) of other covariates changed by > 20%. When all remaining variables were significant at p≤0.05 or were confounders, variables that were not significant in the univariable analysis were introduced one at a time and evaluated for significance. Biologically plausible
interaction terms for the resulting model were assessed. The assumption of proportional hazards was evaluated by the link test, scaled Schoenfeld residuals, interaction terms for covariates and time, Cox proportional hazard plots stratified by each level of a covariate after adjusting for other covariates, and Kaplan-Meier versus predicted survival plots for each level of covariates. Outliers and influential observations were identified with Cox-Snell residuals and assessed for biological plausibility.

Results

There were 317 laboratory-confirmed cases during the study period and 78.9% (n=250) were successfully contacted and agreed to participate resulting in questionnaire data collected from 51 cases in PD and 199 cases in WDG.

Demographic data from this study have been previously reported [17]. Briefly, 140 cases were male and 109 were female. Cases had a median age of 27.4 (mean=29.0) years. The mean duration of illness was 10 days with a median of 8 days and a range of 0.5 to 77 days with a right-skewed distribution (Figure 3.1). Fourteen cases (5.6%) had ongoing symptoms at the time of the telephone interview.

One hundred and sixty-five cases (66.0%) were unable to carry out most regular activities during their illness. Of 150 cases attending school or working outside of the home, 135 (90.0%) took time off due to their illness (mean and median of 4 days, range: 0.5 to 14 days) (Table 3.2). Thirty-five cases (14.0%) had relatives/friends who missed a mean of 1.8 days of work (median:
1, range: 1 to 7 days) in order to care for them (Table 3.2). The mean number of days that cases were unable to perform their usual activities including going to work or school significantly increased as severity of illness increased (P=0.001) (Figure 3.2). Self-reported severity of illness according to defined categories resulted in 24 cases (9.6%) with Quite Mild, 61 cases (24.4%) with Fairly Mild, 87 cases (34.8%) with Moderate, 56 cases (22.4%) with Fairly Severe, and 22 cases (8.8%) with Quite Severe illness (hospitalized) (Figure 3.2).

Symptoms reported by more than 70% of patients were diarrhea, fatigue, stomach cramps, loss of appetite, and fever (Table 3.1). Forty-eight per cent of cases reported blood in the stool (Table 3.1) but as previously reported, the proportion of cases with blood in the stool was significantly higher for those under 5 years of age (p=0.001) [17]. In this study, 3 cases reported diarrhea as the only symptom of their illness and 230 cases (92%) reported at least 3 symptoms. All cases in this study were laboratory-confirmed and therefore accessed the health care system. Of the two hundred and forty-nine cases who reported the type of health care accessed during their illness, most (182, 73.1%) utilized one route, most commonly the family doctor (162, 65.1%), followed by emergency rooms (114, 45.8%) and walk-in clinics (38, 15.3%). In smaller communities, walk-in clinics may not have been available.

Ninety cases (36%) took medication other than antimicrobials in the 4 weeks prior to their illness, most commonly for gastroesophageal reflux (28 cases), pain (16 cases), high blood pressure (11 cases), immunosuppression (9 cases), and hyper/hypo thyroid (7 cases). Three cases took a laxative in the 4 weeks prior to illness and one case took an antidiarrheal drug in that time period.
Twelve cases (4.9%) took antimicrobials in the 4 weeks prior to their illness; including amoxicillin (5 cases), cefaclor (1 case), clarithromycin (1 case), norfloxacin (1 case), tetracycline (1 case), trimethoprim-sulfamethoxazole and penicillin (1 case), vancomycin (1 case) and unknown (1 case). The reason for antimicrobial use prior to illness was not known.

The PHL received isolates from 124 cases for speciation and susceptibility testing. Isolates from the remaining 126 cases were discarded in error at the primary laboratory or were unable to be matched with case data. Of the 124 isolates, 121 (97.6%) were *C. jejuni* and 3 (2.4%) *C. coli*. Antimicrobial resistance in these isolates has been previously reported [17]. Briefly, no resistance to AMC, CHL, or GEN was found. Six isolates (4.8%) were resistant to NAL and CIP, 2 (1.6%) were resistant to erythromycin and 58 (46.8%) isolates were susceptible to all antimicrobials tested.

The most common antimicrobials taken during illness were ciprofloxacin (43 cases, 17.6%), azithromycin (32 cases, 13.1%), erythromycin (29 cases, 11.8%), and clarithromycin (11 cases, 4.5%). Antimicrobials not expected to be clinically effective for campylobacteriosis that were taken by cases included amoxicillin, metronidazole, trimethoprim-sulfamethoxazole, sulfa drugs, and tetracycline. Ten cases took more than one antimicrobial for their illness. As previously reported, cases treated with antimicrobials did not significantly differ from those that were not treated with antimicrobials with regards to age category, severity of illness, chronic medical condition or history of recent international travel (p>0.05) [17].

In this study 22 cases began taking antimicrobials within 3 days of the onset of symptoms and 108 cases began taking antimicrobials more than 3 days from the onset of symptoms. The
number of days between the onset of symptoms and the initiation of antimicrobial treatment ranged from 0 to 37 with a median of 6 days. Thirty-two cases (24.4%) began taking antimicrobials after their symptoms had stopped.

Fifty-one cases took a macrolide and provided a start date. Of these, seven (13.7%) took the recommended course and were classified as complete macrolide and 44 were incomplete macrolide. Thirty-nine cases were incomplete because their symptoms ended prior to the completion of the recommended course of treatment. Although antimicrobial use after symptoms ended was not included in the analysis, it is worth noting that of these 39 cases, 29 continued to take the macrolide after their symptoms had ended and until at least the recommended course of treatment was completed.

Thirty-four cases took ciprofloxacin and provided a start date. Seventeen were classified as complete ciprofloxacin and 17 were incomplete ciprofloxacin. Of these, 14 cases were incomplete when their symptoms ended prior to the completion of the recommended course of treatment. Eleven cases continued to take ciprofloxacin after their symptoms had ended for at least the recommended course of treatment.

**Survival analysis**

Variables that were assessed in the univariable analysis are indicated in (Table 3.2). Resistance to erythromycin was not included in the analysis because the prevalence was less than two per cent of cases. None of the explanatory variables significantly differed by health unit. The use of
rehydration fluids was significant in the univariable survival analysis but this variable was significantly collinear with the consumption of pain medications (r=0.82). Since more cases took the latter and it was more significantly associated with duration of illness, the rehydration fluid variable was not submitted to the multivariable model (Table 3.2). Having a chronic infection that could weaken the immune system and taking a non-antimicrobial medication in the 4 weeks prior to illness were both significant in the univariable survival analysis but were significantly collinear (r=0.84)(Table 3.2). Taking a non-antimicrobial medication in the 4 weeks prior to illness was more significantly associated with duration of illness than chronic illness and was more reliable. Therefore taking a non-antimicrobial medication in the 4 weeks prior to illness was submitted to the multivariable model. Variables for gastroesophageal reflux, blood pressure, immunosuppression, and thyroid medications in the four weeks prior to illness were not significant on univariable analysis. There was no significant time-varying effect of AMU. Therefore the impact of antimicrobial use on the resolution of symptoms was consistent regardless of when in the course of illness the antimicrobial use began.

The following variables were included in the initial multivariable model: gender, antimicrobial use during campylobacteriosis, taking an antidiarrheal medication during campylobacteriosis, taking an analgesic during campylobacteriosis, taking any non-antimicrobial medication in the 4 weeks prior to illness, and whether the campylobacter isolate was resistant to ciprofloxacin. The general variable for any non-antimicrobial medications and the variable for pain medications in the four weeks prior to illness were significant on univariable analysis. Each of these variables was submitted to separate model building processes. The multivariable model that included the pain medications variable had a smaller number of observations but the same final outcome as
the model which included the general variable. Therefore, the model with the general variable for the use of any non-antimicrobial medications prior to illness was utilized.

None of the non-significant variables in the univariable analysis were significant when added to the preliminary model. No biologically plausible interaction terms were significant. The evaluation of the model indicated that the proportional hazard assumption was met. One case identified as an outlier was omitted from the analysis due to a very prolonged duration of illness of quite mild severity and a chronic illness where diarrhea was a symptom. All other outliers and influential observations were biologically plausible and therefore were retained in the analysis.

The final model included antimicrobial use during illness and the use of non-antimicrobial medication in the 4 weeks prior to illness (Figure 3.5). When compared to cases who did not take antimicrobials, the rate of symptom resolution was increased for cases who took a macrolide for less than the recommended number of days (350\% greater rate of symptom resolution), ciprofloxacin for at least the recommended number of days (214\%) and cases who took an antimicrobial not recommended for campylobacteriosis (225\%) (Figure 3.4, Figure 3.5). Since the increased rate of symptom resolution is consistent regardless of when during the illness antimicrobial use began, it would result in a shorter duration of illness at the population level. Taking ciprofloxacin for less than the recommended number of days and taking a macrolide for at least the recommended number of days was not significantly associated with the rate of symptom resolution and therefore did not have a significant impact on duration of illness. When cases had taken non-antimicrobial medication in the four weeks prior to illness, the rate of symptom resolution was decreased by 33\% when compared to cases who did not take medication
in the four weeks prior to illness. Therefore the duration of illness at the population level will be longer for cases taking non-antimicrobial medication in the four weeks prior to illness.

Discussion

The median and range of duration of illness in this study is comparable with previously reported research [14, 22]. Duration of diarrhea was not specifically recorded in this study. Although duration of diarrhea is commonly reported in both observational studies and clinical trials, the overall duration of illness is less frequently reported [15, 22-25]. It is important to note that the overall duration of illness is not necessarily equivalent to the duration of diarrhea. Overall duration better represents the total burden of illness due to campylobacteriosis on both the individual and society [14, 24, 26].

In this study the distribution of the duration of illness was right-skewed with a median duration of illness of 8 days but a range of 0.5 to 77 days. It is difficult to determine the factors impacting the duration of illness when the natural course of the illness is relatively short in a substantial proportion of cases. As well, 6% of cases still had symptoms at the time of the telephone interview. The Cox proportional hazard model was used to investigate the factors associated with duration of illness since it is a semi-parametric method and therefore does not include assumptions on the distribution of failure times. Since Cox proportional hazard models are essentially a series of conditional logistic regression models for each day where at least one case resolves, only cases that still have symptoms on a specific day are compared. For example, the
effect of complete or incomplete antimicrobial treatment on the odds of symptoms ending seven
days after symptoms began is based only on cases that still had symptoms at the beginning of the
seventh day. Due to the natural short course of illness in cases of campylobacteriosis, there was
a small number of observations in these data once the duration of illness exceeded 17 days.

For cases taking a non-antimicrobial medication in the 4 weeks prior to their illness the rate of
symptom resolution was significantly lower, resulting in an overall longer duration of illness.
This may be due at least in part to effects of the underlying conditions that necessitated the
medication, particularly in cases of chronic illness. These medications were taken orally and
therefore may also have affected the gastrointestinal flora [27]. Reporting of medication use in
our study appeared to be more reliable than reporting of chronic illness. A substantial number of
cases replied “No” when asked if they had a chronic condition yet reported the use of ongoing
medications for chronic illness (e.g. anti-hypertensives) in a separate question. This may be due
to the successful clinical management of the chronic illness with the ongoing medication. Since
these variables were collinear, the previous use of non-antimicrobial medications variable was
retained for modelling. Although medications taken for non-chronic conditions were also
included in the previous medications variable, the direction and magnitude of the effect was
similar regardless of whether these cases were included. Therefore the more inclusive version of
the variable was used.

Antimicrobial use prior to illness was not significantly associated with duration of illness in this
analysis, although it was associated with an increased risk of campylobacter infection in previous
research [28]. Our study may not have had sufficient power to detect a significant effect, since
less than 5% of cases took an antimicrobial in the 4 weeks prior to illness in this study. The potential role of prior antimicrobial use on risk or duration of illness needs to be further investigated in order to better understand the epidemiology of *Campylobacter*.

Previous observational and clinical studies have evaluated the impact of antimicrobial use on the duration of campylobacteriosis by comparing the mean duration of diarrhea or illness in cases who did or did not take antimicrobials, with variable results [14, 15]. A meta-analysis that examined 11 clinical trials on the effects of antimicrobial treatment of campylobacteriosis on duration of diarrhea found that results from individual studies were variable, but overall antimicrobial treatment significantly decreased the mean duration of diarrhea when compared to a placebo using a random effects model [15]. This is consistent with the effect on duration of illness of some antimicrobial treatment in the Cox proportional hazard model from this study. It should be noted, however, that there may have been an over-representation of some erythromycin treatment results in the meta-analysis due to the possible inclusion of some of the same cases in more than one paper [23, 26]. Some of the variability in effect that has been seen in previous work may be due to the use of mean duration of diarrhea/illness as an outcome since the natural short course of illness in many cases, and the non-normal and right-censored nature of the data may have impacted the results. The increased rate of symptom resolution and therefore shorter duration of illness in cases with incomplete macrolide treatment was largely due to symptoms ending before the recommended course of treatment was complete. This may be the result of the specific macrolide and dosage used. The dosages of each antimicrobial were not recorded in this study. The specific macrolide used was captured in the questionnaire; however there were insufficient cases to analyze these separately. Although a complete course of
treatment with azithromycin was considered 3 days for this analysis, some research has demonstrated that a single treatment is effective with higher dosages [29]. It appears that the effect of macrolides is quite rapid, regardless of when in the course of the disease they are initiated. Therefore if symptoms have not ceased by the end of the complete course of treatment, there is no treatment effect.

Ciprofloxacin is frequently recommended for the treatment of campylobacteriosis when antimicrobials are indicated [16, 18], however increasing prevalence of resistance to ciprofloxacin is an international concern [16, 19]. The apparent effectiveness of ciprofloxacin in this study is likely due to the relatively low prevalence of resistance to ciprofloxacin seen in isolates from this study and in previously reported Canadian data [30-32]. Cases with campylobacter isolates resistant to ciprofloxacin had longer durations of illness in several studies [33-35] but not in others [22, 36]. Although infections with a ciprofloxacin resistant isolate were identified as significant in the univariable Cox proportional hazard analysis (p<0.2), this variable was not significant in the multivariable model and did not act as a confounder. The results of this study also indicate that a complete course of ciprofloxacin is required in order to have a significant impact on the rate of symptom resolution and therefore duration of illness.

Further research is necessary to investigate the effect of antimicrobials not expected to be clinically effective against Camplyobacter on the rate of symptom resolution and therefore duration of illness. However, it has been previously reported that duration of illness is decreased by antimicrobial therapy in cases of diarrhea, even when no bacterial pathogen was isolated [23, 37], perhaps due to suppression of undetected pathogens or other effects on the intestinal flora.
Although it has been frequently reported that antimicrobial treatment is most effective when given early in the course of campylobacteriosis [15, 16], there was no time-varying effect of antimicrobial use in this study. Therefore the impact of antimicrobial use on the resolution of symptoms was consistent regardless of when in the course of illness the antimicrobial use began. This may be due to the lack of direct comparison between early and late treatment in previous studies.

The consumption of anti-diarrheal medications and analgesics during illness were significant in the univariable survival analysis, but they were not retained in the multi-variable model. Antidiarrheal drugs are recommended in some cases of infectious diarrhea and have been reported to decrease the duration of diarrhea [38-40]. However a large American study actually found a significantly increased duration of diarrhea in cases taking an antidiarrheal [34]. The persistence of symptoms other than diarrhea may also prevent these medications from having an impact on duration of overall illness. Although analgesics would be expected to have an impact on the rate of resolution of fever and headache symptoms, they should not impact other clinical symptoms of campylobacter infections. The majority of cases in this study reported at least 3 symptoms for their illness.

