University of Alberta

EPIDEMIOLOGICAL ASPECTS OF *CAMPYLOBACTER* INFECTIONS IN SOUTHERN ALBERTA

by

Julie Yuriko Mori Johnson 

A Thesis Submitted to the Faculty of Graduate Studies and Research
in partial fulfilment of the degree of Doctor of Philosophy

in

Department of Public Health Sciences

and

Department of Agriculture, Food and Nutritional Science

Edmonton, Alberta

Fall 2007

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-32986-3
Our file Notre référence
ISBN: 978-0-494-32986-3

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABSTRACT

The incidence of *Campylobacter* infections in southern Alberta is relatively high. A case-control study was conducted in 2004-2005 in the Chinook and Calgary Health Regions to investigate risk factors for ciprofloxacin resistance in *Campylobacter* infections. Among a study sample of 229 patients, 28.8% of *Campylobacter* isolated from patients were ciprofloxacin resistant. The risk factor of greatest concern was recent overseas travel, including travel to Latin America, Asia, Africa, and Europe. Age and possession of antibiotics for future use were also risk factors for resistance. Participation bias may have led to an overestimation of risk associated with foreign travel, but the risk was still strong after adjusting for this bias. Duration of diarrhoea in *C. jejuni* infections was prolonged among study participants who travelled overseas and had diarrhoea that persisted up to 30 days. Delays in antibiotic treatment did not affect the duration of diarrhoea following initiation of treatment. The putative virulence plasmid pVir was detected in 13.3% of a sub-sample of *C. jejuni* strains (n = 73). pVir presence was not predictive of diarrhoea duration, but was associated with travel-related infections. These results indicate that there are some risk factors associated with infections caused by *Campylobacter* afflicting travellers. To assess how well the travelling population understands travellers' diarrhoea, a knowledge survey of travellers departing for Mexico was conducted. Most understood the risks of consumption of specific foods during travel. Many obtained travel health information prior to travel and those who had information on travellers' diarrhoea scored better on the survey.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DEDICATION

This thesis is dedicated to my parents, Minoru and Midori Mori, to my husband, Daniel Johnson, and to my sons, Dexter and Scott.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ACKNOWLEDGEMENTS

I am very grateful for the guidance provided by my thesis supervisory committee: Dr L. Duncan Saunders, Dr Lynn McMullen, Dr Paul Hasselback, Dr Marie Louie, and Gian Jhangri. Duncan remained a positive, pragmatic, and energetic supporter throughout the development of this thesis. Lynn taught me about standing up for academic priniciples. Paul was instrumental to the development of the case-control study and provided an abundance of insight and feedback. Marie provided an expert's perspective as a public health microbiologist and unwavering encouragement. Gian was the calm voice of statistical reason. Thank you, all.

I am also extremely appreciative of the generous cooperation and support of the following people, who assisted in data collection and in providing laboratory data. Without their time and effort, this thesis would not exist.

Chapters 3 and 4:

- Calgary Health Region, Environmental Public Health: Dr Judy MacDonald, Norm Carlson, Larry Crowe, Jennifer Jacobsen, Kenning Eng, Sujata Patel, Sarah Nunn.
- Calgary Laboratory Services: Heather Semeniuk
- Chinook Health Region, Health Protection: Karen Thomas and the Public Health nursing staff.
- Public Health Agency of Canada, Lethbridge Laboratory: Dr Victor Gannon, William Mauro

Chapter 5:

- Calgary International Airport Authority: Chris Armstrong, Shali Ramdeen

Finally, I am grateful for the financial support provided through an Alberta Heritage Foundation for Medical Research Health Research Studentship.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE OF CONTENTS

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF TABLES

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABBREVIATIONS

Term	Abbreviation
Minimum inhibitory concentration	MIC
Pulsed-field gel electrophoresis	PFGE
National Collection of Type Cultures	NCTC
Clinical and Laboratory Standards Institute	CLSI
Centers for Disease Control and Prevention	CDC
Campylobacter Sentinel Surveillance Scheme Collaborators	CSSSC
Foreign-related (infection)	F
Domestically-acquired (infection)	D
Mixed; foreign and domestic infections	M
Odds ratio	OR
Confidence interval	CI
Matched odds ratio	mOR
Interquartile range	IQR
Hazard ratio	HR
Analysis of variance	ANOVA
Traveller's diarrhoea	TD

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 1:

INTRODUCTION

Campylobacter infection in humans is a complex and relatively poorly understood disease, even though it is one of the most common types of foodborne bacterial infection in the developed world (1-3). There are many critical unanswered questions, both epidemiological and microbiological in nature, and from these knowledge gaps arise considerable public health challenges. Accordingly, in this thesis, the investigation of *Campylobacter* infections in southern Alberta was approached in a holistic manner, with several studies from various perspectives. Each study contained within the thesis was an attempt to address specific epidemiological, microbiological, or public health questions.

1.1 The Case-Control Study

The core research of the thesis was a case-control study that explored risk factors for ciprofloxacin resistance in *Campylobacter* infections. Worldwide, the level of resistance to ciprofloxacin, a first-line therapy for *Campylobacter* infections, is increasing among human-infective strains and those isolated from food animals and foods (4-8). The incidence of *Campylobacter* infections is high in southern Alberta, relative to the rest of Canada (9). A recent report in the scientific literature stated that the proportion of ciprofloxacin resistance among a sample of human *Campylobacter* infections in Alberta was only 2% (n = 203) (10). However, personal communications with public health officers in southern Alberta suggested that resistance levels may be higher, and some surmised that the regional livestock industry may have a role.

Others in the U.S.A. and the U.K. have conducted case-control studies of risk factors for ciprofloxacin resistance in human *Campylobacter* infections; three key studies were conducted by Smith *et al.* (11), the *Campylobacter* Sentinel Surveillance Scheme Collaborators (12), and the Foodborne Disease Active Surveillance Network (13). The primary findings included higher risk for people who had travelled abroad, had consumed pre-cooked cold meat or poultry in a commercial establishment, or had used a

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

fluoroquinolone prior to stool sample submission (11-13). These studies represented valid research, but two important issues, antibacterial use and misuse of antibiotics, were not addressed.

Although antibiotic use prior to stool sample submission has been investigated in two of the studies mentioned above (11, 13), exposure to household and personal hygiene antibacterial products prior to *Campylobacter* infection was not included as a potential risk exposure in any of the case-control studies. Some evidence has been recently presented that suggests the use of household and personal hygiene antibacterial products may increase the development of antibiotic resistant bacterial populations (14, 15). Bacteria utilise efflux systems in order to resist toxins and compounds used in antibacterial products, and efflux mechanisms allow for the potential for antibiotic-antibacterial cross-resistance to develop (16). The possibility that ingestible antibacterial products, dishwashing soap and toothpaste could increase risk of resistance seemed an interesting hypothesis for this project.

There are some arguments in the literature for antibiotic misuse by patients as a factor in the development of antibiotic resistance, thus, this issue was also queried in the thesis work. The potential impact of self-medication with antibiotics has been presented by a Working Party of the British Society for Antimicrobial Chemotherapy (17). The potential is so important, they suggest, that regulatory instruments should be put in place in the U.K. to link licensing of antibiotics to resistance surveillance and to change the over-the-counter availability of some antibiotics. The tendency for people to keep leftover antibiotics for future use has been well-documented in the literature (18-20), and some suggest this type of misuse could select for resistance among bacterial populations in the community (21). Factors that have been found to contribute to misuse or self-medicating include access to veterinary drugs (22) and living outside of urban areas (23).

It is common practice in many food-risk retrospective studies to ask participants if they consumed specific foods 10 – 14 days before becoming ill (24-26). One goal of the case-control study was to look for a risk gradient for food exposures to improve the precision

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of the risk estimate. So, rather than follow the common practice, study participants for this study were asked for an estimate of the number of times in a 2-week period specific foods were likely to be consumed.

The case-control study also included many of the questions common to the previously mentioned case-control studies, but the motivation here was to test these additional hypotheses and to examine ciprofloxacin resistance in *Campylobacter* among a diverse study population with a mix of urban and rural citizens.

1.2 Investigation of the Duration of Diarrhoea

In addition to the ciprofloxacin resistance risk factor analysis, the data on diarrhoea duration and antibiotic treatment of case-control study participants was explored. Recently, the effects of foreign travel, antibiotic treatment, and ciprofloxacin resistance were debated in the literature, following the publication of a study conducted by the Centers for Disease Control and Prevention (CDC) that found the key factor in prolonged diarrhoea caused by *Campylobacter* infection was ciprofloxacin resistance (27). Critics argued that the methods used to obtain those results were questionable; that the authors only reported a significant effect within a subset of study participants (29). Furthermore, the critics presented a cross-tabulation of the CDC duration data, grouped by ciprofloxacin resistance and travel which shows no association in the unadjusted data (29). These arguments were countered by the CDC authors by pointing out the critics had not taken into consideration the effect of antidiarrheal use in their crude analysis (28). This effect was so strong, they argued, that subset analysis was the only valid way of controlling for its effect (28). The investigation of diarrhoea duration in this thesis will add to the ongoing discussion of this topic.

In addition to the variables listed above, the effect of a putative virulence factor on duration of diarrhoea was examined. Among reports on conserved *C. jejuni* virulence-associated genes *cadF, flaA, cdtA, cdtB*, prevalence is very high and without much variation, but the prevalence of the plasmid pVir, which is responsible for host cell invasion in some *C. jejuni* strains, is more variable (30-32). With the laboratory

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

assistance of the Public Health Agency of Canada, polymerase chain reaction (PCR) assays for pVir were undertaken to address this question, and it was hypothesized that the resulting data could, in part, explain variation in duration of diarrhoea.

1.3 Survey of Knowledge of Travellers' Diarrhoea

Preliminary investigation of the case-control results suggested clear differences between *Campylobacter* infections acquired while at home and those acquired during travel outside of Canada and the U.S.A. Ciprofloxacin resistance and duration of diarrhoea were found to be greater among travel-related infections.

It seemed, therefore, that education of the traveller, specifically education targeting the improvement of their knowledge of risks for travellers' diarrhoea, which is commonly caused by *Campylobacter* infection, was an important element in the public health epidemiology of *Campylobacter*. There are many ways by which travellers can obtain information about these risks. Travel clinics, health care practitioners, travel agents, personal contacts, and internet sites can all provide written or oral information, but it is unclear how effective the information is in knowledge improvement. The most valid method of assessing effectiveness of learning material is pre and post-education testing, but the survey presented in this thesis provided a cost-effective means of roughly estimating effectiveness.

The following thesis documents the three aforementioned research projects and the background literature.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.4 References

1. Anonymous. Preliminary FoodNet data on the incidence of foodborne illnesses--selected sites, United States, 2002. MMWR Morb Mortal Wkly Rep 2003;52:340-3.
2. Bowman C, Flint J, Pollari F. Canadian integrated surveillance report: *Salmonella, Campylobacter*, pathogenic *E. coli* and *Shigella*, from 1996 to 1999. Can Commun Dis Rep 2003;29 Suppl 1:i-vi, 1-32 .
3. Fisher IS, Meakins S. Surveillance of enteric pathogens in Europe and beyond: Enter-net annual report for 2004. Euro Surveill 2006;11:E060824 3.
4. Gaudreau C, Gilbert H. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. Antimicrob Agents Chemother 1998;42:2106-8.
5. Gaudreau C, Gilbert H. Antimicrobial resistance of *Campylobacter jejuni* subsp. jejuni strains isolated from humans in 1998 to 2001 in Montreal, Canada. Antimicrob Agents Chemother 2003;47:2027-9.
6. Gupta A, Nelson JM, Barrett TJ, et al. Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. Emerg Infect Dis 2004;10:1102-9.
7. Luber P, Wagner J, Hahn H, Bartelt E. Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001-2002 from poultry and humans in Berlin, Germany. Antimicrob Agents Chemother 2003;47:3825-30.
8. Lucey B, Cryan B, O'Halloran F, Wall PG, Buckley T, Fanning S. Trends in antimicrobial susceptibility among isolates of *Campylobacter* species in Ireland and the emergence of resistance to ciprofloxacin. Vet Rec 2002;151:317-20.
9. Alberta Health and Wellness Health Surveillance. Health trends in Alberta 2000. (Edmonton): Alberta Health and Wellness, 2000. (http://www.health.gov.ab.ca/resources/trends_index.html).
10. Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, Taylor DE. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance. Antimicrob Agents Chemother 2004;48:3442-50.

11. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N Engl J Med 1999;340:1525-32.
12. Campylobacter Sentinel Surveillance Scheme Collaborators. Ciprofloxacin resistance in *Campylobacter jejuni*: case-case analysis as a tool for elucidating risks at home and abroad. J Antimicrob Chemother 2002;50:561-8.
13. Kassenborg HD, Smith KE, Vugia DJ, et al. Fluoroquinolone-resistant *Campylobacter* infections: eating poultry outside of the home and foreign travel are risk factors. Clin Infect Dis 2004;38 Suppl 3:S279-84.
14. Levy SB. Antibacterial household products: cause for concern. Emerg Infect Dis 2001;7:512-5.
15. Aiello AE, Larson E. Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. Lancet Infect Dis 2003;3:501-6.
16. Poole K. Mechanisms of bacterial biocide and antibiotic resistance. Symp Ser Soc Appl Microbiol 2002:55S-64S.
17. Reeves DS, Finch RG, Bax RP, et al. Self-medication of antibacterials without prescription (also called 'over-the-counter' use). A report of a Working Party of the British Society for Antimicrobial Chemotherapy. J Antimicrob Chemother 1999;44:163-77.
18. Vanden Eng J, Marcus R, Hadler JL, et al. Consumer attitudes and use of antibiotics. Emerg Infect Dis 2003;9:1128-35.
19. Pechere JC. Patients' interviews and misuse of antibiotics. Clin Infect Dis 2001;33 Suppl 3:S170-3.
20. Richman PB, Garra G, Eskin B, Nashed AH, Cody R. Oral antibiotic use without consulting a physician: a survey of ED patients. Am J Emerg Med 2001;19:57-60.
21. Carey B, Cryan B. Antibiotic misuse in the community--a contributor to resistance? Ir Med J 2003;96:43-4, 46.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

22. Erramouspe J, Adamcik BA, Carlson RK. Veterinarian perception of the intentional misuse of veterinary medications in humans: a preliminary survey of Idaho-licensed practitioners. J Rural Health 2002;18:311-8.
23. Edwards DJ, Richman PB, Bradley K, Eskin B, Mandell M. Parental use and misuse of antibiotics: are there differences in urban vs. suburban settings? Acad Emerg Med 2002;9:22-6.
24. Schorr D, Schmid H, Rieder HL, Baumgartner A, Vorkauf H, Burnens A. Risk factors for *Campylobacter* enteritis in Switzerland. Zentralbl Hyg Umweltmed 1994;196:327-37.
25. Neimann J, Engberg J, Molbak K, Wegener HC. A case-control study of risk factors for sporadic campylobacter infections in Denmark. Epidemiol Infect 2003;130:353-66.
26. Rodrigues LC, Cowden JM, Wheeler JG, et al. The study of infectious intestinal disease in England: risk factors for cases of infectious intestinal disease with *Campylobacter jejuni* infection. Epidemiol Infect 2001;127:185-93.
27. Nelson JM, Smith KE, Vugia DJ, et al. Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. J Infect Dis 2004;190:1150-7.
28. Nelson JM, Tauxe RV, Angulo F. Reply to Cox et al. J Infect Dis 2005;191:1566-7.
29. Cox LA, Jr., Copeland D, Vaughn M. Ciprofloxacin resistance does not affect duration of domestically acquired campylobacteriosis. J Infect Dis 2005;191:1565-6; author reply 1566-7.
30. Bacon DJ, Alm RA, Burr DH, et al. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infect Immun 2000;68:4384-90.
31. Bang DD, Nielsen EM, Scheutz F, Pedersen K, Handberg K, Madsen M. PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. J Appl Microbiol 2003;94:1003-1014.
32. Datta S, Niwa H, Itoh K. Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. J Med Microbiol 2003;52:345-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 2;
LITERATURE REVIEW

2.1 Introduction to Campylobacter

2.1.1 Taxonomy and Characterisation of Campylobacteriaceae

The family of Campylobacteraceae bacteria is within the epsilon subdivision of the Proteobacteria. Spiral bacteria have been observed for over a century, and those that are presently recognized as *Campylobacter* species were likely similar to bacteria isolated and identified as belonging to the genus *Vibrio* by McFadyean and Stockman in 1913. *V. jejuni* was identified as the infective organism in bovine dysentery by Jones *et al.* in 1931 (1) and *V. coli* were identified in 1943 from swine faeces by L.P. Doyle (2). Several *Vibrio* species were moved into the newly named genus *Campylobacter* in 1963, with *C. fetus* as the type species (3).

C. jejuni and *C. coli* are associated with serious human infections; *C. jejuni* is by far the most prevalent human pathogen (4). There are two genetically distinct subspecies of *C. jejuni, C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei;* the latter is not associated with animal reservoirs or human infection (5). In 1989, the stomach pathogen previously known as *C. pylori* was found to have a fatty acid structure uncharacteristic of the genus *Campylobacter* and was renamed *Helicobacter pylori* (6). Presently, there are 17 species of *Campylobacter* including *C. fetus, C. hyointestinalis, C. sputorum, C. mucosalis, C. consisus, C. curvus, C. rectus, C. coli, C. jejuni, C. lari, C. upsalensis, C. gracilis, C. showae, C. helveticus, C. hominis, C. insulaenigrae,* and *C. lanienae* (7)

Campylobacter are gram negative rods from 0.5 to 5 μm in length with variable forms. They may be observed as single curved spiral rods under normal growth conditions, as coccoid forms in old cultures, as gull-winged forms when two cells are joined as a chain, and as corkscrew forms as they propel themselves with their polar flagella (8). Most are microaerophilic; however, *C. fetus* and *C. rectus,* can use fumarate respiration and grow

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

in anaerobic conditions (9, 10). *Campylobacter* grow at 37°C, but not at 10°C. *C. jejuni, C. coli, C. lari,* and *C. upsalensis* are thermophiles, able to grow at 42°C (9).

To date, the genomes of three strains of *C. jejuni* have been sequenced: NCTC11168, RM1221, and 81-176, and results indicate these genomes contain from 1.6 to 1.8 million base pairs (11-13). Parkhill and colleagues reported that *C. jejuni* NCTC11168 has only four repeated sequences in its genome (13). They pointed out that given the apparent high potential for genetic variability among *C. jejuni*, it may be a quasi-species with no definitive genomic sequence. Hyper-variable sequences responsible for biosynthesis of surface proteins and the ability to use alternative respiratory pathways have been identified as important tools that allow *C. jejuni* to colonize variable intestinal environments (12, 13).

Others have documented the extensive genetic diversity among *Campylobacter* species. A study of 156 isolates of four species demonstrated that the likelihood of differences in alleles at loci for 11 different enzymes is between 61 and 90% (14). Campylobacters, particularly *C. jejuni* and *C. coli* are capable of readily sharing genetic information across species (14) and may lead to instability with respect to taxonomic divisions.

2.1.2 Laboratory Methods for Campylobacter

Selective Culture and Species Identification Methods. Campylobacter can be isolated from blood and faecal samples from humans and food animals and from foods. Although stool cultures are frequently requested for patients presenting symptoms of diarrhoeal infection in the U.S.A., the proportion of tests that yield identifiable pathogens is low; only 2% of over 30,000 specimens tested in 10 states during 1990 through 1992 were positive for the presence of *Campylobacter,* which was the most common pathogenic bacteria detected (15). In Alberta it has been recommended that stool cultures should only be ordered for patients who a) have had diarrhoea for $\geq$ 5 days and have not used antibiotics recently or b) have severe, bloody diarrhoea (16).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Various selective media are available for the isolation of *Campylobacter* from faecal samples and foods, including Skirrow's media, Preston media (or Campylobacter Agar Base), modified charcoal cefoperazone deoxycholate agar (mCCDA), cefoperazone-amphotericin-teicoplanin agar (CAT), and Karmali agar. Preston media and Karmali agar are used for the isolation of *Campylobacter* from foods (17, 18). Both mCCDA and CAT agars are used for testing of clinical isolates, and their *Campylobacter* isolation rates are very similar, although mCCDA medium is somewhat more effective for the isolation of *C. jejuni* (19, 20).

Incubation temperature can influence the sensitivity of detection of some species. For example, although *C. jejuni* and *C. coli* isolates can be isolated when incubated at 42°C the sensitivity of detection of these species is increased when incubated at 37°C (21). Micro-aerobic conditions are required for the isolation of campylobacters. The optimal gas composition for growth and isolation of *Campylobacter* is 5% O_2, 10% CO_2, and 85% N_2 (17). *Campylobacter* are slow-growing bacteria and it is recommended that 72 hours incubation is required for the absence of growth to be reported (22).

Campylobacter isolates can be identified at species level with a series of biochemical assays. Criteria for the differentiation of *C. jejuni, C. coli,* and *C. lari* from other campylobacters include positive catalase test results, and growth at 42°C, but not at 25°C (17). *C. jejuni* can be usually differentiated from *C. coli* and *C. lari* strains on the basis of a positive hippurate hydrolysis test, although *C. jejuni* subsp. *jejuni* are occasionally hippuricase-negative (17, 23, 24).

Two *C. jejuni* serotyping schemes have been developed by Penner and Hennessy and Lior *et al.* (25, 26). The Lior scheme groups *C. jejuni* strains into 21 serogroups based on their heat-labile antigens while the Penner method consists of 23 serogroups based on heat-stable antigens. The latter, which was developed in 1980, is the most commonly used serotyping method, but its utility is limited by the high frequency of nontypeable strains among campylobacters (27).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Methods for Epidemiologic and Molecular Genetic Investigations. Due to the existence of problematic hippuricase-negative *C. jejuni* strains, molecular methods are required to differentiate this species from *C. coli.* Molecular methods can also permit more rapid detection of *Campylobacter* than can culture methods. There are a number of PCR-based assays that have been developed for the identification of some species of *Campylobacter.* Primer sets have been developed for specific target genes that encode structural proteins or enzymes such as hippuricase (28-30). Primers that use random gene targets (31) or those that amplify fragments of the 23S rRNA and 16S rRNA of campylobacters have also been developed (29, 32). Due to the genetic variability and diversity within *Campylobacter* species, single PCR assays are not adequate for reliable speciation (5).

Amplified fragment length polymorphism (AFLP)-based profiling has been shown to be a robust method of species discrimination and an effective means of clustering isolates of different sources from similar outbreak events (33). *Campylobacter* strains of 97% AFLP profile similarity have been accurately identified as genetically or epidemiologically related (33).

Pulsed-field gel electrophoresis (PFGE) can also be used to link various cases and sources to *Campylobacter* food or waterborne outbreaks, and is the primary method used by PulseNet, the U.S. network for molecular subtyping of foodborne pathogens, including *C. jejuni.* In investigations of *Campylobacter* outbreaks, including those associated with specific foods, water sources, school gatherings, and populations, *Campylobacter* outbreak strains had identical PFGE profiles, that were unlike non-outbreak *Campylobacter* strains (34-37). Some have suggested, however, that the level of precision in genetic discrimination that can be measured by PFGE is excessive when resources are limited, and that many field epidemiological investigations do not require high levels of precision (38, 39).