Although rehydration fluids are very commonly recommended for acute gastroenteritis in general and campylobacteriosis in particular, there is no published information on the impact of this intervention on the duration of illness [12, 38].

Unexpectedly, there was no significant association between severity and duration of illness in this study population. Although severity was assessed through specific questions on activity
limitations, this assessment was self-reported and did not include descriptors such as the number of stools per day. However, the number of days cases were unable to perform normal activities and unable to attend work or school in this study was similar to previous research [14, 34].

Treatment with antimicrobials significantly accelerated the resolution of symptoms when analyzed using a Cox proportional hazards model. This effect was not altered by the timing of the antimicrobial treatment. Although decreasing the duration of illness has a positive impact on the burden of illness for both the individual and society, the use of antimicrobials in the treatment of campylobacteriosis should be considered in the context of the self-limiting nature of this illness, the potential impact on antimicrobial resistance, and the impact of previous medication usage. In particular, the low prevalence of ciprofloxacin resistance in Canada is advantageous and should be protected through the judicious use of fluoroquinolones. The use of macrolides may provide the best opportunity to affect duration of illness without a substantial risk of antimicrobial resistance development. Further investigation into the effect on duration of illness of antimicrobials not expected to be clinically effective against campylobacter is necessary.

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speciation and antimicrobial susceptibility testing, and D. Pearl and Z. Poljak for statistical advice.
References


Figure 3.1: Duration of illness in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units (n=249).
Figure 3.2: Self-reported severity of illness according to a defined severity scale and mean number of days of limited activity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units.

Quite mild: feeling slightly unwell but able to do all normal activities
Fairly mild: feeling quite unwell but able to do most normal activities
Moderate: having to stay at home but able to get out of bed for limited activities
Fairly severe: confined to bed at home and unable to do any normal activities
Quite severe: hospitalized
Figure 3.3: Reported symptoms by severity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units.
Figure 3.4: Cox proportional hazard model for duration of illness in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units (n=227): Hazard ratios* and 95% Confidence intervals.

- Significant effect on duration of illness
- Non-significant effect on duration of illness

CIP: ciprofloxacin

* A hazard ratio > 1 indicates an increased rate of symptom resolution and therefore a decreased duration of illness.

* A hazard ratio < 1 indicates a decreased rate of symptom resolution and therefore an increased duration of illness.
Figure 3.5: Predicted survival curves of significant antimicrobial use variables from Cox proportional hazard model for duration of illness in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units (n=227) with adjustment for prior use of non-antimicrobials.
Figure 3.3: Reported symptoms by severity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units.
Table 3.1: Summary of reported symptoms by severity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>n/N* (%)</th>
<th>Quite Mild</th>
<th>Fairly Mild</th>
<th>Moderate Severe</th>
<th>Fairly Severe</th>
<th>Quite Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>248/250 (99.2)</td>
<td>23/24</td>
<td>61/61</td>
<td>87/87</td>
<td>55/56</td>
<td>22/22</td>
</tr>
<tr>
<td>Stomach Cramps</td>
<td>212/235 (90.2)</td>
<td>13/17</td>
<td>48/56</td>
<td>78/84</td>
<td>55/56</td>
<td>18/22</td>
</tr>
<tr>
<td>Fatigue</td>
<td>221/246 (89.8)</td>
<td>12/24</td>
<td>51/60</td>
<td>81/85</td>
<td>55/55</td>
<td>22/22</td>
</tr>
<tr>
<td>Loss of Appetite</td>
<td>208/247 (84.2)</td>
<td>9/24</td>
<td>49/61</td>
<td>79/86</td>
<td>54/55</td>
<td>17/21</td>
</tr>
<tr>
<td>Fever</td>
<td>180/246 (73.2)</td>
<td>11/23</td>
<td>39/61</td>
<td>65/85</td>
<td>48/55</td>
<td>17/22</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>147/228 (64.5)</td>
<td>4/22</td>
<td>24/57</td>
<td>57/75</td>
<td>46/53</td>
<td>16/21</td>
</tr>
<tr>
<td>Headache</td>
<td>142/224 (63.4)</td>
<td>6/13</td>
<td>24/55</td>
<td>54/81</td>
<td>42/53</td>
<td>16/22</td>
</tr>
<tr>
<td>Nausea</td>
<td>149/235 (63.4)</td>
<td>6/17</td>
<td>25/58</td>
<td>57/83</td>
<td>45/55</td>
<td>16/22</td>
</tr>
<tr>
<td>Blood in Stool</td>
<td>114/237 (48.1)</td>
<td>13/24</td>
<td>28/60</td>
<td>31/79</td>
<td>31/53</td>
<td>11/21</td>
</tr>
<tr>
<td>Bloating</td>
<td>86/235 (36.6)</td>
<td>4/24</td>
<td>14/58</td>
<td>31/78</td>
<td>21/54</td>
<td>5/21</td>
</tr>
<tr>
<td>Vomit</td>
<td>71/247 (28.7)</td>
<td>1/24</td>
<td>11/61</td>
<td>28/85</td>
<td>26/55</td>
<td>5/22</td>
</tr>
<tr>
<td>Other **</td>
<td>79/248 (31.9)</td>
<td>3/24</td>
<td>13/61</td>
<td>33/85</td>
<td>20/56</td>
<td>10/22</td>
</tr>
</tbody>
</table>

* number of cases with responses varied by symptom

** symptoms most commonly reported under “Other” included joint pain (11 cases), muscle pain (21 cases), dizziness (8 cases), and rash (4 cases).
Table 3.2 Summary of reported days of missed work or school by severity in laboratory-confirmed cases of campylobacteriosis in Perth District and Wellington-Dufferin-Guelph health units

<table>
<thead>
<tr>
<th>Severity</th>
<th>Case – days off</th>
<th></th>
<th>Caregiver – days off</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Quite Mild</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fairly Mild</td>
<td>2.7</td>
<td>2</td>
<td>27</td>
<td>2.2</td>
</tr>
<tr>
<td>Moderate</td>
<td>3.8</td>
<td>3</td>
<td>50</td>
<td>1.6</td>
</tr>
<tr>
<td>Fairly Severe</td>
<td>5.2</td>
<td>5</td>
<td>39</td>
<td>1.6</td>
</tr>
<tr>
<td>Quite Severe</td>
<td>4.3</td>
<td>4</td>
<td>11</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Table 3.3: Potential covariates evaluated in univariable survival analysis in laboratory-confirmed cases of campylobacterosis in Perth District and Wellington-Dufferin-Guelph health units.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Present/Total</th>
<th>Percentage</th>
<th>Significant**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>122 / 249</td>
<td>49.0</td>
<td>No</td>
</tr>
<tr>
<td>Year 2</td>
<td>127 / 249</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>Season:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>30 / 249</td>
<td>12.0</td>
<td>No</td>
</tr>
<tr>
<td>Spring</td>
<td>42 / 249</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>101 / 249</td>
<td>40.6</td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>76 / 249</td>
<td>30.5</td>
<td></td>
</tr>
<tr>
<td>Severity of Illness:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quite mild</td>
<td>23 / 249</td>
<td>9.2</td>
<td>No</td>
</tr>
<tr>
<td>Fairly mild</td>
<td>61 / 249</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>87 / 249</td>
<td>34.9</td>
<td></td>
</tr>
<tr>
<td>Fairly Severe</td>
<td>56 / 249</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>Quite Severe</td>
<td>22 / 249</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Age: median</td>
<td>27.4</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>140 / 249</td>
<td>56.2</td>
<td>Yes</td>
</tr>
<tr>
<td>Female</td>
<td>109 / 249</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>Chronic health conditions*</td>
<td>74 / 250</td>
<td>29.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Antimicrobial use prior to illness</td>
<td>12 / 244</td>
<td>4.9</td>
<td>No</td>
</tr>
<tr>
<td>Non antimicrobial use prior to illness</td>
<td>90 / 249</td>
<td>36.1</td>
<td>Yes</td>
</tr>
<tr>
<td>Antimicrobial use during illness***</td>
<td>130 / 249</td>
<td>52.2</td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-diarrheal medication during illness</td>
<td>129 / 244</td>
<td>52.9</td>
<td>Yes</td>
</tr>
<tr>
<td>Analgesic during illness</td>
<td>180 / 248</td>
<td>72.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-nausea medication during illness</td>
<td>57 / 245</td>
<td>23.3</td>
<td>No</td>
</tr>
<tr>
<td>Rehydration fluids during illness*</td>
<td>59 / 244</td>
<td>24.2</td>
<td>Yes</td>
</tr>
<tr>
<td>Campylobacter isolate resistant to ciprofloxacin</td>
<td>6 / 124</td>
<td>4.8</td>
<td>Yes</td>
</tr>
<tr>
<td>Campylobacter isolate resistant to ≥ 1 of the antimicrobials tested</td>
<td>66 / 124</td>
<td>53.2</td>
<td>No</td>
</tr>
</tbody>
</table>

* not submitted to multi-variable model due to collinearity
** significant at p≤0.2 on at least one of Log rank or Wilcoxon tests, or in a univariable Cox proportional hazard model
*** Time-varying antimicrobial use during illness variables (complete ciprofloxacin, incomplete ciprofloxacin, complete macrolide, incomplete macrolide, other antimicrobial) used in analysis but cannot be represented in table format
Chapter 4: Prevalence and antimicrobial resistance in *Campylobacter* spp. isolated from retail chicken in two health units in Ontario

Abstract

*Campylobacter* is an important enteric pathogen of humans which can cause diarrhea, fever, and abdominal pain. *Campylobacter* infections have frequently been associated with the handling and consumption of raw and undercooked poultry. Antimicrobial resistance in *Campylobacter* organisms is of concern in the treatment of campylobacteriosis in vulnerable populations. A multi-year project was conducted in the Perth and Wellington-Dufferin-Guelph public health units in Ontario, Canada to investigate the prevalence and antimicrobial resistance of *Campylobacter* spp. in retail chicken. Retail chicken was sampled from randomly selected stores in these health units. Resulting *Campylobacter* isolates were tested for susceptibility to amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), tetracycline (TCY), and trimethoprim-sulfamethoxazole (SXT) using the E-test®. The prevalence of *Campylobacter* in 1256 retail chicken samples was 59.6%. Of these, 9% were *C. coli*, 1% *C. lari* and 90% *C. jejuni*. The number (%) of chicken isolates resistant to one or more antimicrobial agents was: 301 (40%; one); 374 (50%; two); 39 (5%; three); 20 (3%; four), and 6 (1%; five). Nine isolates (1%) were susceptible to all antimicrobial agents tested. All isolates were susceptible to AMC, CHL, and GEN. Less than 10% of isolates were resistant to NAL, CIP, CLI, ERY, and AMP. Resistance to TCY was common (56%). There were no isolates with a resistance pattern that included all three antimicrobials important in the treatment of human
campylobacteriosis (CIP, ERY, and TCY) however 24 isolates (3.2%) were resistant to at least two of these antimicrobials.

**Introduction**

*Campylobacter* is an important enteric pathogen of humans which can cause diarrhea, fever, and abdominal pain. The diarrhea is often severe and may contain blood (34). In Canada, the Notifiable Diseases Program reported 30.2 isolations of *Campylobacter* per 100,000 people nationally and 31.8 per 100,000 in Ontario in 2004 (15). There were 3,945 campylobacteriosis cases reported in Ontario that year (15). Also in 2004, the FoodNet Surveillance system in the United States reported 5,684 cases of campylobacteriosis and a rate of 12.79 per 100,000 people across their sites (14). FoodNet data from 2010 indicated a similar rate of 13.5 per 100,000 people (22). Since gastrointestinal illness is significantly under-reported, the true number of cases is likely substantially higher (13, 31). The case fatality rate for *Campylobacter* was reported as 1.0 per 1,000 in the United States in 1999 (41) and approximately one in one thousand cases can also progress to the neurological manifestation, Guillian-Barré syndrome (2). *Campylobacter* infections have frequently been associated with the handling and consumption of raw and undercooked poultry (10, 14, 4). However, in poultry *Campylobacter* is a commensal organism, seldom causing disease (41). *Campylobacter jejuni* is the most common species isolated from poultry in North America and is also the most common species of zoonotic agent associated with human clinical disease (16, 48, 26, 18, 3, 2).
Campylobacteriosis is usually a self-limiting infection (3, 4) however, particularly in vulnerable populations it can become systemic and require treatment with antimicrobials. Erythromycin is commonly used in both adults and children and fluoroquinolones, such as ciprofloxacin can be used in adult cases (2). Tetracycline can also be utilized to treat these infections in adults (4). Although resistance to these antimicrobials may have immediate clinical significance, resistance in Campylobacter to other antimicrobials is also of concern due to co-selection (6). There is also evidence that antimicrobial resistance in Campylobacter may be associated with increased virulence and adverse outcomes in human cases (20) as well as prolonged duration of diarrhea (39, 11, 35). Therefore resistance in Campylobacter organisms to antimicrobials in general, and these clinically important antimicrobials in particular, is of concern. This multidisciplinary project was conducted from 2001 to 2004 in the Perth and Wellington-Dufferin-Guelph (WDG) health units in Ontario, with the objective of determining the prevalence of Campylobacter contaminated retail chicken sold in these regions. A second objective of this study was to describe the prevalence of antimicrobial resistance and the antimicrobial resistance patterns of the Campylobacter isolates from retail chicken in these health units.

Materials and Methods

Sample collection

A sampling frame of retail outlets selling fresh chicken within the borders of the Perth and Wellington-Dufferin-Guelph (WDG) health units in Ontario was generated using telephone
directory “yellow pages”, retail store listings from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), retail store listings from the participating health units, store listings in the Ontario Business Directory, and exploration of the communities. In each retail outlet, the amount of cooler space allocated to retail chicken was utilized as a surrogate measure for store size. This was then used to generate a weighted sampling list. Fresh retail chicken was sampled from stores that were randomly selected with probability proportional to their size. Fresh chicken was purchased at approximately two week intervals throughout the study period; July 2001 to January 2004. Samples were placed into a cooler and transported directly to the Public Health Agency of Canada laboratory.

Fresh chicken in Canada is defined as at or below 4°C but above -2°C by the Canadian Food Inspection Agency (17). Sampling alternated between the two study health units. Bone-in chicken legs and thighs with skin were the designated sample based on prevalence and cost of purchase (16) but if these were not available, alternate chicken parts were purchased. Epidemiological information on the samples was collected including; sample type, store name, date sampled, store size (large, medium, small), independent or chain, and store location (address and city).

Sample size was calculated to allow detection of prevalence as low as 2% with a precision of 1.5% and a confidence of 99% in order to detect rare antimicrobial resistance patterns e.g. fluoroquinolone resistance.