The flagella of *Campylobacter* have a wide variety of surface antigens that, in some strains, has a role in their serotyping (40). Consequently, flagella typing, which is based on the *fla*A and *fla*B genes is effective for preliminary grouping of strains for

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

epidemiological purposes (41). The entire sequence of *flaB, flaA*, or the short variable region of the latter sequence have been shown to be effective tools for clustering a group of outbreak strains or strains from the same source population since the *fla* genes are relatively stable and the assays are relatively inexpensive (42-44).

Greater levels of genetic information are required in clinical microbiology, when searching for genes or regions that have not yet been characterised. Whole genome methods, such as cDNA microarray assays, have been developed for *Campylobacter* that allow for a gene presence/absence comparison of one strain to the genetic signals of another. Microarrays based on the NCTC strain 11168 have been used to identify regions of the genome of other *C. jejuni* strains that are responsible for specific characteristics, or to assess the level of genetic diversity among a group of strains (45-47). This method, however, is too costly for routine epidemiological purposes. In the near future, however, high-throughput genomic methods will enable cost-effective, highly detailed genomic comparisions of specific gene clusters of clinical interest or of the whole *C. jejuni* genome (48).

Antibiotic Susceptibility Testing. The susceptibility of a *Campylobacter* strain to an antibiotic can be determined from its minimum inhibitory concentration (MIC) as interpreted from agar and broth dilution test results, or from inhibition zone diameter results of disk diffusion test.

The agar dilution method involves the inoculation of 10^4 cfu of a cultured strain suspended and adjusted to a 0.5 MacFarland using a Steers or Cathra replicator into Mueller-Hinton agar with 5% blood and serial dilutions of an antibiotic (49-51). Broth dilution involves the inoculation of strain suspensions into Mueller-Hinton broth with serial dilutions of antibiotic. Plates and tubes are incubated at 37°C for 24 to 48 h in micro-aerophilic conditions. The MIC is read as the lowest concentration at which there is no visible growth on, or in, the incubated agar or broth. Dilution methods are used by some as a reference method, but both are too time-consuming to be used for routine laboratory purposes (49, 52). Mechanised systems with prepared micro-titre plates are

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

commonly used to expedite the broth dilution testing process. Micro-broth dilution testing is a time-efficient method of obtaining MIC data, and the sensitivity of this method is near that of agar dilution (49).

The disk diffusion method is an inexpensive and simple method to measure antibiotic sensitivity. A colony of a strain is grown in a nutrient broth for 2 to 5 hours until the broth becomes cloudy, then suspensions are swabbed onto Mueller-Hinton agar with 5% blood and a paper disk containing a known amount of antibiotic (for example, 5 μg of ciprofloxacin) is placed onto the plate (51, 53). Following incubation at 35°C for 48 hours under micro-aerophilic conditions, the diameter of the zone of inhibition, in which there is no bacterial growth due to the inhibitory concentration of antibiotic, is read to the nearest millimetre. The zone of inhibition is then used for the interpretation of antibiotic susceptibility [susceptible, intermediate, or resistant (SIR)].

Dilution MIC results are more informative than are disk diffusion SIR results because there are more ordinal intervals in the MIC range than there are in the SIR range. Nevertheless, disk methods are convenient in routine surveillance where the incidence of *Campylobacter* is high, and nalidixic acid disks are often used as a surrogate to assess fluoroquinolone susceptibility in campylobacters. Gaudreau and Gilbert found that ciprofloxacin resistance in *Campylobacter* strains was correctly predicted in 86.7% of 15 nalidixic acid disk diffusion tests, and all 129 strains that were susceptible to nalidixic acid were susceptible to ciprofloxacin (51).

Another commonly used testing method is the Etest method, which has been used to obtain MIC values of various genera of bacteria since the early 1990's. The Etest uses a plastic strip with a gradient of antibiotic on one surface and a MIC reading scale on the other. The strip is placed on Mueller-Hinton agar with 5% blood that has been covered with a 1.0 MacFarland suspension of a strain that had been cultured on blood agar. For the testing of *C. jejuni* and *C. coli* the agar and strip are incubated for 42°C for 18 to 24 h or at 35°C for 24 to 72 h (55). The Etest is faster than agar dilution testing, but it is expensive. Ge and colleagues found high agreement between Etest results and agar

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

dilution results for *Campylobacter* isolates (50). Ciprofloxacin MICs determined by Etest results for 85.2% isolates (n = 108) were within one dilution of the MIC determined by agar dilution.

Resistance is typically assessed relative to the cut-offs for resistance determined and published by the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards, NCCLS). Currently there are no standards for antibiotic susceptibility testing of *Campylobacter,* although standard protocols and interpretive standards are being developed (55). Commonly, resistance in *Campylobacter* has been classified using CLSI breakpoint guidelines for aerobic human isolates (22, 56-59). CLSI recommends that Enterobacteriaceae and other aerobic organisms whose ciprofloxacin MIC is ≥4 μg/ml should be interpreted as resistant to ciprofloxacin (55). In the absence of guidelines for *Campylobacter* some have simply stated they assumed ≥4 μg/ml as ciprofloxacin resistance breakpoint (60, 61), which is the breakpoint for *Campylobacter* developed by the National Antimicrobial Resistance Monitoring System (NARMS) (62). Still others use the recommended ciprofloxacin resistance breakpoint of ≥ 1mg/L set by the British Society of Antimicrobial Chemotherapy (63, 64). CLSI recommends zones of inhibition ≤ 15mm indicate resistance to ciprofloxacin, and zones ≥ 21mm indicate susceptibility to ciprofloxacin.

2.2 Campylobacter Infections

2.2.1 Clinical Infection

Bacteria of the *Campylobacter* genus have tropisms for various tissues and organs; the preferred targets differ among species and sub-species. Consequently, *Campylobacter* species are variously associated with infections and diseases such as gingival and gastrointestinal infections, septic abortion, and autoimmune disorders such as reactive arthritis, and Guillain-Barré syndrome (65-68). *Campylobacter* associated with gingival infections include *C. rectus, C. consisus, C. curvus, C. showae* (9, 65). *C. fetus* strains have been associated with sporadic cases of septic abortion and neonatal mortality in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

humans (66, 69, 70). *Campylobacter* are one of the more common causes of traveller's diarrhoea and sporadic enterocolitis (71-75). Species definitively or putatively associated with intestinal infection and *Campylobacter* colitis in humans include *C. hyointestinalis, C. sputorum, C. jejuni, C. coli, C. lari,* and *C. hominis* (76, 77). This review will focus on species involved with gastrointestinal illness in humans.

Fever and abdominal pain that accompanies diarrhoea are common clinical features of *Campylobacter* spp. infections; bloody stool and vomiting are less frequent (78). Typically, the incubation period for infection is two to three days, and duration of diarrhoea is 7 to 11 days (79-81).

Severity and duration of gastroenteritis caused by *Campylobacter* varies between people from developed and developing countries; differences in antibody levels may have an important role. In developing countries, immunity may develop as individuals are repeatedly exposed, and it is often initiated in childhood (82). For travellers from developed countries, those who have lower levels of antibodies to *C. jejuni* before travel are more likely to have a higher risk of diarrhoea during travel to a developing country (83).

2.2.2 *Campylobacter* Infection Treatment and Prophylaxis

Typically, gastroenteritis caused by *Campylobacter* is self-limiting. In uncomplicated cases of gastrointestinal infection in those who are immunocompetent, standard therapy involves electrolyte and fluid replacement, and antibiotics are not required. Initial treatment can also involve the use of over-the-counter anti-diarrhoeal medications (84, 85). When illness coincides with bloody stool, fever, hospitalization, antibiotic use, or dehydration, faecal culture is recommended (78). Others argue that while testing faecal specimens for pathogen identification and antibiotic susceptibility prior to treatment may be prudent, it may increase the duration of illness, hence, empirical treatment with a fluoroquinolone, erythromycin, or azithromycin may be more beneficial in some cases (85).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Duration of diarrhoea, fever, vomiting, and abdominal pain in gastroenteritis patients, including individuals infected with *Campylobacter,* can be significantly shortened with 500 mg ciprofloxacin, administered twice daily (86, 87). Nevertheless, some authorities have recommended that ciprofloxacin treatment be reserved for those with frequent diarrhoea for more than three days, and for those with fever, vomiting, myalgia, abdominal pain, or complications (87, 88). Ciprofloxacin is associated with few adverse reactions, and is generally well-tolerated (86, 89). Some authorities list erythromycin as the only recommended treatment in immunocompetent and immunocomprimised patients (90). Erythromycin has good absorption in the intestine, but it may not be effective if treatment is delayed after the onset of symptoms (91). Azithromycin is also an effective alternative to fluoroquinolones when traveller's diarrhoea is caused by *Campylobacter* (92).

Fluoroquinolones are sometimes used as prophylaxis against traveller's diarrhoea. Juckett reports that 500 mg of ciprofloxacin can be taken once daily for up to three weeks; however, with increasing resistance to ciprofloxacin among many bacteria associated with traveller's diarrhoea, he advises against the use of this drug for prophylaxis (84). Alternative prophylaxis drugs, bismuth subsalicylate and rifaximin, have lower protection rates than those of fluoroquinolones, but the latter is the preferred prophylaxis, as there is little concern regarding rifaximin resistance (93). Rifaximin, a non-absorbable rifamycin derivative, may become a commonly used traveller's diarrhoea prophylaxis for travellers in areas where *Campylobacter* infection is endemic, because it is equivalent to ciprofloxacin in effectiveness, the likelihood of developing resistance is low, and there are few adverse effects associated with it (94).

Occasionally, *Campylobacter* illness can lead to conditions beyond gastroenteritis. Bacteraemia can develop in patients with *Campylobacter* infections, particularly in immunocompromised individuals (95). Infections among homosexual men have been problematic for decades and even more so among those with human immunodeficiency syndrome (96-98).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

C. jejuni and *C. coli* are the *Campylobacter* species most commonly involved in acute disease and chronic sequela in humans. Systemic infections and auto-immune disorders such as reactive arthritis, Fisher syndrome, and Guillain-Barré syndrome (GBS) may follow *C. jejuni* infections. Although less than two out of 10,000 of *Campylobacter* infections are followed by GBS, it is associated with serious clinical outcomes such as paralysis in the arms, legs, and face, and can result in severe disability or death (99, 100). Compared with others in the community, those with *Campylobacter* infections have an increased risk of short-term (30 day) and long-term (up to 1 year) mortality, which may be due to sequela of infections (101). Fatalities have been recorded in the literature, and the estimated of annual death rate for Colorado was <0.1 per 100,000 (102).

Recently, *C. jejuni* have been identified as putative causative organisms for immunoproliferative small intestinal disease (IPSID), a mucosa-associated lymphoid tissue lymphoma (103, 104). IPSID lymphomas are strongly associated with geography, with many cases reported from Africa and the Middle East. Speculative models involve chronic re-infection leading to sustained IgA-producing plasma cells, and *C. jejuni* cytolethal distending toxin causing DNA breaks that lead to B cells mutating and becoming atypical plasma cells (104, 105).

Post-infection irritable bowel syndrome (PI-IBS) is diagnosed as persistent change in bowel habits following gastroenteritis. Along with *Shigella, Campylobacter* is one of the more common agents of precursory infection that leads to PI-IBS (106).

2.2.3 Incidence of *Campylobacter* Infections

Campylobacter are one of the most common agents of foodborne disease in the developed world. In the U.S., following declines in incidence during 1996 through 2002, the CDC estimated the incidence of *Campylobacter* infections in 2002 was 13.4 per 100,000 persons, which was the second most common foodborne infection (72). In Alberta, estimated incidence of *Campylobacter* in 1997 was 41.6 per 100,000 persons (107).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Although *Campylobacter* are not as commonly associated with foodborne outbreaks as are other enteric pathogens, there are many recorded in the literature. An early outbreak associated with unpasteurised milk occurred in Red Deer, Alberta, in 1980, in which 14 cases were confirmed following a religious camp event (108). Unpasteurised milk has been identified as the source of three other *Campylobacter* outbreaks in Europe and the U.S. (109-111).

Water has been associated with various outbreaks of *Campylobacter* infections in North America and Europe (39, 112-114). *Campylobacter* and *Escherichia coli* O157:H7 infections were concurrently identified in two North American waterborne outbreaks (39, 112). Other reports include one outbreak associated with barbequed chicken in Germany (34), two associated with salad in Northern Ireland and South Australia (115, 116), and three associated with other foods or food handling (36, 117, 118). Source of infection has not been identified in several other outbreaks of infection (119-122). In these investigations, there was no source tracking, no food source identified epidemiologically to the outbreak, or no remaining food to be examined. A nosocomial *Campylobacter* outbreak occurred in Spain in 2000, and a farming, work-related outbreak was reported in Ontario in 1994 (123, 124).

Although the number of laboratory-confirmed cases in some *Campylobacter* outbreaks is less than 10, it ranges from 10 to over 100 in most others. The largest number of confirmed cases of *Campylobacter* in an outbreak event was 116, which occurred in the Walkerton, Ontario waterborne disease outbreak, during May of 2000 (39). Almost all reported outbreaks were very short in duration, although an outbreak associated with a self-serve salad bar at a training facility in South Australia persisted for 11 weeks (115). In that outbreak, six clinical cases presented over one week in August of 1995, and 68 were symptomatic within October and November of that year. The overall attack rate was 26%, with 74 ill, but only 16 culture-positive. Among 20 reported *Campylobacter* outbreaks, 16 were associated with *C. jejuni*, *C. coli* was associated with one outbreak in Belgium, and speciation of several other outbreaks was not conducted.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.2.4 Aetiology of *Campylobacter* Infection

Animal Sources of Campylobacter. Animals are believed to be an important reservoir for *Campylobacter;* livestock and companion animals are both potential sources of human pathogenic species. The thermophilic species of *Campylobacter* are suited to growth in poultry, which have a body temperature typically 41°C or 42°C. Over 40% of broilers at slaughter in Canada harbour *Campylobacter* (125). Poultry in retail stores is more commonly contaminated with *Campylobacter* than are other retail meats. In a recent study, 62% of raw chicken legs (n = 100) from supermarket chains in Alberta were contaminated with *Campylobacter,* and 79% of those strains were of the species *C. jejuni* (18). Reports from the U.S. and Europe have recorded levels of contamination ranging from 29 to 84% (58, 126-133). In those reports, between 85 and 95% of campylobacters isolated from poultry belong to the species *C. jejuni* (125, 134).

Campylobacter are commonly shed by live beef cattle and diary cows. Prevalence estimates are between 22 and 62% (20,135,136). The detection of *Campylobacter* is much lower from beef carcasses and retail meats than from bovine faeces. Less than 1% of beef carcasses processed in Australia were found to harbour *Campylobacter* (137). Contamination of beef retail products with *Campylobacter* is rare (1.3%, n = 151) (135). Recent estimates of *Campylobacter* in raw milk range from 0.47% (n = 1720) to 9.2% (n = 131) in North American bulk milk tanks (138,139). Campylobacters in beef products and dairy cows are largely of the species *C. jejuni* (135,136).

Similar to cattle, cows, and their food products, the majority of pigs shed *Campylobacter,* but these organisms are rarely detected in pork from retail stores (58,135,140). A recent survey found no contamination among raw pork chops (n = 98) purchased in Alberta stores (18). Among processed pork and retail pork products in the U.S. between 1.3 and 6.7% are contaminated with *Campylobacter* (133,141). The majority of campylobacters in pigs and pork are *C. coli* (58,135,142,143).

Companion animals are also a reservoir of *Campylobacter,* and this source is often associated with *C. upsaliensis* (144-148). Among healthy animals, between 6 and 43% of

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

dogs, and between 5 and 13% of cats shed *C. upsaliensis*, while between 3 and 22% of dogs, and between 0% and 4% of cats shed *C. jejuni* (144-146, 148). In a population-based study of *Campylobacter* species from humans, less than one percent of campylobacters detected (n = 492) were identified as *C. upsaliensis,* while 97% were *C. jejuni / C. coli,* thus, the pet-associated species appear to not be a major contributor to *Campylobacter* infections in humans (149). Although human contact with dogs and cats is relatively common, the risk of acquiring *Campylobacter* infection from pets is not great, since the species most commonly shed by pets is apparently only weakly associated with human infections.

Campylobacter species have also been isolated from wild birds at a relatively high frequency. Investigations in Scandinavia report the prevalence of *Campylobacter* among wild birds ranges between 12 and 28%, and the majority of those are *C. jejuni* (150, 151). Kapperud and Rosef found strong variation in carriage among 26 bird species; 90% (n = 48) of samples from hooded crows (*Corvus corone cornix*) and 51% (n = 76) of puffins (*Fratercula arctica*) were positive for *Campylobacter* presence (150), while only four percent (n = 71) of domestic pigeons (*Columba livia*) were positive. Wild birds may serve as a means of transporting *Campylobacter* from one livestock ecosystem to another.

Other Sources of Campylobacter. C. coli, C. lari, and *C. jejuni* have been reported in river water, roof water, agricultural runoff, and piped drinking water in developed countries (152-154). Although the concentration of *C. lari* reported in drinking water in New Zealand was low (0.3 per 100 ml), the number of positive samples was relatively high (29% of 24) (152), which may suggest the possibility that this species is quite ubiquitous and is more able to withstand water treatment processes than are other species. It is apparent from such reports that consumption of untreated water can increase one's risk of infection by *C. jejuni.* This risk is difficult to measure in retrospective studies of *Campylobacter* infection, due to the fact that few people in the developed world drink untreated water. *Campylobacter* are negatively affected by sunlight (155); however, other questions remain about environmental campylobacters with respect to their

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

survival, as well as their virulence and the relative contributions of wild and domesticated animals.

Secondary transmission of *Campylobacter* is seldom reported in the literature; however, a food handler was identified as the source of one outbreak in the U.S.A., and there is one report of nosocomial transmission in a neonatal ward in a hospital in Spain (36, 123).

2.3 Pathogenesis and Virulence Determinants

The pathogenesis of *Campylobacter* infection is a rapidly developing field of research. Similar to other enteric pathogens such as *Salmonella* and *E. coli* O157:H7, *Campylobacter* infect hosts and cause disease in broadly defined stages. Typically, the first stage of enteric infection requires adhesion to the epithelium of the intestine. Other stages that may or may not occur include the release of toxins, the invasion of the epithelial cells, the induction of an immune or inflammatory response, and the translocation across the epithelial layer and transportation throughout the body via the lymphatic system. Disease is caused by the infection itself or autoimmune responses to the infection (156).

2.3.1 Adhesion

Adhesion is critical to the ability of an enteric pathogen to cause infection and survive; if they are not firmly attached to the epithelium they will be readily forced through the gastrointestinal system and will be without an opportunity to colonise and establish a population. Presently, it appears that *Campylobacter* possess a variety of structures and proteins that allow attachment to epithelial cells; two mechanisms involve flagella and fibronectin.

The ability of *C. jejuni* to autoagglutinate, a process that requires flagella, is associated with various levels of adherence to intestinal cells (157,158). The *flaA* gene of is largely responsible for motility in *C. jejuni* (159). *C. jejuni* preferentially bind to finger-like

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

projections of human intestinal cells called fibronectin (160), and this binding is encoded by the gene *cadF* (161). Fibronectin is a glycoprotein present in the basement membrane underlying the epithelial cells and serves as a binding site for various pathogens including *Salmonella enterica* servar Typhimurium (162).

2.3.2 Invasion and Toxin Production

In the process of invasion of host cells, *C. jejuni* align themselves with the microtubules of the host cell, which are surface strands on the cytoskeleton of eukaryotic cells (163). Once aligned, *C. jejuni* can gain access into the intracellular region of the host cell by taking advantage of normal cellular energy conversion processes (163).

When the *Campylobacter* invasion antigen protein named CiaB that is produced by *C. jejuni* was identified, it was thought to be involved in the internalisation of the bacteria in host cells via a type III secretion system (164). The role of Cia protein secretion in virulence has been confirmed (165); however, the subsequent publication of the *C. jejuni* NCTC 11168 genome provided no evidence of a type III secretion system (13).

While the genome of *C. jejuni* may not possess genes encoding a type III secretion system, a plasmid found in the *C. jejuni* strain 81-176, pVir, contains genes with homology to a type IV secretion system (166). Mutations in two genes in this system, *virB10* and *virB11,* strongly reduced this strain's adhesion on and internalisation of intestinal cells (166). The *virB11* gene of *C. jejuni* was considered to be important for virulence as it is a homolog of the *virB11* of *Helicobacter pylori,* in which it encodes a type IV secretion system (167).

Cytolethal distending toxin (CDT) production has been characterised as a virulence factor of *C. jejuni* (168-170). The toxicity of CDT was observed early in the history of *Campylobacter* virulence research (171). *Campylobacter* CDT appears to be destructive on a number of fronts. Once inside intestinal epithelial cells, *C. jejuni* CDT survives attacks from human monocytes, and induces apoptosis of intestinal cells (172). In addition, there appears to be an interdependency or interaction between CDT proteins and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the release of interleukin-8, and other pro-inflammatory chemokines, which are promoters of localised immune response (170, 173).

2.4 Fluoroquinolones and Resistance in *Campylobacter*

2.4.1 Fluoroquinolone activity

Quinolone antibiotics, which include fluoroquinolone antibiotics, are synthetic compounds that have bactericidal activity against gram negative and gram positive bacteria. Nalidixic acid, developed in 1962, was the forerunner to other quinolones. Fluoroquinolones were introduced in the mid 1980's to improve activity against *Pseudomonas aeruginosa* through the addition of fluorine at the 6-position of the quinolone nucleus. These second-generation quinolones include ciprofloxacin, ofloxacin, and norfloxacin. They are indicated for urinary tract infections, sexually transmitted diseases, and soft tissue infections (174). Third-generation quinolones, including levofloxacin and gatifloxacin, have greater activity against gram positive bacteria than do second-generation quinolones; however, they are less effective against *Pseudomonas* spp. Of all the fluoroquinolones, ciprofloxacin has the greatest activity against gram negative bacteria, such as *Salmonella, E. coli, Shigella,* and *Campylobacter* (175).

The activity of quinolones against bacteria is based on their ability to inhibit DNA gyrase, which is involved in the negative supercoiling of DNA following DNA replication (175, 176). This enzyme is comprised of two types of subunits; A-subunits are encoded by the *gyrA* gene and their function is to hydrolyse the separate strands of DNA and wrap the strands around themselves, and B-subunits are involved in re-sealing the DNA strands.

2.4.2 Fluoroquinolone Use in Humans

Fluoroquinolone prescribing has increased in Canada in the past decade (177). A recent report estimates the annual number of quinolone prescriptions dispensed in Canada increased from 60 to 90 per 1000 persons during 1997 through 2004, and prescribing in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Alberta was the third highest among all provinces (178). In the United States, between 200,000 and 250,000 prescriptions for ciprofloxacin were written weekly in 2000, which is, on average, 40.2 prescriptions/1000 persons/year (179). In October 2001, following cases of anthrax inhalation in some regions of the U.S., the number of ciprofloxacin weekly prescriptions increased by 160,000 from October 2000 (178). Nevertheless, compared to other types of antibiotics, the consumption of quinolones and fluoroquinolones is low. In Manitoba, only 4.0% of antibiotic prescriptions in 1997 were for fluoroquinolones, while almost 50% of antibiotics used were penicillins (179). Quinolone prescriptions only accounted for 2.5% of all antibiotic prescriptions in the U.K. in 1996 (180). Among oral and intra-muscular antibiotics prescribed to U.S. adults with common infectious conditions, quinolones accounted for 8% in 1991 and 16% in 1999 (181).