**Isolation**
All *Campylobacter* isolates from retail chicken were obtained utilizing the method previously described by Valdivieso-Garcia, et al (5). Briefly, 25 g of chicken skin was sampled from each package in order to obtain a standardized sample regardless of package size. The chicken skin was added into 100 ml of Rosef’s enrichment broth and incubated using the ramping temperature: 30°C for 4 hours, 37°C for 2 hours and 42°C for 48 hours. Then 200 μl was placed on a paper filter disc on a hydrophobic grid membrane filter (HGMF) sitting on semi-solid media with FBP (ferrous sulphate, sodium metabisulfite, and sodium pyruvate) and no antibiotics. Presumptive *Campylobacter* colonies were streaked on a Mueller–Hinton blood agar plate and incubated microaerobically at 42°C for 24 h to obtain pure cultures. *Campylobacter* strains were identified by: dark–field microscopy for motility and morphology; ELISA reactivity to specific monoclonal antibodies to thermophilic *Campylobacter*; hydrolysis of sodium hippurate; and PCR using specific primers for *Campylobacter jejuni* and *Campylobacter coli* (28, 25).

**Minimum inhibitory concentrations**

The E test® was used for the determination of the minimum inhibitory concentration (MIC) of amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), tetracycline (TCY), and trimethoprim-sulfamethoxazole (SXT) in the chicken isolates as indicated in the manufacturer’s instructions and described in a previous communication, including test conditions and control strains (44) (E test, bioMérieux, Stockholm, Sweden). The breakpoints utilized for this study were AMC, ≥ 32/16 μg/ml; AMP,CHL, ERY, and NAL, ≥ 32 μg/ml; CIP and CLI, ≥ 4 μg/ml; GEN and TCY ≥ 16 μg/ml and SXT, ≥ 4/76 μg/ml.
Data analysis

All bacteriological and store data were entered into a database (Microsoft® Access 2000, Microsoft Corporation, Redmond, Washington, USA) and exported to Stata (Stata Intercooled version 9, Stata Corporation, College Station, Texas, USA) for manipulation and analysis. Differences between *Campylobacter* prevalence and isolate susceptibility in the two health units were evaluated utilizing two-tailed Pearson’s Chi-square or the Fisher’s exact tests (p≤0.05).

Results and Discussion

Fresh chicken parts purchased included: legs (670), thighs (271), drumsticks (128), quarters (122), breasts (48), halves (12), wings (4), and backs (1), for a total of 1256 packages of chicken. Samples were collected from 44 different stores, 31 in the Wellington-Dufferin-Guelph health unit area and 13 in the Perth health unit area. Thirty-six stores belonged to 14 different retail chains associated with 5 different corporations. The other 8 stores were independents. Processing plant information was available on 444 (35.4%) of the sampled packages and 29 different processing plants were represented. As the majority of packages sampled did not indicate the processing plant of origin, the total number of processing plants represented in this research is unknown.

*Campylobacter* was isolated from 749 of 1256 samples (59.6%, 95% CI[56.86%-62.36%]). The prevalence of *Campylobacter* in the two health units was 60.1% (395 of 657) in Perth and 58.1% (354 of 599) in Wellington-Dufferin-Guelph. These prevalence estimates were not significantly
different when compared utilizing a Pearson Chi-square test (p=0.712). There were no significant differences between the proportions of each *Campylobacter* species isolated from chicken from the two health units. As well, there was no significant difference in the prevalence of *Campylobacter* by store size or sample type (p>0.05) (Table 4.2).

There is a large range in the reported prevalence of *Campylobacter* on retail chicken that may be due to differences in isolation methodologies, season of sampling, geographical scope of project, type and size of meat sample, fresh versus previously frozen chicken sampled, and utilization of a sampling frame, as well as actual differences in prevalence. The reported prevalence of *Campylobacter* on retail chicken in recent studies ranged from 49.5% to 93.2% (6, 9, 42, 24, 19, 21, 47, 29, 33, 37). The results of this study are consistent with the results from other structured sampling schemes in North America (48, 16, 43, 44).

*Campylobacter jejuni* was the most common species found in the retail chicken sampled in this study (90.4%) although *C. coli* (8.7%) and *C. lari* (0.9%) were also recovered. These results are consistent with international research (32, 46, 47, 29, 48, 38, 26, 18) as well as the CIPARS and National Antimicrobial Resistance Monitoring System (NARMS) surveillance systems (16, 43, 44). The results from this study however are in contrast to those from retail chicken sampled in Korea between 2000-2006 where the distribution of *C. jejuni* and *C. coli* was approximately equal (21, 19, 24). Differences in the proportion of species isolated could be due to differences in the proportion of species in the broiler population as well as differences in the conditions of retail chicken processing, transport, and storage due to environmental factors influencing survival (23, 45). Antimicrobial resistance profiles can differ substantially between the various
Campylobacter species and therefore the proportion of each species in the pool of isolates tested can impact the overall results (12). Another factor contributing to the observed differences among studies in the proportions of each species isolated and consequently susceptibility testing results, is the lack of standardization of isolation protocols (8, 42, 38, 18, 16, 48, 44, 26, 30, 4, 7, 9, 46).

In this study, the proportion of isolates resistant to clindamycin and erythromycin was significantly higher in C. coli than in C. jejuni as was also found in the NARMS data and for erythromycin in Ontario abattoir data (Table 4.2) (26, 44) Also, significantly more C. coli exhibited resistance patterns that included both ERY and TCY.

The range of MIC values observed in this study exceeded the ranges reported in a Canadian abattoir study for all antimicrobials common with this study (18). This is likely due to the substantially larger sample size in the current study. All isolates were susceptible to amoxicillin/clavulanic acid, chloramphenicol, and gentamicin. Less than ten percent of isolates were resistant to nalidixic acid (14 isolates, 1.9% [1.03%-3.12%]), ciprofloxacin (14 isolates, 1.9% [1.03%-3.12%]), clindamycin (24 isolates, 3.2% [2.06%-4.73%]), erythromycin (25 isolates, 3.3% [2.17%-4.89%]), and ampicillin (55 isolates, 7.3% [5.58%-9.45%]). Resistance to tetracycline was common (56.1% isolates [52.44%-59.67%]), but the overwhelming majority of resistance in the Campylobacter isolates was to trimethoprim/sulfamethoxazole (724 isolates, 96.7% [95.11%-97.83%]). However, trimethoprim was utilized in the isolation protocol for this study which may have biased these results.
Fluoroquinolones are utilized in the treatment of severe campylobacteriosis in adults and therefore the emergence of fluoroquinolone resistance in isolates from retail chicken is of concern for public health. Six of the seven C. lari isolates (85.7%) were resistant to ciprofloxacin. This was significantly different than the 6/677 (0.89%) seen in the C. jejuni isolates and the 2/65 (3.08%) seen in the C. coli isolates (p<0.001) (Table 4.2). All of the isolates in this study that were resistant to nalidixic acid were also resistant to ciprofloxacin. Cross-resistance between these two antimicrobials is a common finding in Campylobacter where, in contrast to some other foodborne pathogens, a single mutation in the gyrA gene can result in a high level of resistance to both antimicrobials (36). The proportion of isolates resistant to nalidixic acid and ciprofloxacin (1.9%), was substantially smaller than in recent results from the United States the United Kingdom, Korea and Japan (Table 4.3). Our findings are, however, consistent with other Canadian studies where less than 4% of Campylobacter isolates were resistant to ciprofloxacin (Table 4.3). These differences between countries may be due in part to differences in antimicrobial usage within their respective broiler chicken industries.

Fluoroquinolones were approved for use in broiler chickens in the United States in 1995 and that approval was withdrawn in 2005 due to concerns regarding antimicrobial resistance and public health (16). Fluoroquinolones have not been approved for use in broiler chickens in Canada (30), although extra-label drug use is not prohibited.

Erythromycin is the treatment of choice when antimicrobials are indicated in cases of campylobacteriosis involving children. It has also become the treatment of choice for severe campylobacteriosis in adults when resistance to fluoroquinolones is encountered (2, 1). Therefore resistance to this drug is of concern. In this work, 3.3% of isolates were resistant to
erythromycin and none of the ciprofloxacin resistant isolates were also resistant to erythromycin. This is substantially lower than previous Canadian studies but similar to NARMS data and results from the U.K. (Table 4.3). There was less resistance in a Danish study and Korean study (4, 19) but substantially more in another Korean and Japanese studies (24, 38) (Table 4.3). Interestingly, there was no resistance observed in either C. jejuni or C. coli in an American abattoir study (40). In the EUCAST summary of antimicrobial wild-type distributions there was no resistance to erythromycin demonstrated in C. jejuni or C. coli isolates utilizing the 32 μg/ml breakpoint (12). This differs from these results where significantly more C. coli (12.3%) were resistant to erythromycin than C. jejuni (2.5%) (two-sided, p=0.001) and that of Larkin and collaborators (27). Human campylobacteriosis is more frequently associated with C. jejuni, however clinical infections with C. coli also occur and therefore resistance to erythromycin in C. coli is of concern.

There was a moderate amount of resistance to tetracycline in these isolates (56.1%) which is consistent with other Canadian studies and surveillance data (30, 18, 16) (Table 4.3). The wild type distributions reported by EUCAST show no resistance to tetracycline for either C. jejuni or C. coli (12). Resistance to tetracycline is commonly seen in both pathogenic and commensal bacteria isolated from food animals and their products (16, 44). This is likely due at least in part to the extensive history of tetracycline use in food animal production in North America.

Previous work in Canada has reported 21.8% of isolates resistant to ampicillin (18) and international work has reported a range from 21.8% to 56.2% (Table 4.3). These estimates are substantially higher than the 7.3% found in the current study.
There was also a small amount of resistance to clindamycin (3.2%) in this study which is similar to abattoir studies and CIPARS Ontario retail data (Table 4.3).

A summary of the antimicrobial minimum inhibitory concentrations for the isolates in this study is presented in Table 4.3. There was no significant difference in the proportion of resistant isolates by store size (p>0.05). In this work, six isolates (0.8%) were resistant to five antimicrobials and no isolates were resistant to more than five antimicrobials (Table 4.4). The majority of the resistant isolates were resistant to one or two antimicrobials (301 isolates, 40.2%; 374, 49.9%), respectively. One percent of isolates (9 isolates) were susceptible to all ten antimicrobials tested. Of these nine, eight were C. jejuni and one was C. coli. This is similar to American (40) and Korean studies (24) where 0.5% and 1.2% respectively were susceptible to all antimicrobials tested. In another Korean study (19), there were no C. jejuni isolates that were susceptible to all antimicrobials tested. The result in the current study is substantially different than a previous Ontario study in provincial abattoirs where 32% of chicken isolates were susceptible to all ten antimicrobials tested (26). However, SXT was not included in the panel of the previous Ontario study and if SXT results are excluded from the current study, then the percentage of isolates susceptible to the other nine antimicrobials is 39.5%. This illustrates the point that comparisons between studies for multi-resistance frequencies are problematic due to differences in the antimicrobial resistance panels utilized and should be attempted or interpreted only with extreme caution. For instance, in the studies cited here the antimicrobial resistance panels varied between six (24), seven (40, 19) and ten (26) antimicrobials tested with only 4 antimicrobials common to all panels.
There were 15 different antimicrobial resistance patterns observed in these isolates. The most frequent was TCY-SXT (355 isolates, 48.0%). The second most common pattern of resistance was SXT (287 isolates, 38.8%). Other patterns were much less frequent and are described in Table 4.5. There were no isolates with a resistance pattern that included CIP and ERY and TCY. Andersen and collaborators in Denmark found 0.4% (2/767) of isolates of *C. jejuni* that were resistant to ciprofloxacin, erythromycin and tetracycline (4). Lévesque and co-workers in Québec, Canada found 1.8% (1/56) of *C. jejuni* isolates from chicken were resistant to all three antimicrobials (30). Interestingly, CIPARS 2003 data showed resistance to all three antimicrobials in one of 94 isolates from retail chicken in Québec but none of the 78 isolates from retail chicken in Ontario (18). There were 21 isolates (2.8%) with a resistance pattern that included both ERY and TCY. Of these, 15 were *C. jejuni* and 6 were *C. coli*. These results indicate that the proportion of resistant *C. coli* isolates with this pattern is significantly higher than the proportion of resistant *C. jejuni* isolates with this pattern (two-sided, p=0.002). The significantly greater proportion of *C. coli* isolates with the ERY-TCY pattern is likely due in part to the higher level of resistance to erythromycin in these isolates in general. Three isolates (0.4%) that were resistant to CIP were also resistant to TCY but no isolates were resistant to both CIP and ERY. The three isolates with the CIP-TCY pattern were *C. jejuni*. This is similar to CIPARS 2003 results from Ontario where 3.8% (3/78) of isolates were resistant to two of the three antimicrobials but substantially lower than the 18.1% (17/94) found in Québec isolates in that year.

This study contains a large collection of *Campylobacter* isolates from the systematic sampling of fresh retail chicken in Canada. The prevalence of antimicrobial resistant and susceptible
*Campylobacter* in retail chicken in the present study in Ontario was largely consistent with previous Canadian work, primarily in Ontario and Québec. However, apparent differences were observed particularly with resistance to erythromycin and nalidixic acid, both of which have human health implications (Table 4.3). The basis of these differences may include geographical differences in the source(s) of retail chicken, sampling intensity, inter-laboratory variation, and isolation methodologies. This reinforces the importance of designing retail sampling to be representative of the population of interest. The differences in prevalence of resistance and resistance patterns between *Campylobacter* species emphasize the importance of reporting this information in all studies investigating antimicrobial resistance in *Campylobacter*. There was resistance in these isolates to each of the three primary antimicrobials utilized for the treatment of human campylobacteriosis. Although there were no isolates resistant to all three of these antimicrobials, there were isolates that demonstrated resistance to two of the three antimicrobials and these included both *C. jejuni* and *C. coli*.

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12. European Committee on Antimicrobial Susceptibility Testing. Data from the European


Table 4.1: Prevalence of *Campylobacter* by sample type from fresh retail chicken in two health units in Ontario, Canada.