From the perspective of pharmacoeconomic analyses, fluoroquinolones are cost-effective and important treatments for many common illnesses. For example, ofloxacin is one of the most cost-effective empiric treatments of urinary tract infections (182). Another example is the use of ciprofloxacin for the treatment of acute exacerbations of chronic bronchitis. While the net cost of ciprofloxacin treatment is greater than that of other antibiotics (such as amoxycillin, cefuroxime, erythromycin, cefaclor), based on cost per quality-adjusted life-year gained, ciprofloxacin is more efficacious in the treatment of bronchitis (183).

Nevertheless, there are some clinical issues surrounding fluoroquinolone use. There have been cautions and restrictions against the use of fluoroquinolones in children, largely based on quinolone cartilage toxicity observed in immature animals (184). However, recent research indicates that the proportion of children on ciprofloxacin who experience joint pain is low and the proportion is similar to that observed in adults on ciprofloxacin (185, 186). Another concern surrounding the use of broad-spectrum antibiotics, such as fluoroquinolones, is that they affect not only pathogenic bacteria, but also commensal organisms of the gastrointestinal tract. The gut microbiota plays an important role as it provides colonisation resistance against exogenous pathogenic organisms through the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

production of volatile fatty acids, nutrient competition, and attachment site competition (187). These endogenous populations are protective against gastrointestinal colonisation of *Campylobacter* (188). Two meta-analyses reported strong suppression of Enterobacteriaceae in the gastrointestinal flora following ciprofloxacin exposure, and, fortunately, very little evidence of antibiotic resistance among the endogenous bacteria following treatment (189, 190).

2.4.3 Utilization in Animals

In Canada, the notice of compliance in Canada for enrofloxacin (a fluoroquinolone) used by turkey producers as an egg dip was withdrawn in October, 1997, and presently, no fluoroquinolone is permitted for use in poultry (191). Since 2004, the subcutaneous use of enrofloxacin was approved for use in cattle in Canada (192). Also, the use of enrofloxacin, marbofloxacin, and orbifloxacin is permitted for therapeutic purposes in dogs and cats (192). In the U.S., injectable enrofloxacin and danofloxacin are approved for the treatment of bovine respiratory disease in cattle (193). The approval for use of enrofloxacin in poultry in the U.S. was withdrawn in 2005 (193). Extra-label use fluoroquinolones in food animals is not permitted in the U.S. (Extra-label use is the use of a product not indicated on the product label). As in Canada, the use of orbifloxacin and enrofloxacin to treat some diseases in dogs and cats is permitted in the U.S. There is no veterinary antibiotic use monitoring in Canada or the U.S., therefore, the extent of fluoroquinolone use in these countries is unknown. Furthermore, sales of antibiotics for animal use are considered to be proprietary information and thus, data on animal fluoroquinolone use is unavailable in the literature.

Published literature on antibiotic usage in animals in developing countries is sparse (194, 195), but there are indications that the lack of regulatory controls on such usage in some of these countries is a contributary factor in antibiotic resistance (196).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.4.4 Causes and Effects of Fluoroquinolone Resistance

Clinical Effects. Several groups have attempted to quantify the effects of fluoroquinolone resistance on the outcomes of *Campylobacter* infections. Investigators of the FoodNet surveillance system in the U.S.A. reported that among domestically-acquired *Campylobacter* infections not adjunctively treated with anti-motility or anti-diarrhoea medication, duration of diarrhoea was significantly longer among those who had fluoroquinolone-resistant *Campylobacter* than among those infected with a fluoroquinolone-susceptible strain (197). Travers and Barza suggested that these results demonstrated increased virulence in *Campylobacter* that are resistant to fluoroquinolones (198); however, they did not consider the possibility that the increase in duration of diarrhoea could have been caused by delays in prescribing appropriate antibiotics for those patients with fluoroquinolone-resistant infections.

Recently, others have suggested that antibiotic resistance acquired through genetic mutation comes at a cost to the virulence properties of gram-negative bacteria (199). Under selective antibiotic pressure, resistance to nalidixic acid is associated with an initial loss of virulence among *S.* Typhimurium and *E. coli,* followed by the acquisition of compensatory mutations that restored virulence (200-203).

Resistance Mechanisms. Presently, two mechanisms by which *Campylobacter* species acquire fluorquinolone resistance have been identified. The most commonly implicated involves mutation in gyrase-encoding sequences. Mutations in *gyrA* at positions Asp-90, Ala-70, and Thr-86, have been linked to fluoroquinolone resistance, although the first two mutation sites are associated with lower levels of resistance (204) As noted above, DNA gyrase, encoded by *gyrA,* is a type II DNA topoisomerase involved in replication. In 1993, Wang and colleagues discovered that a substitution of cytosine for thymine at nucleotide 256 in the *gyrA* gene of *C. jejuni* results in a Thr-86 to Ile substitution (a substitution of isoleucine for threonine in codon 86) (205). Since then, others have also found this mutation is important for mediating decreased susceptibility to ciprofloxacin among *C. jejuni, C. coli,* and *C. lari* (206-211).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The consistency of this phenomenon was illustrated by the work of Hakanen and colleagues, who reported ciprofloxacin MICs and amino acid substitutions in the quinolone resistance-determining region of 162 *C. jejuni* strains (207). All strains with the Thr-86 to Ile substitution were resistant to ciprofloxacin (MIC from 8 to >64 μg/ml) and all strains without the substitution were susceptible to ciprofloxacin (MIC from 0.064 to 1 μg/ml). Other workers have also found the Thr-86 to Ile substitution is always present in clinical *C. jejuni* isolates with high level resistance to ciprofloxacin (MIC ≥ 16 μg/ml) (212, 213). Some have suggested that a mutation in *parC,* which encodes topoisonerase IV is also responsible for quinolone resistance in *C. jejuni* (208), but other laboratories were unable to detect *parC* sequences from clinical strains of *C. jejuni* (209, 212, 214).

Another mechanism involved in fluoroquinolone resistance in *Campylobacter* is the activity of efflux pumps, which can transport toxic substances out of normally functioning gram negative bacteria. For example, exposure of a bacterium to a chemical compound that is toxic to that organism could lead to the expression of a specific efflux pump system. If a certain antibiotic is transportable via this efflux pump, then once the system is triggered, the bacterium would be resistant to that antibiotic.

In 1995, Charvalos and colleagues found *C. jejuni* mutants with defective efflux pumps accumulated fluoroquinolones and other unrelated antibiotics intracellularly (215). Later, Pumbwe and Piddock characterised the *Campylobacter* multi-drug efflux protein, Cme in *C. jejuni* (216). Others have reported that the susceptibility of a *cmeABC* mutant of strain *C. jejuni* 81-176 to many structurally unrelated antimicrobials is 2 to 256 times greater than that of the wild-type strain (217). In addition, without a functioning CmeABC pump, fluoroquinolone resistance in *gyrA* mutants drops below levels of clinical significance, suggesting that the CmeABC efflux pump is required for high-level fluoroquinolone resistance (214).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.4.5 Prevalence of Resistance to Fluoroquinolones in *Campylobacter*

Campylobacter Isolated from Humans. There are many reports of increasing trends in resistance to fluoroquinolones among *Campylobacter* strains from humans, foods, and food animals (57, 218-221). There are particularly high levels of resistance among strains in developing parts of the world, and among strains isolated from travelers who have returned from a developing country (61, 222-225).

Information presented in Table 2.4.1 indicates a strong regional variation and increasing trends over time. The reports from Reina (226) and Hoge (225) demonstrate an upward trend in resistance among *Campylobacter* from Spain and Thailand, respectively. There are similar trends documented in Canada, the U.S., and Ireland. All studies reported negligible levels of resistance at the beginning of the study period, followed by a rapid increase: in Montreal, an increase of resistance of 13% over 12 years was reported (57). In Pennsylvania, Nachamkin reported an increase of 33% within five years (60), and an increase of 34% over a four year period was documented in Ireland (221). However, it is unclear if the upward trend of resistance in all infections reflects trends of both domestic and travel-related infections, since there are few studies which consistently report stratified results over time.

It should be noted that the laboratories of Gaunt and the *Campylobacter* Sentinel Surveillance Scheme Collaborators used the British Society for Antimicrobial Chemotherapy breakpoint of 1 mg/L (64, 227). These labs may, therefore, have had higher numbers of resistant strains than if they had used the more commonly used 4 mg/L National Committee for Clinical Laboratory Standards breakpoint for Enterobacteriaceae. Another limitation with the data presented in Tables 2.4.1 and 2.4.2 is the constraint on comparability due to variation among sampling and laboratory methods used in individual studies.

Continuous antibiotic resistance monitoring schemes such as the National Antimicrobial Monitoring System (NARMS), the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), and the Canadian Integrated Program

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

for Antimicrobial Resistance (CIPARS) contribute to some of the data presented in Tables 2.4.1 and 2.4.2. Surveillance and monitoring systems are an invaluable source of longitudinal, nation-wide data.

Campylobacter Isolated From Foods and Food Animals. Similar to trends in fluoroquinolone resistance among *Campylobacter* isolated from humans, there appears to be some geographic variation in the frequency of resistance among strains from animals and their food products (Table 2.4.2). Levels reported in three Canadian publications are very low (228-230), while levels in Spain, Iran, and Korea are over 75% (58, 231, 232). Two European studies found fluoroquinolone resistance in *Campylobacter* from domestic meat products was lower than that from imported meat products (233, 234), and a Swiss study reported that *Campylobacter* from imported products were five times more likely to be resistant to ciprofloxacin than strains from domestic products (235). Trends over time of fluoroquinolone resistance in *Campylobacter* from animal sources are largely unreported in the literature, except for the small decrease from 1997 to 2000 in ciprofloxacin resistance among *Campylobacter* isolated from chicken reported in Northern Ireland (236). Consistently, fluoroquinolone resistance is more common among *C. coli* than among *C. jejuni,* within a single animal source (61, 237-240).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 2.4.1. Quinolone or fluoroquinolone resistance in *Campylobacter* isolated from humans in travel-related, domestically-acquired, or mixed (travel and domestic) infections; grouped by country and listed by initial sampling year.

First author (citation)	Years of data	Location of sampling	Species	Travel ‡	Resistance %	n
Gaudreau (57)	1985-1986	Montreal, Canada	*C. jejuni*	M	0	47
	1995-1997			M	13	158
Guevremont (229)	1998-1999	St-Hyacinthe, Canada	*C. jejuni*	M	9	23
Gaudreau (218)	1998	Montreal, Canada	*C. jejuni*	M	10	62
	2000				26	72
	2001				47	51
	1999-2001			D	9	109
				F	66	74
Gibreel (241)	1999-2002	Alberta, Canada	*C. jejuni*	M	2	203
Smith (61)	1992	Minnesota, USA	*C. jejuni*	M	1	NA
	1998				10	NA
Nachamkin (60)	1996	Pennsylvania USA	*C. jejuni*	M	8	48
	2001				41	47
Gupta (219)	1997-2001	USA	*C. jejuni*	M	16	1471
			C. coli	M	30	63
Kassenborg (242)	1998-1999	USA	spp.	D	9	582
				F	42	64
Gaunt (227)	1991	Plymouth, UK	spp.	M	4	2209
CSSSC (64)	2000-2001	UK	*C. jejuni*	D	10	2783
				F	53	653
Lucey (221)	1996-1998	Ireland	*C. jejuni*	M	0	36
	2000				34	96

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

First author (citation)	Years of data	Location of sampling	Species	Travel ‡	Resistance %	n
Moore (236)	1996	Northern Ireland	spp.	M	9	175
	1999				23	262
	2000				17	333
Hakanen (224)	1995-1997	Helsinki, Finland	*C. jejuni*	F	40	205
	1998-2000				60	149
Sjogren (243)	1992-1995	Sweden	spp.	D	6	36
				F	26	274
Sjogren (244)	1998-1999	Sweden	*C. jejuni*	M	1	110
			C. coli	M	0	27
Osterlund (245)	2000-2002	Sweden	*C. jejuni* and *C. coli*	D	4	164
Aarestrup (56)	1995-1996	Denmark	*C. jejuni*	M	12	75
Engberg (223)	2001-2002	Funen, Denmark	spp.	D	10	526
				F	50	152
Endtz (247)	1982	The Netherlands	spp.	M	0	78
	1989				11	143
Hein (245)	1998	Styria, Austria	*C. jejuni*	M	39	239
Krausse (248)	1980-1982	Germany	*C. jejuni* and *C. coli*	M	0	30
	1997-1998				29	93
	2001				30	99
Luber (220)	1991	Berlin, Germany	*C. jejuni*	M	6	68
	2001				46	65
	1991		*C. coli*		0	14
	2001				41	17
Prats (249)	1985-1987	Barcelona, Spain	*C. jejuni*	M	1	660
	1995-1998				82	909

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

First author (citation)	Years of data	Location of sampling	Species	Travel ‡	Resistance %	n
Reina (226)	1987	NA – possibly Spain	*C. jejuni*	M	0	104
	1989				3	146
	1991				30	154
Gallardo (75)	NA	Barcelona, Spain	*C. jejuni*	F	13	24
				D	38	88
Unicomb (250)	1999-2001	New South Wales, Australia	spp.	D	0	144
				F	43	7
Hoge (225)	1987	Thailand	spp.	D	0	14
	1990				0	63
	1993				40	52
	1995				84	27
Bodhidatta (222)	1998-2000	Bangkok, Thailand	*C. jejuni*	D	88	138
			C. coli		97	36

‡ D indicates domestically-acquired strains only; F indicates strains from foreign travel-related strains only; M indicates mixed strains with no stratification of strains by travel.

NA indicates information not given in the publication.

CSSSC: *Campylobacter* Sentinel Surveillance Scheme Collaborators

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 2.4.2. Quinolone or fluoroquinolone resistance in *Campylobacter* isolated from foods and food animals, grouped by country and listed by initial sampling year.

First author (citation)	Years of data	Source of samples	Source of strains	Species	Resistance %	n
Guevremont (229)	1998-1999	Quebece, Canada	Broiler faeces	*C. jejuni*	1	180
Inglis (230)	1999	Alberta, Canada	Cattle faeces	*C. jejuni*	1	NA
Kos (228)	2001	Alberta, Canada	Broilers, retail	*C. jejuni*	8	104
Smith (61)	1996 - 1997	Minnesota, U.S. A.	Retail chicken	*C. jejuni*	19	67
				C. coli	26	19
Ge (237)	1999-2000	Washington D.C., U.S.A	Poultry, beef, and pork, retail	*C. jejuni*	25	88
				C. coli	40	75
Price (251)	2003	Baltimore, U.S.A.	Chicken, raw, retail	spp.	40	36
Sãnchez (252)	NA	Missouri, U.S.A.	Immersion-chilled chicken	spp.	58	73
		Nebraska, U.S.A.	Air-chilled chicken		18	59
Wilson (234)	1995, 2000	Northern Ireland	Chicken, raw, retail	spp.	11	208
		Imported from developing countries	Chicken, frozen, retail		14	42
Moore (236)	1997 2000	Northern Ireland	Chicken	spp.	9 5	327 44

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

First author (citation)	Years of data	Source of samples	Source of strains	Species	Resistance %	n
Fallon (253)	1999	Ireland	Broiler chickens	*C. jejuni*	18	78
Avrain (238)	1999	France	Chicken caecal samples	*C. jejuni*	25	297
				C. coli	43	96
Aarestrup (142)	1995 - 1996	Denmark	Broilers	*C. jejuni*	2	97
			Cattle		11	47
Andersen (233)	1996 - 2003	Denmark imported	Retail poultry	*C. jejuni*	6	367
					13	88
	1999	Denmark & imported			9	NA
	2003				2	NA
Pedersen (239)	1998 - 1999	Denmark	Chicken cloacal swabs	*C. jejuni*	11	2785
				C. coli	29	321
Van Looveren (254)	1998	Belgium	Broilers	*C. jejuni*	44	285
			Layers		28	105
			Turkeys		35	94
Frediani-Wolf (255)	2001	Switzerland	Poultry at slaughter	*C. jejuni*	2	195
Ledergerber (237)	2002	Switzerland & imported	Retail poultry	spp	29	87
Pezzotti (135)	2000 - 2001	Northeastern Italy	Broilers	*C. jejuni*	42	64
			Pigs	*C. coli*	36	47
			Beef cattle	*C. jejuni*	25	12
Sãenz (58)	1997 - 1998	Spain	Broilers	*C. jejuni*	99	59
			Retail chicken products		74	41
Taremi (231)	2004	Tehran, Iran	Retail chicken & beef	spp.	75	72

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

First author (citation)	Years of data	Source of samples	Source of strains	Species	Resistance %	n
Cardinale (240)	2001 - 2002	Dakar, Senegal	Chicken carcasses	*C. jejuni*	33	120
				C. coli	37	85
Sackey (256)	NA	Accra, Ghana	Retail chicken	*C. jejuni*	0	7
Kang (232)	2000 - 2002	Korea	Retail chicken	spp.	88	594

NA indicates information not given in the publication.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.5 Risk Factor Analyses of Fluoroquinolone Resistance in *Campylobacter* Infections

To date there are only a handful of research papers in the literature that report on the effects of risk exposures on the likelihood of fluoroquinolone resistance in *Campylobacter* infections in humans. There is variation in the risk exposures investigated, study design, and presentation of the results. The following is an evaluation of methods and presentation of results. Papers were identified from abstracts found in the MedLine database using the search terms: *Campylobacter* and resistance.

2.5.1 Gaunt and Piddock Study

The sample population for the first risk factor analysis of ciprofloxacin resistance included all *Campylobacter* patients identified by routine diagnostic testing at a public health laboratory in the U.K. over six months in 1991 (227). Controls matched to cases by age, sex, month of onset, and place of residence were obtained (maximum two controls per case) from among patients with ciprofloxacin-susceptible *Campylobacter* infections. Questionnaires administered by environmental health workers included questions on travel history, quinolone use, food history, family contacts, and exposure to animals. A three-month exposure risk period was assumed. There were only 15 cases and 24 controls in this study. None had precedent quinolone drug exposure, but foreign travel was considered a highly significant risk factor ($p = 0.01$). Recent poultry consumption was not associated with resistance. The main weakness of this study is the small sample size, and the lack of information on data analysis methods. There was, apparently, no attempt to control for confounding or to conduct any multivariate analysis.

2.5.2 Smith *et al* Study

The study of quinolone resistance in *Campylobacter* infections by Smith *et al.* (61) is very commonly cited in discussion sections of other research articles. It is a very wide-ranging paper that presents a survey of *Campylobacter* from retail chicken, results of a six-year surveillance of *Campylobacter* from humans reported in Minnesota, and results

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

from a molecular subtyping analysis of human and chicken strains, as well as a comparative study of nalidixic acid resistant and nalidixic acid susceptible *C. jejuni.* From 1996 through 1997, there were 142 infections caused by nalidixic acid resistant *C. jejuni* reported in Minnesota, and 1576 infections from susceptible strains. The research team was fortunate to obtain a 92% participation rate from cases. Two controls infected with nalidixic acid-susceptible *C. jejuni* were matched to each case by age, residence, and date of stool collection. Participants were questioned about clinical history, antibiotic use one month prior to infection, recent diarrhoea, use of antibiotics by household members, history of food consumption, animal contact, and travel history. The exposure risk period for all exposures except for antibiotics was seven days.

The final multivariate logistic regression model from the data included variables for quinolone use [odds ratio (OR) = 8; 95% confidence interval (CI) for odds ratio (OR) = 3, 21] and for travel to Mexico [OR = 26; 95% CI (OR) = 9, 79], Caribbean countries [OR = 46; 95% CI (OR) = 10, 214], Asia [OR = 41; 95% CI (OR) = 10, 163], and Spain [OR = 49; 95% CI (OR) = 4, 570]. No foods were identified as risk factors for resistance. Unfortunately, this paper is frequently cited for its evidence that chicken is largely to blame for the increase in quinolone resistance in human *Campylobacter* strains, which the authors extrapolated from their subtyping results; yet their own results from the case-comparison study suggested that food consumption had no role in resistance risk.

2.5.3 Campylobacter Sentinel Surveillance Scheme Collaborators Study

In a more thoroughly documented study, a collective of *Campylobacter* researchers in the U.K. reported an investigation that involved 3489 patients from whom *C. jejuni* was isolated over a one-year period (64). Risk exposures evaluated by the CSSSC included travel history, exposure to foods and water, the environment, and animals two weeks prior to onset of illness. Antibiotic exposure was not included as it was felt that questions about this topic are typically poorly answered. Fortunately, for readers who are interested in methodological details, the questionnaire was provided in a file supplemental to the published report. The list of food types included in the questionnaire

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

is quite extensive, and possible responses are "never", "once" or "more often". They also covered waterborne risk exposures very thoroughly.

The prevalence of ciprofloxacin resistance among the 3489 patients with *C. jejuni* in the study population was 19%. Separate analyses were conducted for travel-related infections and indigenous infections. Infections caused by strains that were ciprofloxacin-susceptible but resistant to more than one other antibiotic were excluded from all analyses (n = 1110); the risk factor analysis was conducted on the remaining 2379 participants. There was no difference in age or gender between ciprofloxacin-resistant *C. jejuni* infection case and ciprofloxacin-susceptible *C. jejuni* infection control groups, which may call into question the need for matching on age and gender in the previously discussed studies. Among those who had recently travelled (n = 495), independent risk factors for ciprofloxacin resistance included travel to Spain [OR = 7; 95% CI (OR) = 4, 13], Portugal [OR = 22; 95% CI (OR) = 4, 115], or Cyprus [OR = 12; 95% CI (OR) = 1, 108], chicken consumption [OR = 5; 95% CI (OR) = 2, 12], and bottled water consumption [OR = 4; 95% CI (OR) = 2, 8]. Protective exposures included contact with pet birds [OR = 0.1; 95% CI (OR) = 0.02, 0.6], travel to Africa [OR = 0.1; 95% CI (OR) = 0.02, 0.7], and consumption of tap water [OR = 0.2; 95% CI (OR) = 0.1, 0.5]. Among indigenous cases and controls (n = 1884), only one independent risk factor was identified: consumption of cold meats [OR = 2; 95% CI (OR) = 1, 3]. Consumption of tap water [OR = 0.4; 95% CI (OR) = 0.2, 0.9], and summer infection versus other seasons [OR = 0.5; 95% CI (OR) = 0.3, 0.7] were found to be protective factors. The most salient points were: 1) foreign travel to certain countries was a strong risk factor, 2) chicken consumption was a risk factor, but only among travel-related infections, and 3) there was a difference in the risk factors for ciprofloxacin resistance in *C. jejuni* among those infections acquired abroad and those acquired domestically. It is unfortunate that the authors did not examine the effect of antibiotic exposure, which is uncommon among the general population, given that they had a very extensive sample population.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.5.4 Kassenborg *et al.* Study

The Foodborne Disease Active Surveillance Network in the U.S. interviewed 646 *Campylobacter* patients over a one-year period (242); this included 64 cases with fluoroquinolone-resistant *Campylobacter* infections and 582 controls with fluoroquinolone-susceptible infections. Among potential cases and controls eligible for this study, the participation rates were 68% and 76%, respectively. In addition, 62 healthy controls were obtained for comparison purposes. Study participants were questioned about antibiotic and antacid use and specific medical conditions four weeks prior to onset. They were also asked about food and water consumption, child care and animal exposure, travel, and food handling practices one week prior to onset.