<table>
<thead>
<tr>
<th>Sample</th>
<th># isolates</th>
<th># samples</th>
<th>prevalence</th>
<th>95% Confidence Interval</th>
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<tbody>
<tr>
<td>Back</td>
<td>1</td>
<td>1</td>
<td>1.000</td>
<td>0.025</td>
</tr>
<tr>
<td>Breast</td>
<td>25</td>
<td>48</td>
<td>0.521</td>
<td>0.372</td>
</tr>
<tr>
<td>Drumstick</td>
<td>82</td>
<td>128</td>
<td>0.641</td>
<td>0.551</td>
</tr>
<tr>
<td>Half</td>
<td>7</td>
<td>12</td>
<td>0.583</td>
<td>0.277</td>
</tr>
<tr>
<td>Leg</td>
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<td>670</td>
<td>0.625</td>
<td>0.587</td>
</tr>
<tr>
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<td>122</td>
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</tr>
<tr>
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<td>271</td>
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<td>0.496</td>
</tr>
<tr>
<td>Wing</td>
<td>3</td>
<td>4</td>
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<td>0.194</td>
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Table 4.2: Antimicrobial resistance in *Campylobacter* isolates from fresh retail chicken in two health units in Ontario, Canada

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>n</th>
<th>%R</th>
<th>Distribution of MICs</th>
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<td>0.0</td>
<td>0 0 5 68 251 284 123 12 6 0 0 0 0 0 0 0 0</td>
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<td>677</td>
<td>0.8</td>
<td>0 0 5 67 239 262 91 6 1 0 0 0 0 0 0 0 0</td>
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<tr>
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<td>65</td>
<td>0.6</td>
<td>0 0 0 0 0 8 14 32 0 0 0 0 0 0 0 0 0</td>
</tr>
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<td>0.3</td>
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<td>1.9</td>
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<td>0 2 0 0 5 92 459 192 26 7 1 1 0 0 0 5</td>
</tr>
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<tr>
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<td>0.6</td>
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</tr>
<tr>
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<td>1 1 2 14 114 339 180 44 28 0 1 0 0 25</td>
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<td>677</td>
<td>2.5</td>
<td>0 1 2 11 33 161 66 23 7 0 1 0 0 17</td>
</tr>
<tr>
<td>C. coli</td>
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<td>0.3</td>
<td>0 0 0 0 3 10 21 21 0 0 0 0 0 0</td>
</tr>
<tr>
<td>C. lari</td>
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<td>0.6</td>
<td>0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0</td>
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<td>0.8</td>
<td>1 0 3 37 368 298 0 0 0 0 0 0 0 0 0 0 0</td>
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<td>3.1</td>
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<tr>
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<td>C. jejuni</td>
<td>677</td>
<td>4.5</td>
<td>0 0 0 0 0 0 0 1 3 5 3 9 6 4 0 0 44</td>
</tr>
<tr>
<td>C. coli</td>
<td>65</td>
<td>0.5</td>
<td>0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 44</td>
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<tr>
<td>C. lari</td>
<td>7</td>
<td>0.0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>749</td>
<td>0.0</td>
<td>0 2 0 2 0 46 401 219 54 23 2 0 0 0</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>677</td>
<td>0.8</td>
<td>0 2 0 2 0 44 291 160 38 11 1 0 0 0 0</td>
</tr>
<tr>
<td>C. coli</td>
<td>65</td>
<td>0.5</td>
<td>0 0 0 0 0 0 2 3 24 26 16 0 0 0 0 0</td>
</tr>
<tr>
<td>C. lari</td>
<td>7</td>
<td>0.0</td>
<td>0 0 0 0 0 0 1 5 0 0 1 0 0 0 0 0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>749</td>
<td>56.1</td>
<td>1 37 183 57 36 10 3 2 0 0 1 11 10 398</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>677</td>
<td>5.5</td>
<td>1 25 30 49 22 9 3 2 0 0 1 11 9 35</td>
</tr>
<tr>
<td>C. coli</td>
<td>65</td>
<td>4.9</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>C. lari</td>
<td>7</td>
<td>3.2</td>
<td>0 0 1 2 1 0 0 0 0 0 0 0 0 0 0 1 0</td>
</tr>
</tbody>
</table>

Note: * Roman numerals I-III indicate the ranking of human health Importance (Veterinary Drugs Directorate). The unshaded fields indicate the range tested for each antimicrobial on the E-test strip. % R is the percentage of resistant isolates. Solid bars represent the resistance breakpoints.

* Resistance in *C. coli* significantly higher than resistance in *C. jejuni*.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study Location</th>
<th>Sample Location</th>
<th>Species(^c)</th>
<th>n</th>
<th>AMC</th>
<th>AMP</th>
<th>CIP</th>
<th>CLI</th>
<th>CHL</th>
<th>ERY</th>
<th>GEN</th>
<th>NAL</th>
<th>SXT</th>
<th>TCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>2002-2004 ON, Canada</td>
<td>Retail – fresh</td>
<td>J, C, L</td>
<td>749</td>
<td>0</td>
<td>7.3</td>
<td>1.9</td>
<td>3.2</td>
<td>0</td>
<td>3.3</td>
<td>0</td>
<td>1.9</td>
<td>96.7</td>
<td>56.1</td>
<td></td>
</tr>
<tr>
<td>CIPARS</td>
<td>2003 ON, Canada</td>
<td>Retail – fresh</td>
<td>J, C, s</td>
<td>78</td>
<td></td>
<td></td>
<td>3.9</td>
<td>7.8</td>
<td>1.3</td>
<td>10.3</td>
<td>0</td>
<td>10.3</td>
<td>57.7</td>
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<td></td>
</tr>
<tr>
<td>CIPARS</td>
<td>2003 QC, Canada</td>
<td>Retail–fresh</td>
<td>J, C, s</td>
<td>94</td>
<td></td>
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<td>3.2</td>
<td>20.4</td>
<td>0</td>
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<td>1.1</td>
<td>5.3</td>
<td>70.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lévesque</td>
<td>2007 QC, Canada</td>
<td>Retail – fresh</td>
<td>J</td>
<td>56</td>
<td></td>
<td></td>
<td>1.8</td>
<td></td>
<td></td>
<td>16</td>
<td></td>
<td>58.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larkin</td>
<td>2006 ON, Canada</td>
<td>Abattoir–swabs</td>
<td>J, C</td>
<td>435</td>
<td></td>
<td></td>
<td>3.7</td>
<td></td>
<td></td>
<td>0</td>
<td>6.7</td>
<td>0.2</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guévremont</td>
<td>2006 QC, Canada</td>
<td>Abattoir–caecal</td>
<td>J, C</td>
<td>188</td>
<td></td>
<td>21.8</td>
<td>1.1</td>
<td>6.9</td>
<td>0</td>
<td>6.9</td>
<td>0</td>
<td></td>
<td>65.4</td>
<td></td>
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<tr>
<td>NARMS</td>
<td>2003 USA</td>
<td>Retail–fresh</td>
<td>J, C</td>
<td>469</td>
<td></td>
<td></td>
<td>14.1</td>
<td></td>
<td></td>
<td>0</td>
<td>2.8</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Son</td>
<td>2007 USA</td>
<td>Abattoir–carcass</td>
<td>J, C</td>
<td>215</td>
<td></td>
<td></td>
<td>27</td>
<td>1.4</td>
<td></td>
<td>0</td>
<td>0</td>
<td>27</td>
<td></td>
<td>99.1</td>
<td></td>
</tr>
<tr>
<td>Kang</td>
<td>2000-2002 Korea</td>
<td>Retail d</td>
<td>J, C</td>
<td>594</td>
<td></td>
<td>87.9</td>
<td></td>
<td>1.3</td>
<td>19.4</td>
<td></td>
<td></td>
<td>91.4</td>
<td></td>
<td>87.2</td>
<td></td>
</tr>
<tr>
<td>Han</td>
<td>2004 Korea</td>
<td>Retail d</td>
<td>J</td>
<td>116</td>
<td></td>
<td>43.1</td>
<td></td>
<td>92.2</td>
<td>2.6</td>
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<td>6.9</td>
<td>92.2</td>
<td></td>
<td>99.1</td>
<td></td>
</tr>
<tr>
<td>Sallam</td>
<td>2006 Japan</td>
<td>Retail b</td>
<td>J, C</td>
<td>195</td>
<td></td>
<td>39</td>
<td></td>
<td>51.8</td>
<td>1.5</td>
<td>18.5</td>
<td>2.6</td>
<td>62.1</td>
<td></td>
<td>34.9</td>
<td></td>
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<tr>
<td>Andersen</td>
<td>1999-2003 Denmark</td>
<td>Retail d</td>
<td>J</td>
<td>460</td>
<td></td>
<td>7.4</td>
<td></td>
<td>0.3</td>
<td>0.7</td>
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<td></td>
<td></td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Little</td>
<td>2003-2005 UK</td>
<td>Retail – fresh</td>
<td>J, C</td>
<td>89</td>
<td></td>
<td>56.2</td>
<td></td>
<td>16.9</td>
<td>4.5</td>
<td>2.3</td>
<td>1.1</td>
<td>19.1</td>
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<td>47.2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) results not cited where breakpoints differed from current study and raw data not presented

\(^b\) included chicken by-products containing internal organs

\(^c\) J: *Campylobacter jejuni*; C: *Campylobacter coli*; L: *Campylobacter lari*; s: *Campylobacter* spp.

\(^d\) no differentiation made between fresh and/or previously frozen chicken
Table 4.4: Multiple resistance in Campylobacter isolates from retail chicken in two health units in Ontario, Canada

<table>
<thead>
<tr>
<th># of Antimicrobials in</th>
<th># Isolates (%)</th>
<th># C. jejuni isolates (%)</th>
<th># C. coli isolates (%)</th>
<th>C. lari isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9 (1.2)</td>
<td>8 (1.2)</td>
<td>1 (1.5)</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>301 (40.2)</td>
<td>286 (42.3)(^a)</td>
<td>15 (23.1)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>374 (49.9)</td>
<td>335 (49.5)</td>
<td>38 (58.5)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>3</td>
<td>39 (5.2)</td>
<td>30 (4.4)</td>
<td>5 (7.7)</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td>4</td>
<td>20 (2.7)</td>
<td>13 (1.9)</td>
<td>5 (7.7)(^b)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>5</td>
<td>6 (0.8)</td>
<td>5 (0.7)</td>
<td>1 (1.5)</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Resistance in C. jejuni significantly higher than resistance in C. coli, p<0.05
\(^b\) Resistance in C. coli significantly higher than resistance in C. jejuni, p<0.05
Table 4.5: Antimicrobial resistance patterns in *Campylobacter* isolates from fresh retail chicken in two health units in Ontario, Canada (n=749)

<table>
<thead>
<tr>
<th>Antimicrobial Resistance Pattern</th>
<th># of Isolates</th>
<th>% of Resistant Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCY-SXT</td>
<td>355</td>
<td>48.0 %</td>
</tr>
<tr>
<td>SXT</td>
<td>287</td>
<td>38.8 %</td>
</tr>
<tr>
<td>AMP-TCY-SXT</td>
<td>25</td>
<td>3.4 %</td>
</tr>
<tr>
<td>AMP-SXT</td>
<td>17</td>
<td>2.3 %</td>
</tr>
<tr>
<td>CLI-ERY-TCY-SXT</td>
<td>15</td>
<td>2.0 %</td>
</tr>
<tr>
<td>TCY</td>
<td>13</td>
<td>1.8 %</td>
</tr>
<tr>
<td>CIP-NAL-SXT</td>
<td>9</td>
<td>1.2 %</td>
</tr>
<tr>
<td>AMP-CLI-ERY-TCY-SXT</td>
<td>6</td>
<td>0.8 %</td>
</tr>
<tr>
<td>CIP-NAL-TCY-SXT</td>
<td>3</td>
<td>0.4 %</td>
</tr>
<tr>
<td>AMP-CIP-NAL-SXT</td>
<td>2</td>
<td>0.3 %</td>
</tr>
<tr>
<td>AMP-ERY-SXT</td>
<td>2</td>
<td>0.3 %</td>
</tr>
<tr>
<td>AMP-TCY</td>
<td>2</td>
<td>0.3 %</td>
</tr>
<tr>
<td>CLI-ERY-SXT</td>
<td>2</td>
<td>0.3 %</td>
</tr>
<tr>
<td>AMP</td>
<td>1</td>
<td>0.1 %</td>
</tr>
<tr>
<td>CLI-TCY-SXT</td>
<td>1</td>
<td>0.1 %</td>
</tr>
</tbody>
</table>

Chapter 5: Molecular epidemiology of *Campylobacter jejuni* human and chicken isolates from two health units in Ontario

Abstract:

A population-based study was conducted over a two year period in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario, with an objective of using Comparative Genomic Fingerprinting with a 40 gene assay (CGF40) to investigate the association between human cases of campylobacteriosis and *Campylobacter* isolates from retail chicken. CGF results were available for isolates from 115 human cases and 718 retail chicken samples. These data were combined with CGF results from a large reference database of *Campylobacter* isolates. Isolates were categorized into types based on ≥90% CGF40 fingerprint similarity (CGF-90%). CGF-90% types were categorized as chicken associated (CA90) when the proportion of animal isolates in the given type that originated from chicken was at least 80% and was statistically significant. Risk factor data were collected from cases by questionnaire. Urban cases were significantly more likely than rural cases to be CA90 and there were significantly fewer CA90 cases in the second year of the study. Due to the population distribution in Canada and most industrialized countries, the majority of *Campylobacter* cases are urban dwellers. Therefore, the association between urban cases and chicken associated types of *Campylobacter* emphasizes the importance of educational and food safety efforts to reduce the impact of *Campylobacter* from retail chicken on public health. Sources other than chicken may be more important for rural dwellers.
Introduction

Although *Campylobacter* has been identified as a cause of disease in animals for many years, the identification of *Campylobacter* as the most common cause of bacterial gastroenteritis in humans is relatively recent [1, 2]. *Campylobacteriosis* in humans typically occurs as sporadic infection and outbreaks are uncommon [1, 2]. A large number of zoonotic reservoirs has been identified for human campylobacteriosis including poultry, ruminants, and wild birds [2-4]. Exposure routes include direct contact, food, contaminated milk, and contaminated water [2-5]. International travel is also a commonly identified risk factor [2, 4, 6]. Case-control studies have consistently identified chicken consumption as a major risk factor for campylobacteriosis [2, 4, 6-9]. Additional research has estimated that the proportion of human campylobacteriosis cases that can be attributed to the consumption of chicken ranges from 20 to 80 percent [3, 10, 11].

In order to fully investigate the epidemiology of *Campylobacter* in the human population, it would be useful to be able to distinguish epidemiologically-linked isolates by molecular subtyping methods. However, the development of useful molecular sub-typing techniques for *Campylobacter* has been complicated by the high genetic diversity and weak clonal population structure of this genus [12, 13]. Currently available methods, including PFGE, *fla* typing, amplified fragment length polymorphism (AFLP), and multilocus sequence typing (MLST), have been problematic due to issues with their discriminatory ability as well as the financial resources and expertise they require [12-14]. The recently-developed Comparative Genomic Fingerprinting (CGF) method is a rapid, low cost and highly discriminatory alternative to Multi-
locus Sequence Typing (MLST) [13] that has been recently used to identify genomic clusters of
*C. jejuni* and *C. coli* from surveillance data [14].

Accordingly, a population-based study was conducted over a two year period in the Perth
District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario, with an objective
of investigating the relationship between sporadic cases of campylobacteriosis in humans and
*Campylobacter* from retail chicken based on CGF molecular sub-typing.

Methods

Data collection and laboratory methods for this project have been previously described [Chapter
3: 15, 16]. In brief, laboratory-confirmed human cases of campylobacteriosis during the study
period were eligible for inclusion in the study. Health unit personnel contacted cases, obtained
consent for participation and administered a questionnaire. Case isolates were forwarded to the
Public Health Ontario Laboratory (PHL) for confirmation, speciation, and antimicrobial
susceptibility testing. Over the same time period and geographical area, retail chicken samples
were obtained according to a representative sampling plan. These samples were cultured at the
Laboratory for Foodborne Zoonoses, Public Health Agency of Canada (LFZ-PHAC) and the
resulting isolates were speciated and tested for antimicrobial susceptibility. Minimum inhibitory
concentrations were determined by the E test for 9 antimicrobials including ciprofloxacin (CIP;
breakpoint: > 4 μg/ml).
Comparative Genomic Fingerprinting utilizing a 40 gene assay (CGF40) was performed at the LFZ-PHAC on the study isolates from human cases and retail chicken in order to provide molecular sub-typing information [13]. A reference database (RD) of over 6000 *Campylobacter* isolates from environmental, animal, food and clinical sources from multiple surveillance and research initiatives was assembled by LFZ-PHAC and analyzed by CGF40. Isolates were categorized into types based on ≥ 90% (CGF-90%) and ≥ 95% (CGF-95%) fingerprint similarity as previously described [13]. This database included the retail chicken isolates from this study as well as another 369 retail chicken isolates, primarily from the C-Enternet program [17] and the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) [18] and 138 chicken fecal isolates from British Columbia, Alberta, and Ontario. The RD was made available for inclusion in the analyses of this project.

Potential risk factor data for cases including age, gender, date of illness, income, chicken contact, chicken consumption, urban/rural residence, health unit of residence, and international travel in the 10 days prior to illness were collected as part of the telephone questionnaire [Chapter 3; 15]. In addition, the severity of illness was assessed based on a five point scale via the questionnaire [Chapter 3; 15]. The University of Guelph Research Ethics Board approved this research project.

**Statistical analysis**

Stata Intercooled version 12 (Stata Corporation, College Station, Texas, USA) was used for the analysis.
Using animal-origin isolates from the RD, CGF-90% types were classified as “Chicken Associated” (CA90) when the proportion of isolates in the given type that originated from chicken was at least 80% and was statistically significant with Chi-square or Fisher’s exact analysis as appropriate. This process was repeated to classify “Chicken-Associated” types at the CGF-95% level (CA95). Not included in this analysis were environmental isolates from the RD including those from water, sand, fluff and dust samples, and human clinical isolates.

Exact logistic regression, with CA90 as the outcome, was used to identify questionnaire-derived risk factors for human infection with “Chicken-Associated” types. Variables significant at a p<0.2 were considered for inclusion into the multivariable analysis. Variables with a prevalence of less than two per cent among cases were omitted from further analysis. When variables were highly collinear (p<0.05), one was selected for inclusion into the multi-variable analysis based on level of significance, number of missing observations and reliability. This analysis was repeated using the CA95 classification as the outcome.

The preliminary main effects exact logistic regression model was developed utilizing manual backward selection. When all remaining variables were significant at p≤0.05 or were confounders (removal changed the coefficient of another covariate by > 20%), variables that were not significant in the univariable analysis were introduced and evaluated for significance. Plausible interaction terms for the resulting model were assessed. The model was evaluated by comparing the observed and the expected values for each covariate pattern.
Results

Demographic and isolate speciation and susceptibility data from human cases and retail chicken from this study have been previously reported [Chapter 3; 15, 16]. Briefly, of the 317 human cases eligible for inclusion in the study, 250 (79%) consented to participate and completed the questionnaire and isolates from 124 cases were received at the laboratory. Isolates from the remaining 126 cases were discarded in error at the primary laboratory or were unable to be matched with case data. One hundred and twenty-one isolates (97.6%) from human cases were *Campylobacter jejuni* and 3 (2.4%) *Campylobacter coli*. *Campylobacter* was isolated from 749 of 1256 (59.6%) retail chicken samples collected in the study catchment areas. These isolates were primarily *C. jejuni* (90.4%) although there were small numbers of *C. coli* (8.7%) and *Campylobacter lari* (0.9%). Resistance to ciprofloxacin was found in 14 isolates from humans (1.9%) and six isolates (4.8%) from retail chicken.

Results from CGF analysis were available from 115 human clinical isolates with 55 CGF-90% types and 75 CGF-95% types. There were 21 CA90 types and 9 of these were found in 30 human cases from this study. There were 32 CA95 types and 10 of these were found in 23 human cases. Eighteen cases had a CGF-90% type that was not present in the RD and thirty cases had a CGF-95% type that was not found in the RD. These cases were dropped from the CA90 and CA95 analyses respectively, since it could not be determined from the available data whether or not they were chicken associated. There were 2922 animal-source isolates from the RD, including 718 chicken isolates from this study, included in the Chi-Square/Fisher’s Exact analysis. The percentage of chicken isolates in CGF-90% types that were significant with Chi-
square/Fisher’s Exact ranged from 56% to 100%, although three-quarters of these types had more than 80% of isolates from chicken. For CGF-95% types that were significant with Chi-square/Fisher’s Exact the percentage of chicken isolates ranged from 62% to 100% but 78% of these types had more than 80% of isolates from chicken.

**Exact logistic regression analysis**

Exact logistic regression was performed with CA90 as the outcome variable. Among cases included in the analysis, the median age was 24.5 (range: 0.3-85.7) years, 78% of cases occurred between June and November, approximately 50% of cases occurred in each year of the study, and 43.8% of cases were in the highest income bracket (> $60,000/yr). The hospitalization rate for cases was 9.3%. The proportion of urban cases was very similar in the two study health units (WDG: 68%, PD: 66%). As well, the proportion of urban and rural cases from the overall study that were included in the model dataset was similar (urban: 33%, rural: 41%, p=0.22). In the univariable analysis, season and year of illness were significantly associated with CA90 but were also collinear (r<-.3, p<0.001). Year was included in the multi-variable model because it was more significantly associated with CA90. Additional variables that were assessed in the univariable analysis are described in Table 5.2. Although handling raw chicken was significantly associated with the outcome on univariable analysis, it was not submitted to the multi-variable model due to the high proportion of missing observations (19.6%) and the small overall sample size. The following variables were included in the multivariable model prior to backward selection: urban/rural residence, living on a farm with chickens, egg consumption in
the 10 days prior to illness, having a *Campylobacter* isolate that was resistant to ciprofloxacin, and year of illness. Following backward selection, the preliminary multivariable model included urban/rural residence and year of illness.

None of the non-significant variables in the univariable analysis were significant when added to the preliminary model. No plausible interaction terms were significant. The evaluation of the model indicated that the observed and expected values were very similar.

Therefore the final model included urban/rural residence and year of illness (Table 5.2). Cases living in a city or town were 6.3 times more likely to have an isolate with a CGF-90% type that was associated with chicken (i.e. CA90) than rural cases. Cases from the second year of the study were 84% less likely to have a CA90 isolate. An urban case in year 2 had a 3% less chance of having a CA90 isolate than an urban case in year 1 and a rural case in year 2 had an 84% less chance of having a CA90 isolate than a rural case in year 1. Although age was not statistically significant in the model, there was a significantly higher proportion of cases under the age of 5 in rural vs. urban cases (35% vs. 4%, p<0.001) and 85% of cases under the age of 5 were rural.

Exact logistic regression was also performed with CA95 as an outcome, however, no risk factor variables remained in the preliminary model, which was also non-significant.

Discussion

Urban cases (those self-identifying as living in a city, town, village or hamlet) were substantially more likely to have an isolate with a CGF-90% type that was chicken-associated than were non-
urban cases (those self-identifying as rural) (OR 6.3 [1.8-26.6]). This suggests that retail chicken may be a more important source of *Campylobacter* infection for those in an urban environment, while other sources may be more important for infection in people living in a rural environment. These findings are consistent with studies in New Zealand and Scotland where urban cases were more likely to be infected with chicken MLST types than rural cases, particularly in children [19, 20]. Since age was not significant on univariable analysis or in the multi-variable model from this study, other factors may also play a role in the CA90 differences seen between rural and urban cases. These factors may include sources of campylobacter other than chicken, such as cattle (direct contact, unpasteurized milk), and drinking or recreational water. While we did not collect samples from these sources in our study, isolates from ruminants were present in the RD and previous research has shown that ruminants may be a significant source of *Campylobacter* in human cases, particularly for young, rural children [10, 19, 20]. Due to the low prevalence of *Campylobacter* on retail beef, it is likely that direct exposure to cattle and/or consumption of untreated water contaminated by cattle manure would be more important pathways of exposure than consumption of contaminated beef [3, 18]. It would be expected that these pathways would put rural residents at a higher risk than urban residents. In this study, 46.9% (15/32) of rural cases and 10% (4/37) of urban cases had contact with cattle in the 10 days prior to illness (p=0.001) and 41% lived on a farm where cattle were kept. In contrast, 21% (7/34) of rural cases and 15% (8/53) of urban cases had contact with chicken in the 10 days prior to illness (p=0.508) and 21% lived on a farm where chickens were kept. Therefore, exposure to cattle was more common in the rural cases than exposure to chicken whereas direct exposure to both cattle and chicken was uncommon in urban cases. This is consistent with the agricultural profile of these
health units where dairy farming is prevalent. It is also consistent with previous findings that exposure to chicken through the food chain is more important than direct exposure in campylobacteriosis [4].

Unpasteurised milk has been implicated as a source of Campylobacter and exposure would be expected to be higher in rural residents [21]. In this study 9/88 (10%) of cases reported drinking unpasteurised milk in the 10 days before becoming ill and all 9 were rural cases.

In Ontario, rural residents are less likely than urban residents to have treated municipal water supplies and untreated water has been identified as a potential source of Campylobacter [2, 4]. In this research, 19/19 (100%) of rural resident cases did not use a municipal water supply, whereas 53/54 (98%) of urban cases did.

Year of illness was a significant variable in this model. The breakdown of cases between years in this study was similar (year 1: 56%, year 2: 44%, p>0.05). There was no significant difference in the proportion of the total cases from each year that were included in the model (year 1-50/110, 45.5%; year 2-39/122, 32.0%, p>0.05). There were approximately twice as many urban as rural cases in both year 1 and year 2. There were also no significant differences between year 1 and year 2 with respect to bovine contact, drinking unpasteurized milk, or living on a farm with cattle. Therefore, the substantial drop in the proportion of cases with a CA90 from year 1 to year 2 may be due to risk factors not included in this study. It may also be due to risk factors that were included in the study questionnaire but due to a high number of missing observations could not be adequately assessed in the analysis. Although the odds of having a
CA90 type were significantly reduced in year 2, urban cases in year 2 were still significantly more likely to have a CA90 type than rural cases.

Variables that assessed exposure to chicken through food were not significant in this model. Handling of raw chicken was significant on univariable analysis but was not included in the multi-variable model due to the percentage of missing observations (19.6%). Several other variables related to chicken consumption also had a substantial percentage of missing observations including fresh chicken consumption (26.8%) and barbequed chicken consumption (23.7%). It is possible that multiple routes of exposure to chicken through food occurred in cases from this study. Previous case-control studies have identified various chicken handling and consumption risk factors to be associated with campylobacteriosis [4, 6, 8]. Multiple routes of exposure combined with the overall high levels of chicken handling and consumption in the population, make the identification of specific risk factors for campylobacteriosis from chicken through the food pathway difficult [7]. This is further complicated by the possibility that older children and adults in rural environments may have higher levels of immunity to *Campylobacter* due to previous repeated exposure, primarily to dairy cattle and may therefore be less susceptible to campylobacteriosis from chicken sources [21, 23].

When the analysis was repeated with CGF-95% categorization, there were no significant results. Due to the strong tendency of *Campylobacter* towards recombination and mutation and the highly discriminatory power of CGF [14], typing based on a lower level of similarity (i.e. 90%) may be more useful when examining the relationship between human cases and potential risk
factors for campylobacteriosis. An incomplete representation of chicken types in the RD at the 95% level or insufficient power may also have contributed to the lack of significant results.

The low cost and high volume capacity of the CGF40 molecular sub-typing method facilitated the analysis of all available chicken and human isolates from this study. The highly discriminatory nature of this method resulted in a large number of types found in chicken and human isolates at the CGF-90% and CGF-95% levels. In order to represent the maximum diversity of CGF types possible, data from the chicken isolates in this study were combined with the RD assembled by the LFZ-PHAC.

Isolates from environmental sources were not included in the analysis used to identify “Chicken-Associated” CGF-90% types since Campylobacter from water, sand, etc. is expected to be the result of contamination from other sources [2, 3, 7]. Although fluff samples may have been collected in poultry barns, they may represent sources other than chicken such as wild birds, rodents, turkey, and other poultry and therefore were excluded. Similarly, isolates from human cases were excluded from this portion of the analysis. The very low level of human-to-human transmission of Campylobacter suggests that humans are not a reservoir for Campylobacter, rather infection is derived from food or other environmental sources [2, 7]. The use of Chi Square/Fisher’s Exact analysis in the first step of screening the CGF types ensured a minimum number of isolates in a specific type and avoided the inclusion of types with very small numbers of isolates, all of which were chicken (eg. 1of 1 = 100%) from being classified as CA90. The criterion that 80% of isolates in that type originate from chicken prevented the loss in specificity.
that would result if types with relatively low proportions of chicken isolates were included in the analysis.

The RD included in the analysis of data from this study is comprised of isolates made available to LFZ-PHAC from a variety of research projects and ongoing surveillance programs. The degree to which it is representative of Campylobacter sources in Canada is unknown. The cost and logistics involved in sample collection and primary isolation make it difficult for reference databases to contain isolates which are representative of the organism in all potential sources, and an improved understanding of Campylobacter epidemiology will require efforts to continually improve the representativeness of the RD. The deployment of new sub-typing methods that are rapid, low cost and highly discriminatory will facilitate the molecular characterisation of isolates from such broad-based sampling efforts.

Conclusions:

Urban cases in this study were 6.3 times more likely to have an isolate with a CGF-90% that was associated with chicken (i.e. CA90) than rural cases. Further research is required into the epidemiology of Campylobacter among rural cases as factors other than exposure to chicken through the food chain may predominate. However, due to the population distribution in Canada and most industrialized countries, the majority of Campylobacter cases are urban dwellers. Therefore, the association between urban cases and chicken associated types of Campylobacter emphasizes the importance of educational and food safety efforts to reduce the impact of Campylobacter from retail chicken on public health.
Acknowledgements:

We wish to acknowledge C. Clarke, J. de Grosbois, for questionnaire administration, participating hospital and private laboratories for providing isolates, S. Brown and K. Harris for speciation and antimicrobial susceptibility testing. We acknowledge R. Bean, N. Bunimov, K. Farrell, C. Gill, R. Imgrund, A. Mather, M. B. Varughese and V. Young, who contributed to the sample processing, isolation and characterization of the *Campylobacter* chicken isolates.
Table 5.1: Potential covariates evaluated in univariable exact logistic regression analysis of "Chicken Associated" Comparative Genomic Fingerprint type in laboratory-confirmed cases of campylobacterosis in Perth District and Wellington-Dufferin-Guelph health units

<table>
<thead>
<tr>
<th>Variable</th>
<th>Present/Total</th>
<th>Percentage</th>
<th>Significant*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>55 male; 42 female</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Live on farm with chickens</td>
<td>7/97</td>
<td>7.2</td>
<td>Yes</td>
</tr>
<tr>
<td>Exposure to chickens</td>
<td>16/95</td>
<td>16.8</td>
<td>No</td>
</tr>
<tr>
<td>Live in city or town</td>
<td>62/96</td>
<td>64.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Household member worked in slaughter plant</td>
<td>4/94</td>
<td>4.3</td>
<td>No</td>
</tr>
<tr>
<td>Handling raw chicken</td>
<td>25/78</td>
<td>32.1</td>
<td>Yes*</td>
</tr>
<tr>
<td>Chicken consumption</td>
<td>67/84</td>
<td>79.8</td>
<td>No</td>
</tr>
<tr>
<td>Undercooked chicken consumption</td>
<td>8/83</td>
<td>9.6</td>
<td>No</td>
</tr>
<tr>
<td>Fresh chicken consumption</td>
<td>25/71</td>
<td>35.2</td>
<td>No</td>
</tr>
<tr>
<td>Chicken consumption outside of the home</td>
<td>37/84</td>
<td>44.1</td>
<td>No</td>
</tr>
<tr>
<td>Barbequed chicken consumption</td>
<td>22/74</td>
<td>29.7</td>
<td>No</td>
</tr>
<tr>
<td>Egg consumption</td>
<td>67/90</td>
<td>74.4</td>
<td>Yes</td>
</tr>
<tr>
<td>Undercooked egg consumption</td>
<td>30/79</td>
<td>38.0</td>
<td>No</td>
</tr>
<tr>
<td>Scrambled egg consumption outside of the home</td>
<td>3/82</td>
<td>3.7</td>
<td>No</td>
</tr>
<tr>
<td>International travel</td>
<td>7/97</td>
<td>7.2</td>
<td>No</td>
</tr>
<tr>
<td>* not submitted to multi-variable model due to high proportion of missing observations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>** significant at p&lt;0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Campylobacter* isolate resistant to ciprofloxacin
Table 5.2: Exact logistic regression model for Chicken-associated CGF* types at the 90% fingerprint similarity level in laboratory-confirmed cases of *Campylobacter* in Perth District and Wellington-Dufferin-Guelph health units (n=89)

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>Sufficient Statistic</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban/Rural</td>
<td>6.32</td>
<td>25</td>
<td>0.0016</td>
<td>1.82</td>
</tr>
<tr>
<td>Year</td>
<td>0.15</td>
<td>6</td>
<td>0.0007</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Model Score: 19.93615; Pr>= score ≤ 0.0001

*Comparative Genomic Fingerprinting with 40 gene assay.
References:


Chapter 6: Summary Discussion and Conclusions

While campylobacteriosis is the most common cause of bacterial gastroenteritis in many countries including Canada [1, 2], there are significant gaps in our understanding of the epidemiology of this disease. The objectives of this thesis were to address some of these gaps by conducting a population-based study in two health unit areas in Ontario to investigate:

1. the clinical signs, symptoms, and severity of clinical cases of campylobacteriosis
2. antimicrobial use and antimicrobial resistance associated with clinical cases of campylobacteriosis
3. the factors associated with duration of illness in clinical cases of campylobacteriosis using survival analysis.
4. the prevalence of *Campylobacter* contaminated retail chicken in these health units
5. antimicrobial resistance among *Campylobacter* isolates from retail chicken
6. the association between isolates from human sporadic cases of campylobacteriosis and *Campylobacter* isolates from retail chicken based on CGF molecular sub-typing.