The fluoroquinolone resistance proportion among 858 *Campylobacter* strains available to the study group was 11%. The only identified risk factor for resistance was foreign travel [OR = 8; 95% CI (OR) = 4, 13]. They reported that antibiotic use was not a risk factor [OR = 0.6; 95% CI (OR) = 0.1, 3].

The remaining analysis, the primary focus of the paper, contrasted domestically acquired infections against healthy controls. The use of healthy controls is not as common as is the use of fluoroquinolone susceptible *Campylobacter* infection controls in this field of research. It is possible that, for this large government-funded research team, it was important to identify risks for acquiring ciprofloxacin resistant *Campylobacter* infections for the majority of U.S. citizens (domestic infections) to enable the development of widely useful, science-based interventions to prevent infection. This information, it could be argued, has a more immediate public health benefit than does the identification of risk factors for ciprofloxacin resistance. The multivariate analysis found eating chicken outside of the home was a risk factor for fluoroquinolone resistant *Campylobacter* infection [matched OR (mOR) = 10; 95% CI (mOR) = 1, 78].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.5.5 Hakanen *et al.* Study

A study by Hakanen *et al.* examined Finnish patients who had returned from travel from 1995 through 2000 and from whom *C. jejuni* had been isolated (224). In this work they estimated, for specific travel destinations, the proportion of travellers who acquired ciprofloxacin resistant *C. jejuni.* They then compared the speculative infection rates for each destination to the infection rate for Thailand, the country associated with the highest infection rate. Risk of infection was lower in infections acquired in Spain [n = 77; (rate ratio (RR) = 0.1; 95% CI (RR) = 0.07, 0.2] and Portugal [n = 11; RR = 0.1; 95% CI (RR) = 0.05, 0.2] compared to infections acquired in Thailand. The value of this analysis is questionable, since their reference population was travellers to Thailand, which is not very informative from a public health perspective.

2.5.6 Engberg *et al.* Study

In a research project similar in structure to that presented in the paper by Smith *et al.* (61), a Danish research group conducted a risk factor analysis for quinolone resistance in *C. jejuni* infections, as well as a survey of *Campylobacter* strains isolated from food animals and foods, and a molecular subtyping of human, food, and animal strains (223). In the study region, the prevalence of quinolone resistance among *Campylobacter* was 18%. The risk factor analysis included 42 cases with resistant-strain infections and 84 matched controls with quinolone susceptible-strain infections. Controls were matched by date of stool sample submission. Multivariate logistic regression analysis indicated that travel abroad [mOR = 17; 95% CI (mOR) = 3, 82], swimming [mOR = 5; 95% CI (mOR) = 1, 22], and consumption of fresh poultry other than chicken and turkey [mOR = 19; 95% CI (mOR) = 2, 167], were risk factors for resistance, while eating fresh chicken was a protective factor [mOR = 0.04; 95% CI (mOR) = 0.004, 0.4].

The authors described in detail the controls of veterinary fluoroquinolone use in Denmark and national surveillance data published elsewhere that found no resistance among *C. jejuni* from Danish broiler chickens. Furthermore, they reported that among domestically acquired infections, subtypes of quinolone-susceptible *C. jejuni* were more likely to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

match the subtypes found among all *C. jejuni* isolated from retail foods and chicken than were subtypes of quinolone-resistant strains. They then suggest that these points could explain why eating fresh chicken is a protective factor for resistance. This would be reasonable if they could also suggest why the balance of the population, people who ate an alternative to fresh chicken (frozen chicken, other meats, or no meats) should have resistant infections. They reported that 5 of 11 subtypes of resistant strains from retail food and chicken matched the subtypes of the resistant strains from humans, and 34 of 88 subtypes from susceptible strains from food and chicken matched those from susceptible strains from humans. It is not clear if these data are strong enough to support the authors' hypothesis that retail foods and chicken could not be a source of resistant infections. The conclusions from this study are problematic, therefore, not only because of the small sample size (42 cases, 84 controls) and the wide confidence interval for the protective effect of eating fresh chicken, but also because of the reasoning about the protective nature of eating fresh chicken.

2.5.7 Concluding Remarks

Among the six papers discussed there is one point of consensus: travel abroad, largely to countries less industrialized than the country in which the study is performed, is a risk factor for fluoroquinolone or quinolone resistance among *Campylobacter* infections. The magnitude of the risk is typically large, and there is little debate about the importance of this variable in the prediction of resistance.

The reports of the importance of personal use of fluoroquinolone drugs prior to stool sample submission and the consumption of poultry products are inconsistent among reviewed publications. Authorities in clinical reviews of infectious diarrhoea treatment caution against the empiric use of fluoroquinolone drugs to treat gastroenteritis, due to the increasing levels of fluoroquinolone resistance among bacterial agents (78, 91). The use of fluoroquinolone drugs prior to stool sample submission is, therefore, probably not very common in most study regions, particularly for immunocompetent cases when symptoms are less severe. With very low numbers of exposed people in a sample population, there is a high probability of numerical problems with stratified or

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

multivariate analyses. Point estimates may indicate significant risk, but the 95% confidence interval for the estimate itself may be wide, indicating an unstable and unreliable estimate of the true risk value. An imprecise estimate indicating a "significant" risk is of less value to scientific knowledge than is a precise estimate indicating a "non-significant" risk (257).

In contrast to fluoroquinolone use risk estimates, the inconsistency of risk associated with poultry consumption may be due, in part, to a very high level of exposure in the sample population. It may be difficult to detect a difference in exposure to poultry products between case and control groups, particularly if the exposure period is longer than a few days. Poultry meat, in particular, chicken, is commonly consumed in meals as whole meat, and in sandwiches, pasta, stir fry, salads, etc. Hence, variability of exposure among the two groups may be very slight. The difficulty to detect a difference increases with increasing exposure period, and exposure period varied among the studies discussed here. Added to this is the problem of inaccurate recall, which can lessen data quality. Researchers in child health and breast cancer have demonstrated that food consumption recall can be poor, although there is little indication of differential exposure misclassification (258, 260).

In conclusion, the papers discussed in this section, apart from that of the CSSSC report, have several shortcomings. Descriptions of data collection methods and statistical methods, in particular, diagnostic or sensitivity analyses, are absent from these papers or are lacking in depth. There is almost no discussion of the validity of the results and there are several examples, discussed above, of questionable concluding statements.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.6 References

1. Jones FS, Orcutt M, Little RB. Vibrios (*Vibrio jejuni,* n.sp.) associated with intestinal disorders of cows and calves. J Exp Med 1931;53:853-65.
2. Doyle LP. The etiology of swine dysentery. Am J Vet Res 1948;9:50-1.
3. Sebald M, Veron M. Teneur en bases de l'ADN et classification des Vibrions. Annales de L'Institut Pasteur 1963;105:897-910.
4. Friedman CR, Neimann J, Wegener HC, Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin I, Blaser MJ, eds. *Campylobacter.* Washington, D.C.: American Society for Microbiology, 2000.
5. On SL, Jordan PJ. Evaluation of 11 PCR Assays for Species-Level Identification of *Campylobacter jejuni* and *Campylobacter coli.* J Clin Microbiol 2003;41:330-6.
6. *Campylobacter pylori* becomes *Helicobacter pylori.* Lancet 1989;2:1019-20.
7. Euzeby JP. List of Prokaryotic Names with Standing and Nomenclature, 1997. (http://www.bacterio.net).
8. Vandamme P, Falsen E, Rossau R, et al. Revision of *Campylobacter, Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int J Syst Bacteriol 1991;41:88-103.
9. Vandamme P. Taxonomy of the Family Campylobacteraceae. In: Nachamkin I, Blaser MJ, eds. *Campylobacter.* Washington, D.C.: ASM Press, 2000:3-26.
10. Lancaster CR, Simon J. Succinate:quinone oxidoreductases from epsilon-proteobacteria. Biochim Biophys Acta 2002;1553:84-101.
11. Fouts DE, Mongodin EF, Mandrell RE, et al. Major structural differences and novel potential virulence mechanisms from the genomes of multiple campylobacter species. PLoS Biol 2005;3:e15.
12. Hofreuter D, Tsai J, Watson RO, et al. Unique features of a highly pathogenic *Campylobacter jejuni* strain. Infect Immun 2006;74:4694-707.
13. Parkhill J, Wren BW, Mungall K, et al. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature 2000;403:665-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

14. Meinersmann RJ, Patton CM, Evins GM, Wachsmuth IK, Fields PI. Genetic diversity and relationships of *Campylobacter* species and subspecies. Int J Syst Evol Microbiol 2002;52:1789-97.
15. Slutsker L, Ries AA, Greene KD, Wells JG, Hutwagner L, Griffin PM. *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. Ann Intern Med 1997;126:505-13.
16. Alberta Clinical Practice Guidelines Program Working Group. Laboratory guideline for ordering stool test for investigation of suspected infectious diarrhea. Edmonton: The Alberta Clinical Practice Guidelines Program, 1997.
17. Medeiros D, Hofmann L. Isolation of thermophilic *Campylobacter* from food. Ottawa: Health Products and Food Branch, Health Canada, 2002.
18. Bohaychuk VM, Gensler GE, King RK, et al. Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. J Food Prot 2006;69:2176-82.
19. Engberg J, On SL, Harrington CS, Gerner-Smidt P. Prevalence of *Campylobacter, Arcobacter, Helicobacter*, and *Sutterella* spp. in human fecal samples as estimated by a reevaluation of isolation methods for Campylobacters. J Clin Microbiol 2000;38:286-91.
20. Corry JE, Atabay HI. Comparison of the productivity of cefoperazone amphotericin teicoplanin (CAT) agar and modified charcoal cefoperazone deoxycholate (mCCD) agar for various strains of *Campylobacter, Arcobacter* and *Helicobacter pullorum*. Int J Food Microbiol 1997;38:201-9.
21. Corry JE, Post DE, Colin P, Laisney MJ. Culture media for the isolation of campylobacters. Int J Food Microbiol 1995;26:43-76.
22. Nachamkin I, Engberg J, Aarestrup FM. Diagnosis and antimicrobial susceptibility of *Campylobacter* spp. In: Nachamkin I, Blaser MJ, eds. *Campylobacter*. Washington, D.C.: ASM Press, 2000:45-66.
23. Burnett TA, M AH, Kuhnert P, Djordjevic SP. Speciating *Campylobacter jejuni* and *Campylobacter coli* isolates from poultry and humans using six PCR-based assays. FEMS Microbiol Lett 2002;216:201-9.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

24. Steinbrueckner B, Haerter G, Pelz K, Kist M. Routine identification of *Campylobacter jejuni* and *Campylobacter coli* from human stool samples. FEMS Microbiol Lett 1999;179:227-32.

25. Lior H, Woodward DL, Edgar JA, Laroche LJ, Gill P. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat- labile antigenic factors. J Clin Microbiol 1982;15:761-8.

26. Penner JL, Hennessy JN. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J Clin Microbiol 1980;12:732-7.

27. Newell DG, Frost JA, Duim B, et al. New developments in the subtyping of *Campylobacter* species. In: Nachamkin I, Blaser MJ, eds. *Campylobacter.* Washington, D.C.: ASM Press, 2000:27-44.