The research described in this thesis makes several key contributions to the understanding of the epidemiology of campylobacteriosis. An association between urban residence of cases and infection with chicken-associated strains of *Campylobacter* was found based on CGF molecular sub-typing, which supports previous research in New Zealand and Scotland where this relationship was identified based on MLST sub-typing [3, 4]. Determining the underlying factors responsible for the difference between urban and rural cases with respect to chicken-associated strains of *Campylobacter* is an important area of future research. Also, Cox proportional hazard
models identified a consistent effect of antimicrobial treatment on duration of illness regardless of the point of its initiation during the course of illness. This is in contrast to previous reports based on the mean duration of illness, which suggested that early antimicrobial treatment was more effective [5, 6]. Our research also found an increased rate of symptom resolution with macrolide treatment for less than the recommended period of time or treatment with ciprofloxacin for the recommended period of time when compared to cases with no antimicrobial treatment. This information provides guidance to physicians in cases where antimicrobial treatment is indicated. Although this research focussed on a two-year snapshot of campylobacteriosis in two health units in Ontario, its findings have wider public health surveillance implications. Incorporating and integrating some of the basic components of this study, such as the collection of antimicrobial use and urban/rural residence data as part of standardized risk factor questionnaires and the routine speciation, antimicrobial susceptibility testing, and molecular sub-typing of isolates, to ongoing surveillance at the national level would provide valuable data for public policy, physician guidance and public education purposes.

National surveillance for clinical Salmonella isolates has been implemented through federal-provincial agreements and is facilitated by the National Microbiology Laboratory and the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). This is not the case for Campylobacter in human cases although surveillance of human cases and retail chicken occurs in sentinel sites through the C-Enternet program [7] and surveillance of retail chicken is approaching national coverage through the CIPARS program [8]. Human campylobacteriosis is currently reportable to the federal government and the annual number of cases is determined. However, there is no system in place to collect either Campylobacter
isolates or basic risk factor information in order to better understand the epidemiology of this disease in Canada. One of the logistical difficulties encountered in this research project was identification of cases from the study area at the primary isolation laboratory. From a logistical standpoint, the forwarding of all isolates or a representative sub-set of isolates (e.g. isolates from the first week of the month or a systematic random selection) would be simple and potentially result in a high level of compliance. The recent development and validation of the rapid and low cost CGF molecular sub-typing method for Campylobacter [9] presents an opportunity for routine molecular characterization of surveillance isolates. The roles of federal and provincial laboratories in speciation, antimicrobial susceptibility testing and molecular sub-typing would need to be determined. Currently, routine collection of risk factor data is the responsibility of local public health authorities but there is large variation in the approach of local public health authorities to this issue. Some jurisdictions administer a detailed questionnaire to laboratory-confirmed Campylobacter cases, while others do not collect any information. Standardization of the basic elements of a questionnaire including but not limited to: travel history, age, medication(s) used, and urban/rural residence would need to be established and implemented in at least a representative sub-set of local public health areas in order to provide coherent national data. In order for an ongoing surveillance system for Campylobacter to become a reality, adequate resources at the local, provincial, and federal level would be required to generate risk factor data and process this influx of isolates. However risk factor data when combined with routine antimicrobial susceptibility testing and molecular sub-typing, would provide very powerful information on campylobacteriosis in Canada. This information would be very
valuable in the effort to decrease the impact of campylobacteriosis on the Canadian public, by identifying specific risk groups and behaviours that should be targeted.

Analysis of questionnaires from the 250 cases of campylobacteriosis included in this research revealed that 165 cases (66.0%) reported staying at home or being hospitalized due to their symptom severity. Severity of illness was significantly associated with the number of days cases were unable to work outside of the home or attend school but was not significantly associated with the duration of illness (Chapter 3). The symptoms and clinical signs most commonly reported included: diarrhea, fatigue, stomach cramps, loss of appetite, and fever. Less than half of cases reported blood in the stool although this was significantly higher for those under 5 years of age. This was consistent with previous findings that symptoms of campylobacteriosis resemble other causes of gastroenteritis and cannot be diagnosed based on clinical symptoms alone [6, 10]. While submission of a fecal sample for culture is currently required to diagnose campylobacteriosis, in this research fecal culture results were not available for 179 cases (78.9%) until the day symptoms ended or later (Chapter 2). This is due to the extended period of time from onset of symptoms, consultation with a physician, submission and testing of the fecal sample, and the reporting of culture results (median 10 days), combined with the often limited natural duration of illness (median 8 days). Consequently, in many cases culture results were not available for use in making treatment decisions. Therefore at the patient level the primary value of culture results rests with management of cases that are prolonged and/or unresponsive to initial treatment. At a population level antimicrobial susceptibility information also helps to guide individual treatment decisions. In areas where the prevalence of ciprofloxacin resistance in clinical Campylobacter is quite high, the utilization of ciprofloxacin for treatment should be
discouraged. Also at a population level, the identification of laboratory-confirmed cases provides information on the incidence, demographics, and risk factors, associated with campylobacteriosis and is therefore a necessary element of surveillance programs and ongoing public health initiatives.

More than half of cases in this research (52% overall, 62% of children) took an antimicrobial for their illness (Chapter 2). This is a concerning finding since in most cases, including children, antimicrobials are not indicated for undifferentiated gastroenteritis or campylobacteriosis. In addition, cases that were treated with antimicrobials did not significantly differ from those that were not with respect to age, severity of illness, chronic medical condition, or international travel (Chapter 2). Cases reporting blood in the stool were also not significantly more likely to be treated with antimicrobials, although cases reporting fever were (Chapter 2). This suggests that other factors are involved in the decision to treat campylobacteriosis with antimicrobials. Since antimicrobials for oral therapy of humans are not available over-the-counter in Canada and all cases in this study accessed the healthcare system, it was assumed that the antimicrobials taken for this illness were prescribed by a physician. Further research is required to verify this assumption and to determine the factors utilized by physicians when determining whether to prescribe antimicrobials for acute onset diarrhea. In addition, the antimicrobial selection criteria used by physicians should also be investigated. Scenario-based physician surveys focused on antimicrobial use in undifferentiated gastroenteritis and laboratory-confirmed campylobacteriosis may be useful in identifying these factors in order to support the development of continuing education materials and promote prudent use practices.
When antimicrobial treatment is indicated for campylobacteriosis, fluoroquinolones and macrolides are the antimicrobials of choice. Among the 124 *Campylobacter* isolates from clinical cases in this research, 6 (4.8%) were resistant to ciprofloxacin and 2 (1.8%) were resistant to erythromycin (Chapter 2). There were no isolates with resistance to both ciprofloxacin and erythromycin. This is in contrast to findings in studies conducted in many other countries and other Canadian provinces where a high prevalence of ciprofloxacin in clinical isolates of *Campylobacter* has diminished its usefulness for treatment [6, 11]. The low prevalence of resistance to ciprofloxacin in this research is advantageous and should be protected through the judicious use of fluoroquinolones. The discrepancy of these results with the higher prevalence of ciprofloxacin resistance found in Saskatchewan and Alberta [11, 12] provides an opportunity to conduct a multi-provincial study to investigate possible regional and other factors driving ciprofloxacin resistance in clinical isolates of *Campylobacter*.

In order to investigate antimicrobial use and other factors that were associated with the duration of illness in clinical cases of campylobacteriosis, multivariate Cox proportional hazard models were developed (Chapter 3). This is the first reported use of these models for this purpose. This type of analysis is well suited to examining the factors associated with duration of illness since it is a semi-parametric method therefore it makes no assumptions about the distribution of failure times, in this case the time to the resolution of symptoms. Although the limited natural course of illness of many cases resulted in a right-skewed distribution of duration of illness, the effect of each potential factor was compared over the entire period. The Cox proportional hazard analysis also accommodated variables that changed over time (time-varying variables) and variables whose effect on duration of illness may have changed over time (time-varying effect), both of
which were potentially important aspects of antimicrobial use. This analysis identified a significantly increased rate of symptom resolution among cases treated with a macrolide for less than the recommended period of time, cases treated with ciprofloxacin for at least the recommended period of time, and cases treated with antimicrobials not recommended for campylobacteriosis (Chapter 3). The significant effect of macrolide treatment for less than the recommended period of time was largely due to the rapid resolution of symptoms in cases taking a macrolide. Of the 44 cases that were in this category, 39 were incomplete due to their symptoms ending prior to the completion of the recommended course of macrolide treatment. In contrast, the complete recommended course of treatment was required before an effect of ciprofloxacin treatment was seen. Overall, the low prevalence of resistance to erythromycin and ciprofloxacin in the clinical Campylobacter isolates from this research may partially explain the positive effect of treatment with these antimicrobials (Chapter 2 & 3). Further investigation into the effect on duration of illness of treatment with antimicrobials not expected to be clinically effective against Campylobacter is necessary. Although decreasing the duration of illness has a positive impact on the burden of illness for both the individual and society, the potential benefits from antimicrobial treatment of campylobacteriosis should be considered in the context of the self-limiting nature of this illness and the potential impact of treatment on antimicrobial resistance. The low prevalence of ciprofloxacin and macrolide resistance in both clinical human Campylobacter cases and Campylobacter isolates from retail chicken in Canada is advantageous and should be protected through the judicious use of these antimicrobials in both the human and agri-food sectors. The low prevalence of resistance to erythromycin observed in this research and the results of the Cox proportional hazard modelling suggest that macrolide treatment may
provide the best opportunity to positively affect duration of illness while minimizing antimicrobial resistance selection. It is important that this information, as well as the results on the current level of antimicrobial use in cases of campylobacteriosis, is communicated to physicians in order to facilitate prudent use practices.

Although it has been frequently reported that antimicrobial treatment is most effective when given early in the course of campylobacteriosis [5, 6], there was no time-varying effect of antimicrobial use found in this study (Chapter 3). Rather, the impact of antimicrobial use on the resolution of symptoms was consistent regardless of when in the course of illness the antimicrobial use began. Thus, fecal culture and sensitivity results can provide useful guidance for antimicrobial treatment in prolonged or complicated cases, even though the results are typically not available early in the course of illness.

Antimicrobial use in the 4 weeks prior to illness was not significantly associated with duration of illness. However, this study may not have had sufficient power to detect a significant effect since less than 5% of cases took an antimicrobial in the 4 weeks prior to illness. This factor was previously associated with an increased risk for campylobacteriosis but the impact on duration of illness was not investigated [13]. Therefore the potential role of prior antimicrobial use on risk and duration of illness requires further research.

The use of non-antimicrobial medications in the 4 weeks prior to illness was significantly associated with a decreased rate of symptom resolution in the multivariate Cox proportional hazard model (Chapter 3). When examined individually, taking medications for gastroesophageal reflux, blood pressure, immunosuppression, and thyroid conditions in the 4
weeks prior to illness were not significantly associated with duration of illness. However, taking medications for pain in the 4 weeks prior to illness was significantly associated with duration of illness on the univariable analysis. The magnitude and direction of the effect were similar for the general use of non-antimicrobial medications and the use of pain medications in the 4 weeks prior to illness, therefore the more inclusive variable was submitted to the multivariate model. Although the general variable for non-antimicrobial drug use was the most appropriate for this model, it would not be expected that all non-antimicrobial medications would have the same biological effect on the gastrointestinal tract in general, or campylobacteriosis in particular. Therefore the impact of specific classes of non-antimicrobials used in the 4 weeks prior to illness should be studied further. It is also possible that non-antimicrobial use is acting as a surrogate for chronic medical conditions that were not well defined by patients in this study. Research involving access to case medical records may help to differentiate the effect of non-antimicrobial medications from chronic medical conditions. This type of study may also help to determine the role of antimicrobial use in the 4 weeks prior to illness on duration of campylobacteriosis.

Cases with an isolate resistant to ciprofloxacin were significantly more likely to have a decreased rate of symptom resolution on the univariable analysis but this factor was not significant and did not act as a confounder in the multivariate analysis. There may have been insufficient power in this study to detect a significant effect if present due to the low prevalence of resistance to ciprofloxacin in clinical isolates as well as the absence of antimicrobial resistance results for 126 of 250 cases. Ciprofloxacin resistance in clinical isolates of Campylobacter has been associated with an increased duration of illness in some studies [14-16], but not in others [17, 18]. Considering the low prevalence of ciprofloxacin resistance in this study area, it may be more
feasible to pursue the investigation of this issue in regions where ciprofloxacin resistance is expected to be higher.

The handling and consumption of chicken is a very commonly identified risk factor for campylobacteriosis [2]. In order to further investigate the role of chicken in campylobacteriosis in this study population, a study of *Campylobacter* contamination of retail chicken from the study area was undertaken. There were 749 (59.6%) *Campylobacter* obtained from the representative, systematic sampling of 1256 fresh retail chicken packages in the study health units (Chapter 4). Of these, 14 isolates (1.9%) were resistant to ciprofloxacin and 25 isolates (3.3%) were resistant to erythromycin. There were no isolates resistant to both erythromycin and ciprofloxacin. The relatively low prevalence of resistance to ciprofloxacin and erythromycin in *Campylobacter* isolates from both retail chicken and campylobacteriosis cases in this study is consistent with the estimations of others that 20-80% of all human campylobacteriosis is the result of exposure through chicken[19-22].

This research represents one of the first reported uses of CGF molecular sub-typing to investigate the epidemiology of *Campylobacter*, in particular the association between isolates from human cases of campylobacteriosis and those from chicken. The resulting multivariate logistic regression model indicated that urban cases in this research were 6.3 times more likely than rural cases to have an isolate with a CGF fingerprint at the 90% similarity level that was associated with chicken (Chapter 5). This relationship was however not found when CGF fingerprints at the 95% similarity level were used. Further research into the difference in outcome for strains identified as chicken-associated on CGF- 90% and CGF-95% is needed. Previous research in
New Zealand and Scotland identified an association between urban (vs. rural) residence of cases and infection with chicken-associated strains of *Campylobacter* using multi-locus sequence typing (MLST) [3, 4]. The most common MLST type of *Campylobacter* found in New Zealand is quite rare internationally [4] and the Scottish study was restricted to children under 5 years of age, so the applicability of the results from these studies to other populations was unknown. The concurrence of the results from the present study, using a different technique in a different country, adds credence to the existence of a true association between urban residency of cases and higher risk of infection with chicken-associated strains of *Campylobacter*. Although exposure to different risk factors among urban and rural cases has been suggested as a basis for this difference, it would be useful to conduct a case-control study including both urban and rural cases in order to determine if an urban/rural effect exists once known risk factors have been controlled. Understanding the differences in risk factors for urban versus rural cases would allow for the refinement of public health education campaigns based on the target population.

An important strength of this research was the high (79%) participation rate among eligible cases of campylobacteriosis. This was due to the involvement of experienced local public health personnel in both the development and application of the questionnaire. The collaboration among scientists from federal, provincial and local public health as well as academia was extremely beneficial to the execution of this research project and to the interpretation of data analysis.

There are some limitations to this research. Although it was population-based and data collection was conducted over a two year period, it was limited to two health unit areas and the wider
applicability of the results is unknown. As well, the data collection occurred between 2001 and 2004 and may not reflect the current situation. Two-hundred and fifty cases consented to participate in this research, however only 124 *Campylobacter* isolates were received by the PHO laboratory and matched to case data. Although campylobacteriosis is a reportable illness in Canada, there is no requirement for the *Campylobacter* isolate to be forwarded to provincial laboratories, with the exception of Saskatchewan. Therefore, the forwarding of isolates for this research required individual agreements with each of the private and hospital laboratories responsible for processing fecal samples in the study area. Due to the routine discarding of isolates immediately after genus confirmation and the difficulty in easily identifying cases residing in the study area, not all eligible isolates were forwarded to the provincial laboratory. Moreover, in order to protect confidentiality, the matching of case data and isolate information was done using health unit-assigned case numbers and laboratory assigned isolate numbers. It was not always possible to reconcile these numbers and isolates that could not be matched with case data were excluded.

Research into the epidemiology of campylobacteriosis would be enhanced by the standardization of isolation methodologies as well as the standardization of antimicrobial resistance panels and international breakpoints. This would allow for valid comparisons of individual and multi-resistance results between specific research studies and between countries.

In summary, although the high proportion of cases taking antimicrobials for their campylobacteriosis (52%) is concerning, this does not appear to have resulted in a high prevalence of resistance to ciprofloxacin or erythromycin in human clinical *Campylobacter*
isolates. The use of macrolides and ciprofloxacin increased the rate of resolution of symptoms regardless of when during the course of illness antimicrobial treatment began, but the use of antimicrobials for treatment should be considered in the context of the self-limiting nature of this disease and the potential impact on antimicrobial resistance over time. Based on CGF molecular sub-typing, urban cases were significantly more likely to have a chicken-associated strain of *Campylobacter* than rural cases. Since the majority of the Canadian population is urban-dwelling, this emphasizes the importance of educational and food safety efforts to reduce the impact of *Campylobacter* from retail chicken.
References:


20. **EFSA Panel on Biological Hazards.** Scientific Opinion on quantification of risk posed by broiler meat to human campylobacteriosis in the EU. *European Food Safety Authority Journal* 2010; **8**: 1437-1526.


Case Identification #: ________________

Consent Script

Hi, my name is ______________________ I am a public health inspector at the Wellington Dufferin Guelph / Perth Health Unit. I am contacting you as part of our investigation of your reported Campylobacter infection.

At this time the health unit is working with Health Canada to conduct a health survey. This survey is part of a Health Canada investigation of the risk to human health of Campylobacter contaminated retail poultry. The results will be used to identify ways in which the illness can be prevented. We are asking for your permission to share information that you provide to the health unit and your Campylobacter isolate with Health Canada. The information that we share will not include your name or any other identifying information.

One set of questions are for our investigation of your reportable disease. You are required to answer those questions. We are asking another set of questions on behalf of Health Canada. You are free to refuse to answer any of those questions. As both sets of questions are intermingled for practical purposes, you may ask at any time whether a question is part of our disease investigation or part of Health Canada’s survey. Your cooperation in answering Health Canada’s questions will not affect services that you receive from the health unit now or in the future.

The entire process will take 15 to 20 minutes of your time. Do you agree to participate in the Health Canada Survey and also agree to have the health unit share information obtained from our disease investigation with Health Canada?”

Yes ☐  No ☐
Date: __________(yy) / ______________(mm) / __________(dd)

Would you like more information on this project? If yes, Please contact Dr. Anne Deckert at (519) 826-2160.

IF NO:

I need 5 to 10 minutes of your time to ask you questions about your reportable disease.

Interviewer: _______________________________________

This consent script must be re-read for each person providing information
February 15, 2002

**RDIS Case Management**

**Enteric Illness Questionnaire – Campylobacter Study**

**Section 1: Case Information**

1.1 RDIS Case #: 

1.2 Central Public Health Lab #: 

1.3. Health Unit: 

   - Perth
   - Wellington-Dufferin-Guelph

Data Transferred from Introduction

1.4) Private Lab Name: 

   Private Lab #: 

1.4) Date the specimen was collected: (yy) / (mm) / (dd)

1.6) Date results received: (yy) / (mm) / (dd)

1.6) D.O.B (yy/mm/dd) 

1.7) Gender 

   - Male / Female

1.5) Postal code 

   

**Section 2: Campylobacter Infection**

[Read] I would like to begin with several questions about your recent Campylobacter infection. I will be asking about specific dates around the time of your illness, so it may be helpful for you to have a calendar or diary in front of you. Do you need a few minutes to go get one?

2.1 For the purposes of this questionnaire, we define diarrhea as 2 or more loose stools or bowel movements in any 24-hour period. Along with your Campylobacter infection,

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
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</thead>
<tbody>
<tr>
<td>a. Did you have diarrhea?</td>
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<tr>
<td>➤ If Yes, go to <strong>Question 2.2</strong></td>
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<td>b. As best you can remember, why was a stool culture obtained?</td>
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<tr>
<td>b.1 Because you had symptoms other than illness</td>
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</tbody>
</table>
b.2 Because *Campylobacter* was cultured from a family member or other close contact
b.3 As part of an outbreak investigation
b.4 To follow up a previously positive culture for *Campylobacter*
b.5 Other reason
    ➤ If other, specify [ ]

**Signs and Symptoms**

2.2 Now I would like to ask you some questions specifically about your recent *Campylobacter* infection.

<table>
<thead>
<tr>
<th>During this infection:</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did you have a fever?</td>
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<td>b. Did you vomit?</td>
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<td>c. Did you have stomach cramps?</td>
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<td>d. Did you have blood in your stool?</td>
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<td>e. Did you have a headache?</td>
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<td>f. Did you feel fatigued?</td>
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<td>g. Did you feel sick (nauseated)?</td>
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<td>h. Did you have loss of appetite</td>
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<tr>
<td>i. Did you have bloating</td>
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<td>j. Did you have weight loss</td>
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<tr>
<td>k. Did you have other symptoms?</td>
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<tr>
<td>➤ If other, please specify: [ ]</td>
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<tr>
<td>l. On what date did your symptoms begin?</td>
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<td>m. Are the symptoms ongoing</td>
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<tr>
<td>➤ If Yes, may we contact you again in 2 weeks time to ask how long the symptoms lasted?</td>
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<tr>
<td>➤ If Yes, When is a good time to call?</td>
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<tr>
<td>➤ If No, on what date were you completely over the symptoms?</td>
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</tbody>
</table>
Calculate 14 days after illness onset  ____/______/______
Yyyy mm dd

If today’s date is BEFORE this date, check YES in the Control box (page 1) and complete all questions.

If today’s date is AFTER this date OR date of illness onset is not known, Then check NO in the Control box (page 1) AND skip All questions involving time (last month, last 10 days, etc.)

2.3 Which of the following best describes the severity of your illness? [Read response choices]

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Check One Box</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quite mild (feeling slightly unwell but able to do all normal activities)</td>
<td>(1)</td>
</tr>
<tr>
<td>Fairly mild (feeling quite unwell but able to do most normal activities)</td>
<td>(2)</td>
</tr>
<tr>
<td>Moderate (having to stay at home but able to get out of bed for limited activities)</td>
<td>(3)</td>
</tr>
<tr>
<td>Fairly severe (confined to bed at home and unable to do any normal activities)</td>
<td>(4)</td>
</tr>
<tr>
<td>Quite severe (hospitalized)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

If yes, for how many nights were you hospitalized? ______________ nights [    ]

DK/NS                                                              (77)
Refused                                                            (99)
Activity Limitations and Health Care Utilization

2.4

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did this infection interfere with your ability to perform your usual activities at home, work or school?</td>
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<tr>
<td>➔ If yes, do you go to school or work outside of the home?</td>
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<tr>
<td>➔ If yes, did you take time off work or school as a result of this illness?</td>
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<tr>
<td>➔ If yes, for how many days? ________________ days</td>
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<tr>
<td>➔ If no, specify # days unable to perform usual activities:______________days</td>
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<tr>
<td>b. Were you looked after by someone (eg relative or friend) during your illness?</td>
<td></td>
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<tr>
<td>➔ If yes, Did this person have to take time off work in order to look after you?</td>
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</tr>
<tr>
<td>➔ If yes, how many days did this person take off work in order to look after you?</td>
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</tbody>
</table>

2.5 As a result of this illness, how many times did you visit:

[Please assign each visit to one category only, and write ☐ for none]

<table>
<thead>
<tr>
<th>[Read]</th>
<th># of Times</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. A family doctor's office?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. A walk in/after hours clinic? [ie not family doctor's office]</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>c. Hospital emergency department</td>
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<tr>
<td>d. Other</td>
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<td></td>
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<tr>
<td>➔ If other, please specify:</td>
<td></td>
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</tbody>
</table>

2.6

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. How do you think you became ill?</td>
<td></td>
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<tr>
<td>b. Before your illness, had you heard of Campylobacter as a problem in food or water?</td>
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<tr>
<td>➔If yes, do you remember what kinds of food or water:</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
## Section 3: Environmental Risk Factors

### Residence

<table>
<thead>
<tr>
<th>3.1 Which of the following describes where you live?</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. City</td>
<td></td>
<td></td>
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<tr>
<td>b. Town / Village / Hamlet</td>
<td></td>
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<tr>
<td>c. Rural</td>
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<tr>
<td>➞ If yes, on a farm?</td>
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<tr>
<td>➞ If yes, do you have livestock on your farm?</td>
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<tr>
<td>➞ If yes, specify:________________________________</td>
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<tr>
<td>3.2. What is the source of water for your home?</td>
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<tr>
<td>a. Municipal</td>
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<tr>
<td>b. Private Well</td>
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<tr>
<td>➞ If yes, Dug?</td>
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<tr>
<td>➞ If no, drilled?</td>
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<tr>
<td>➞ If no, cistern?</td>
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<tr>
<td>c. Is the sewage system: Municipal?</td>
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<tr>
<td>➞ If no, Private?</td>
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<tr>
<td>d. Has there been ponding at the tile bed area?</td>
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</tbody>
</table>
### Other Cases

#### 4.1

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. In the 10 days before becoming ill, did you have contact with any other people outside the household who you know were suffering with diarrhea or vomiting?</td>
<td></td>
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<tr>
<td>b. Did you change a baby’s diapers in the 10 days before becoming ill?</td>
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<tr>
<td>☐ If yes. Did the baby have diarrhea? (2 or more loose or watery stools in a row)?</td>
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<tr>
<td>c. Did you provide personal care to anyone (i.e. aid in toileting) in the 10 days before becoming ill?</td>
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<tr>
<td>☐ If yes. Did the person have diarrhea?</td>
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</table>

For the next few questions, I would like to ask you about events which may have occurred in the month before your illness began, that is from [DATE 4 WEEKS BEFORE ILLNESS BEGAN] through [DATE ILLNESS BEGAN]

d. Do you live alone? |         |        |            |              |
| ☐ If No, in the month before your illness, did anyone else in your household suffer from diarrhea (2 or more loose stools or bowel movements in any 24-hour period)? |         |        |            |              |

### Occupation

#### 5.1

Have you or any other member of your household worked in any of the following workplaces in the past month?

<table>
<thead>
<tr>
<th>Workplace</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Daycare centre</td>
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<tr>
<td>b. Nursing home</td>
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<tr>
<td>c. Hospital</td>
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<tr>
<td>d. Doctor’s office</td>
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<tr>
<td>e. Slaughterhouse</td>
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<tr>
<td>f. Meat processing plant</td>
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<tr>
<td>g. Livestock farm</td>
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<td>h. Horse Stable</td>
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<tr>
<td>i. Kennel/ Animal Shelter</td>
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<tr>
<td>j. Animal Research Facility</td>
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<tr>
<td>k. Veterinary hospital</td>
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<tr>
<td>l. Commercial food preparation (eg chef)</td>
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<tr>
<td>m. Butchershop or meat dept of food store</td>
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</table>
### Animal Exposures

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>R (99)</th>
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</thead>
<tbody>
<tr>
<td>6.1 Did you have any contact with animals in the 10 days prior to becoming ill?</td>
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<tr>
<td> If Yes, did you have close/touching contact with any of the following animals:</td>
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</tr>
<tr>
<td>a. Cattle or Calves</td>
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<td>b. Sheep</td>
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<td>c. Goats</td>
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<tr>
<td>d. Pigs</td>
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<tr>
<td>e. Horses</td>
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<tr>
<td>f. Turkeys</td>
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<tr>
<td>g. Chickens</td>
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<tr>
<td>h. Geese</td>
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<tr>
<td>i. Other farm animals</td>
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<tr>
<td> If yes, specify:</td>
<td>[ ]</td>
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<tr>
<td>j. Reptiles/amphibians</td>
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<tr>
<td> If yes, specify:</td>
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<tr>
<td>k. Kittens (&lt; 1 year old)</td>
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<td>l. Cats</td>
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<td>m. Puppies (&lt; 1 year old)</td>
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<td>n. Dogs</td>
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<tr>
<td>o. Birds</td>
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<td> If yes, specify:</td>
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<td>p. Rabbits or rodents (eg mouse, guinea pig, gerbil)</td>
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<tr>
<td>q. Fish (Aquarium)</td>
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<tr>
<td>r. Wildlife</td>
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<td></td>
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<tr>
<td>s. Other pets</td>
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<tr>
<td> If yes, specify:</td>
<td>[ ]</td>
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<tr>
<td>6.2 Did you clean up any of the above animal’s droppings or clean out the litter box/cage/aquarium/stall, etc. of any animals in the 10 days before becoming ill?</td>
<td></td>
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</tbody>
</table>
6.3 Did any of the above animals have diarrhea in the 10 days before your illness started?  
→ If yes, specify: [ ]

6.4 Are any of the above animals EVER fed raw meat, raw fish, raw milk

6.5 In the 10 days before becoming ill, did you clean wild bird droppings from any surface?

<table>
<thead>
<tr>
<th>Travel and outdoor activities</th>
</tr>
</thead>
</table>

7.1 In the 10 days before becoming ill, did you go on a trip? [Do not include commuting for work, school, shopping etc.]
→ If yes, specify Destination and dates

<table>
<thead>
<tr>
<th>Destination</th>
<th>Date (dd/mm) Departed</th>
<th>Date (dd/mm) Returned</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

7.2 In the 10 days before becoming ill, did you go camping or picnicking?
→ If yes, where did you get drinking water? ________________________________
→ If yes, did you treat the drinking water in any way?
→ If yes, specify: ____________________________

7.3 In the 10 days before the illness began, did you drink [include water swallowed while swimming, brushing teeth etc.] from any other untreated water source such as:

a. Lake, river, stream or pond?

b. Private well

7.4 In the 10 days before becoming ill did you go swimming or participate in other water sports (eg. Windsurfing, waterskiing)?
→ If yes, specify: ____________________________
→ If yes, Do you know if any of the other participants were sick?
## Section 4: Food Risk Factors

### Food and Water

#### 8.1

**a. Are you involved in meal preparation in your household?**

<table>
<thead>
<tr>
<th>Always</th>
<th>Usually</th>
<th>Sometimes</th>
<th>Seldom</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

⇒ If never, ask **NON-BOLD questions only**

**b. Are you a strict vegetarian (never eat meat, poultry, fish)?**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>DK/NS</th>
<th>Refused</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(77)</td>
<td>(99)</td>
</tr>
</tbody>
</table>

⇒ If yes, do you prepare meat, poultry or fish for other members of your household.

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>DK/NS</th>
<th>Refused</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(77)</td>
<td>(99)</td>
</tr>
</tbody>
</table>

⇒ If yes, ask **BOLD questions only**

⇒ If no, Skip to Qu. 8.5

#### 8.2

In the 10 days before your illness began ______________ to ______________

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes</th>
<th>No</th>
<th>DK/NS</th>
<th>Refused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(77)</td>
<td>(99)</td>
</tr>
</tbody>
</table>

**a. Did you handle any raw beef or pork?**

**b. Did you eat any of the following:**

- **c. Beef (including hamburgers)**
  - If Yes: Was any of it cooked rare? (Reddish-pink inside?)

- **d. Pork (minced, chops or roast)**
  - If Yes: Was it cooked rare?

- **e. Did you eat any Deli meats (eg. salami, bologna), hotdogs or sausage?**

- **f. Meat spread (eg. paté, liverwurst)?**

- **g. Was any of this beef or pork cooked on a barbecue?**

- **e. Did you eat any Deli meats (eg. salami, bologna), hotdogs or sausage?**

- **f. Meat spread (eg. paté, liverwurst)?**

⇒ If yes, please specify:
8.3 In those ten days:

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did you handle raw turkey or chicken</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Did you eat any turkey or chicken?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If no turkey or chicken eaten, go to Question 8.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>c. Was any of the turkey or chicken pink inside?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Was any of the turkey or chicken fresh (i.e., not previously frozen?)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Was any of this turkey or chicken cooked somewhere other than at home (e.g., restaurant, grocery store or banquet hall)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If yes, please specify:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Was any of this turkey or chicken cooked on a barbecue?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.4 In the ten days before your illness began,

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did you handle raw fish or seafood?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Did you eat any fish or seafood?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If no fish or seafood eaten, go to Question 8.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Was any of it raw or undercooked?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Was any of this fish or seafood cooked somewhere other than at home (e.g., restaurant or banquet hall)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If yes, please specify:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Was any of this fish or seafood cooked on a barbecue?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.5

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did you drink any raw or unpasteurized milk (e.g., goat’s milk) in the 10 days before the illness began?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. In those 10 days, did you eat any cheese that was made with unpasteurized milk?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes, was any of this cheese soft?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes, was any of this cheese hard?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes, specify name(s) of the cheese:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8.6 I will now ask you some questions about eggs. In the 10 days before the illness began, did you eat any of the following types of eggs?

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Scrambled eggs or omelet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes: Were the eggs runny?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes: Were the eggs eaten outside the home? (eg. Restaurant, cafeteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes, specify,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Other types of eggs (eg fried, boiled, poached)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes: Did any of these eggs have a runny yolk?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Raw eggs (eg in cookie dough, cake batter, homemade eggnog, mayonnaise, homemade caesar salad dressing)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.7

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did you attend any large banquet meal (i.e. wedding, conference) in the 10 days before becoming ill?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Did you attend any gatherings/events where any food and/or beverages were served?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>➞ If Yes, specify:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

General Food Consumption questions

8.8 Which of the following meats do you generally eat?

<table>
<thead>
<tr>
<th>Meat</th>
<th>Frequency (per week)</th>
<th>How is it eaten?</th>
<th>How well is it cooked?</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Turkey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Beef/pork/lamb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Wild fowl or game</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Seafood</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

186
8.9 How often do you eat any meals prepared in these locations in a one week period?

<table>
<thead>
<tr>
<th>[Read]</th>
<th># of times</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. fast food restaurant (e.g. McDonald’s, Taco Bell, KFC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. restaurant other than a fast food restaurant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. cafeteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. someone else’s home</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. daycare</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Section 5: Home Hygiene and Knowledge
9.1

<table>
<thead>
<tr>
<th>b. On what type of surface do you prepare or cut the raw meat, poultry or fish?</th>
<th>Wood (1)</th>
<th>Plastic (2)</th>
<th>Glass (3)</th>
<th>Countertop (4)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c. After you have used a cutting board for cutting raw meat or poultry, how would you normally clean it?</td>
<td>Rinse with water (1)</td>
<td>Wash with detergent (hand/dishwasher) (2)</td>
<td>Wash with disinfectant (bleach) (3)</td>
<td>DK/NS (77)</td>
<td>Refused (99)</td>
<td></td>
</tr>
<tr>
<td>d. During food preparation, do you normally use separate knives for raw meat/poultry/fish and other foods?</td>
<td>Yes (1)</td>
<td>No (2)</td>
<td>DK/NS (77)</td>
<td>Refused (99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. During food preparation, do you normally use separate chopping boards for raw meat/poultry/fish and other foods?</td>
<td>Yes (1)</td>
<td>No (2)</td>
<td>DK/NS (77)</td>
<td>Refused (99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. How do you usually defrost meat, poultry, fish or seafood?</td>
<td>Refrigerator</td>
<td>Water</td>
<td>Microwave</td>
<td>Countertop</td>
<td>Other</td>
<td>DK/NS (77)</td>
</tr>
</tbody>
</table>

⇒ If other, specify:
9.2 Please indicate whether you strongly agree, agree, disagree or strongly disagree with the following statements.

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Strongly Agree (1)</th>
<th>Agree (2)</th>
<th>Disagree (3)</th>
<th>Strongly Disagree (4)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Washing your hands with antibacterial soap is <em>more</em> effective in preventing food poisoning than using regular soap</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Washing your hands with very hot water is <em>as effective</em> in preventing food poisoning as washing with soap.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>c. The <em>most</em> appropriate way to defrost meat is in cold water in the sink.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>d. Eating outside of the home (at a restaurant or cafeteria) <em>decreases</em> the chances of getting food poisoning</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>e. Using the same plate that held raw meat to serve cooked meat when barbequing, <em>increases</em> the chances of food poisoning</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>f. Using the meat marinade as a sauce on the cooked meat <em>does not increase</em> the chances of food poisoning.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

**Section 6: Medical History and Antibiotic Use**

**Relevant Medical History**

10.1 Now I have a few questions about your general health. Please remember that you may choose not to respond to these questions or to any others that I ask you.

<table>
<thead>
<tr>
<th>[Read]</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Do you have a medical condition that could weaken Your immune system, such as leukemia or other cancers, HIV infection, or AIDS?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>➞ If yes, specify condition:</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Do you have any chronic illness in which diarrhea is a major Symptom, such as: Chronic colitis Crohn’s Disease Stomach ulcers Irritable bowel syndrome?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>c. Have you ever had surgery to remove part of your stomach or intestines?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>➞ If yes, specify surgery:</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>d. Do you have any disorder which affects the acid level in your stomach?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>e. Do you have any other chronic illness?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>➞ If yes, specify illness:</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>
### Campylobacteriosis Treatment

11.1

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes (1)</th>
<th>No (2)</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Did you take any antibiotics for this illness?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ If yes, <strong>Can you remember the name of the antibiotic(s)?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ If yes, How is that spelled? [Clearly write name of antibiotic(s) in Table below]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ If no, Do you have the medicine bottle or pharmacy receipt? I can phone back after the interview to give you time to get it. [Tick “antibiotic information” in Section 8 on last page if further details may be available later]</td>
<td></td>
<td></td>
<td></td>
<td>[ ]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>i. Name of antibiotic(s)</th>
<th>ii. On what date did you start taking the antibiotic?</th>
<th>iii. Can you recall the date that you stopped taking the antibiotic?</th>
<th>iv. Can you remember the number of days that you took the medication?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(dd/mm/yy)</td>
<td>(dd/mm/yy)</td>
<td>(dd/mm/yy)</td>
</tr>
<tr>
<td>DK (77)</td>
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<td></td>
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</tr>
</tbody>
</table>
11.2 Did you take any of the following medications for your illness?

<table>
<thead>
<tr>
<th></th>
<th>Yes (1)</th>
<th>If Yes, # of Times</th>
<th>Yes</th>
<th>No</th>
<th>DK/NS (77)</th>
<th>Refused (99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Antidiarrheal (eg. Lomotil)</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Medication for pain or fever (eg. Tylenol)</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>c. Rehydration medications (eg. Pediolyte)</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>d. Medication for nausea or vomiting (eg. Gravol)</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>e. Other medication</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>f. What was the approximate total cost of these medications? $_________________</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>g. Did you receive any reimbursement for the cost of these medications?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>h. Were there any other costs related to this illness that we have not mentioned?</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>
## Previous Medications

### 11.3

<table>
<thead>
<tr>
<th>previous_medication</th>
<th>Yes</th>
<th>No</th>
<th>DK/NS</th>
<th>Refused</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. In the 4 weeks before becoming ill, did you take any antibiotics?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If yes, <strong>Can you remember the name of the antibiotic(s)?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>If yes, How is that spelled? <strong>[Clearly write name of antibiotic(s) in Table below]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>If no, Do you have the medicine bottle or pharmacy receipt? I can phone back after the interview to give you time to get it. <strong>[Tick “antibiotic information” on last page if further details may be available later]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Name of antibiotic(s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For how many days did the doctor say to take the medication?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iii. On what date did you start taking the antibiotic?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv. Can you recall the date that you stopped taking the antibiotic? *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you take the entire amount that the doctor prescribed?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 11.4

In those 4 weeks before the illness started, were you taking any of the following medications?

a. Antacids (medicine for heartburn)
   (eg. Tagamet, Rolaids, Maalox)
   **[Read]**

b. Laxatives (eg. Metamucil, Senakot, Prodiem)
   **[Read]**

c. Antidiarrheal medication (eg. Peptobismol, lomotil)
   **[Read]**
d. Immunosuppressant medication
   (eg Prednisone, other steroids, cyclosporine)
   ➔ If yes, specify: [ ]

e. Were you in radiation therapy or chemotherapy for
   the treatment of cancer?
   ➔ If yes, specify: [ ]
f. Other non-prescription medications
   ➔ If yes, specify: [ ]

Section 7: Demographics

I would now like to ask you a few more questions about you and your family. It should only take another
minute or two.

12.1
   a. Which of the following categories best describes the highest level of
      school that you completed? [Read] Yes No DK/NS Refused
      (1) (2) (77) (99)
   b. Less than grade 9
   c. Attended or completed high school
   d. Attended or completed university/college

12.2. Finally, I’d like to ask you which of the following 4 categories best describes your total household
income before taxes in the last year. I’d like to remind you that your response is voluntary and will be kept
confidential. These four income categories are:

These four income categories are: Yes No DK/NS Refused
   (1) (2) (77) (99)
   a. Less than $20,000
   b. More than $20,000 but less than $40,000
   c. More than $40,000 but less than $60,000
   d. More than $60,000

Section 8: Follow-up and Thank You

☐ Antibiotic Information Available? (if ticked, read): You said that you could find the name of the
   antibiotic, would you like to look for it now?
Yes ☐ No ☐, Can I call back later when you have had a chance to find it?  
⇒ If yes, specify date and time, tick yes in follow up box.

This is the end of the formal interview. Thank you very much for your time and cooperation.

Do you have any questions?

____________________________________________________________________________________

12.3 May we contact you again should the need arise? ☐ Yes ☐ No

Thanks again. Good bye.

[Interviewer: Please complete the following:]

12.4 Sex of case: ☐ male ☐ female

12.5 Date of interview: ___/___/______  12.6 Time of interview: __________

12.7 Interviewer’s Name: (please print)

___________________________________________________________

12.8 Interviewer ID #:____________

IMPORTANT

Please check that the Provincial Laboratory number and Case ID number are clearly written on the front of the Case Questionnaire.
Also, if a matched control is needed check “Yes” in Matched Control box (top of front page) and write deadline date (7 days from case interview date) in space provided.

Thanks!

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Campylobacter Project in Perth and Wellington-Dufferin - Guelph Health Units

Health Unit Protocol

For Campylobacter isolated from residents of:
- Perth County
- Wellington County
- Dufferin County
- City of Guelph

When a report of a positive Campylobacter culture is received from a Primary Laboratory
Report the:

Age
Gender
Primary Laboratory
Primary Laboratory Specimen Number
to Health Canada by fax at (519) 826-2255.

Conduct Case Questionnaire

Inclusion Criteria:

Consent to participation
Only use one case from a known outbreak or cluster of samples
Primary case in a household

195
Cases that can communicate in English
Cases who have a phone
If consent received at time of questionnaire administration
Provide the:  Case questionnaire
             Primary isolation laboratory name
             Laboratory sample number for Campylobacter isolate
to Health Canada

Problems with the study:
The first month of the study will be considered a trial period for the questionnaire and the
flow of information. If there are any major concerns with the questionnaire or the flow of
information then it will be adapted. If there are no major concerns in this period, the current
survey instrument will be utilized for the duration of the study.

Conflicts, Complaints or Questions from Cases:
If there are any conflicts, complaints or questions from cases, please refer them to Dr. Anne Deckert at (519) 826-2160. I would be happy to call them back to save them the long
distance charges.

Definitions
If you have any questions or concerns, please contact Dr. Anne Deckert at (519) 826-2160 or Anne_Deckert@hc-sc.gc.ca

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196

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Campylobacter Project in Perth, Wellington, and Dufferin Counties and the City of Guelph

Laboratory Protocol

For Campylobacter isolated from residents of:

   Perth County
   Wellington County
   Dufferin County
   City of Guelph

- Submit pure Campylobacter cultures to Central Public Health Lab (Toronto) in Amies’ charcoal medium or equivalent.
- Clearly label each tube with the submitting laboratory name, date, and submitting laboratory sample #. This must correspond to the information on the submission form.
- Complete the submission form according to the example provided
- Please use the pre-addressed Purolator forms.

Please contact Dr. Anne Deckert at (519) 826-2160 or Anne_Deckert@hc-sc.gc.ca with any questions or concerns.

Fax the Supply Request Form to (519) 826-2255 when you require additional Purolator slips.
Thank you for your participation in this study!

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