28. Gonzalez I, Grant KA, Richardson PT, Park SF, Collins MD. Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the ceuE gene encoding a putative virulence determinant. J Clin Microbiol 1997;35:759-63.

29. Linton D, Lawson AJ, Owen RJ, Stanley J. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J Clin Microbiol 1997;35:2568-72.

30. Stucki U, Frey J, Nicolet J, Burnens AP. Identification of *Campylobacter jejuni* on the basis of a species- specific gene that encodes a membrane protein. J Clin Microbiol 1995;33:855-9.

31. Day WA, Jr., Pepper IL, Joens LA. Use of an arbitrarily primed PCR product in the development of a *Campylobacter jejuni*-specific PCR. Appl Environ Microbiol 1997;63:1019-23.

32. Fermer C, Engvall EO. Specific PCR identification and differentiation of the thermophilic campylobacters, *Campylobacter jejuni, C. coli, C. lari,* and *C. upsaliensis.* J Clin Microbiol 1999;37:3370-3.

33. On SL, Harrington CS. Identification of taxonomic and epidemiological relationships among *Campylobacter* species by numerical analysis of AFLP profiles. FEMS Microbiol Lett 2000;193:161-9.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

34. Allerberger F, Al-Jazrawi N, Kreidl P, et al. Barbecued chicken causing a multi-state outbreak of *Campylobacter jejuni* enteritis. Infection 2003;31:19-23.

35. Hanninen ML, Haajanen H, Pummi T, et al. Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. Appl Environ Microbiol 2003;69:1391-6.

36. Olsen SJ, Hansen GR, Bartlett L, et al. An outbreak of *Campylobacter jejuni* infections associated with food handler contamination: the use of pulsed-field gel electrophoresis. J Infect Dis 2001;183:164-7.

37. Rennie RP, Strong D, Taylor DE, Salama SM, Davidson C, Tabor H. *Campylobacter fetus* diarrhea in a Hutterite colony: epidemiological observations and typing of the causative organism. J Clin Microbiol 1994;32:721-4.

38. Hedberg CW, Smith KE, Besser JM, et al. Limitations of pulsed-field gel electrophoresis for the routine surveillance of *Campylobacter* infections. J Infect Dis 2001;184:242-4.

39. Clark CG, Price L, Ahmed R, et al. Characterization of waterborne outbreak-associated *Campylobacter jejuni,* Walkerton, Ontario. Emerg Infect Dis 2003;9:1232-41.

40. Harris LA, Logan SM, Guerry P, Trust TJ. Antigenic variation of *Campylobacter* flagella. J Bacteriol 1987;169:5066-71.

41. Petersen L, On SL. Efficacy of flagellin gene typing for epidemiological studies of *Campylobacter jejuni* in poultry estimated by comparison with macrorestriction profiling. Lett Appl Microbiol 2000;31:14-9.

42. Harrington CS, Moran L, Ridley AM, Newell DG, Madden RH. Inter-laboratory evaluation of three flagellin PCR/RFLP methods for typing *Campylobacter jejuni* and *C. coli*: the CAMPYNET experience. J Appl Microbiol 2003;95:1321-33.

43. Meinersmann RJ, Helsel LO, Fields PI, Hiett KL. Discrimination of *Campylobacter jejuni* isolates by fla gene sequencing. J Clin Microbiol 1997;35:2810-4.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

44. Mellmann A, Mosters J, Bartelt E, et al. Sequence-based typing of flaB is a more stable screening tool than typing of flaA for monitoring of *Campylobacter* populations. J Clin Microbiol 2004;42:4840-2.
45. Dorrell N, Mangan JA, Laing KG, et al. Whole genome comparison of *Campylobacter jejuni* human isolates using a low-cost microarray reveals extensive genetic diversity. Genome Res 2001;11:1706-15.
46. Pearson BM, Pin C, Wright J, I'Anson K, Humphrey T, Wells JM. Comparative genome analysis of *Campylobacter jejuni* using whole genome DNA microarrays. FEBS Lett 2003;554:224-30.
47. Carrillo CD, Taboada E, Nash JH, et al. Genome-wide expression analyses of *Campylobacter jejuni* NCTC11168 reveals coordinate regulation of motility and virulence by flhA. J Biol Chem 2004;279:20327-38.
48. van Belkum A. High-throughput epidemiologic typing in clinical microbiology. Clin Microbiol Infect 2003;9:86-100.
49. Luber P, Bartelt E, Genschow E, Wagner J, Hahn H. Comparison of Broth Microdilution, E Test, and Agar Dilution Methods for Antibiotic Susceptibility Testing of *Campylobacter jejuni* and *Campylobacter coli*. J Clin Microbiol 2003;41:1062-8.
50. Ge B, Bodeis S, Walker RD, et al. Comparison of the Etest and agar dilution for in vitro antimicrobial susceptibility testing of *Campylobacter*. J Antimicrob Chemother 2002;50:487-94.
51. Gaudreau C, Gilbert H. Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. J Antimicrob Chemother 1997;39:707-12.
52. Sahm DF, Washington JA. Antimicrobial susceptibility tests: Dilution methods. In: Balows A, ed. Manual of clinical microbiology. Washington, D.C.: AMS Press, 1991:1105-16.
53. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
54. Anonymous. ETest application sheet: *Campylobacter*. Solna: AB Biodisk, 2000.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

55. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard - Second Edition, 2002.

56. Aarestrup FM, Nielsen EM, Madsen M, Engberg J. Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, pigs, cattle, and broilers in Denmark. Antimicrob Agents Chemother 1997;41:2244-50.

56. Gaudreau C, Gilbert H. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. Antimicrob Agents Chemother 1998;42:2106-8.

57. Aquino MH, Filgueiras AL, Ferreira MC, Oliveira SS, Bastos MC, Tibana A. Antimicrobial resistance and plasmid profiles of *Campylobacter jejuni* and *Campylobacter coli* from human and animal sources. Lett Appl Microbiol 2002;34:149-53.

58. Saenz Y, Zarazaga M, Lantero M, Gastanares MJ, Baquero F, Torres C. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. Antimicrob Agents Chemother 2000;44:267-71.

59. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard. Wayne: National Committee for Clinical Laboratory Standards, 2002.

60. Nachamkin I, Ung H, Li M. Increasing Fluoroquinolone Resistance in *Campylobacter jejuni,* Pennsylvania, USA,1982-2001. Emerg Infect Dis 2002;8:1501-3.

61. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N Engl J Med 1999;340:1525-32.

62. Guevremont E, Sirois M, Quessy S. Antimicrobial susceptibility patterns of *Campylobacter coli* isolates from swine and humans. Salinpork 2001. Leipzig, 2001.

63. Thwaites RT, Frost JA. Drug resistance in *Campylobacter jejuni, C coli,* and *C lari* isolated from humans in north west England and Wales, 1997. J Clin Pathol 1999;52:812-4.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

64. Campylobacter Sentinel Surveillance Scheme Collaborators. Ciprofloxacin resistance in *Campylobacter jejuni*: case-case analysis as a tool for elucidating risks at home and abroad. J Antimicrob Chemother 2002;50:561-8.

65. Ihara H, Miura T, Kato T, et al. Detection of *Campylobacter rectus* in periodontitis sites by monoclonal antibodies. J Periodontal Res 2003;38:64-72.

66. Steinkraus GE, Wright BD. Septic abortion with intact fetal membranes caused by *Campylobacter fetus* subsp. *fetus*. J Clin Microbiol 1994;32:1608-9.

67. Peterson MC. Clinical aspects of *Campylobacter jejuni* infections in adults. West J Med 1994;161:148-52.

68. Nachamkin I. Chronic effects of *Campylobacter* infection. Microbes Infect 2002;4:399-403.

69. Sauerwein RW, Bisseling J, Horrevorts AM. Septic abortion associated with *Campylobacter fetus* subspecies fetus infection: case report and review of the literature. Infection 1993;21:331-3.

70. Viejo G, Gomez B, De Miguel D, Del Valle A, Otero L, De La Iglesia P. *Campylobacter fetus* subspecies *fetus* bacteremia associated with chorioamnionitis and intact fetal membranes. Scand J Infect Dis 2001;33:126-7.

71. Anonymous. Canadian Integrated Surveillance Report for 1995 on *Salmonella*, *Campylobacter* and Pathogenic *E. coli*. Canadian Communicable Disease Report 1998;24.

72. Anonymous. Preliminary FoodNet data on the incidence of foodborne illnesses--selected sites, United States, 2002. MMWR Morb Mortal Wkly Rep 2003;52:340-3.

73. Health Canada. Notifiable diseases annual summary, 1996. Canada Communicable Diseases Report: Health Canada, 1998.

74. Bowman C, Flint J, Pollari F. Canadian integrated surveillance report: *Salmonella, Campylobacter*, pathogenic *E. coli* and *Shigella*, from 1996 to 1999. Can Commun Dis Rep 2003;29 Suppl 1:i-vi, 1-32 (eng); i-vi, 1-34 (fre).

75. Gallardo F, Gascon J, Ruiz J, Corachan M, Jimenez de Anta M, Vila J. *Campylobacter jejuni* as a cause of traveler's diarrhea: clinical features and antimicrobial susceptibility. J Travel Med 1998;5:23-6.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

76. van Spreeuwel JP, Duursma GC, Meijer CJ, Bax R, Rosekrans PC, Lindeman J. *Campylobacter* colitis: histological immunohistochemical and ultrastructural findings. Gut 1985;26:945-51.

77. Lawson AJ, On SL, Logan JM, Stanley J. *Campylobacter hominis* sp. nov., from the human gastrointestinal tract. Int J Syst Evol Microbiol 2001;51:651-60.

78. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32:331-51.

79. Kapperud G, Lassen J, Ostroff SM, Aasen S. Clinical features of sporadic *Campylobacter* infections in Norway. Scand J Infect Dis 1992;24:741-9.

80. Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ. Experimental *Campylobacter jejuni* infection in humans. J Infect Dis 1988;157:472-9.

81. Blaser MJ. Epidemiologic and clinical features of *Campylobacter jejuni* infections. J Infect Dis 1997;176 Suppl 2:S103-5.

82. Skirrow MB, Blaser MJ. Clinical aspects of *Campylobacter* infection. In: Nachamkin I, Blaser MJ, eds. *Campylobacter*. Washington, D.C.: American Society for Microbiology, 2000.

83. Walz SE, Baqar S, Beecham HJ, et al. Pre-exposure anti-*Campylobacter jejuni* immunoglobulin a levels associated with reduced risk of *Campylobacter* diarrhea in adults traveling to Thailand. Am J Trop Med Hyg 2001;65:652-6.

84. Juckett G. Prevention and treatment of traveler's diarrhea. Am Fam Physician 1999;60:119-24, 135-6.

85. Thielman NM, Guerrant RL. Clinical practice. Acute infectious diarrhea. N Engl J Med 2004;350:38-47.

86. Pichler HE, Diridl G, Stickler K, Wolf D. Clinical efficacy of ciprofloxacin compared with placebo in bacterial diarrhea. Am J Med 1987;82:329-32.

87. Dryden MS, Gabb RJ, Wright SK. Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin. Clin Infect Dis 1996;22:1019-25.

88. Graninger W, Zedtwitz-Liebenstein K, Laferl H, Burgmann H. Quinolones in gastrointestinal infections. Chemotherapy 1996;42 Suppl 1:43-53.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

89. Auquer F, Cordon F, Gorina E, Caballero JC, Adalid C, Batlle J. Single-dose ciprofloxacin versus 3 days of norfloxacin in uncomplicated urinary tract infections in women. Clin Microbiol Infect 2002;8:50-4.
90. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32:331-51.
91. Oldfield EC, 3rd, Wallace MR. The role of antibiotics in the treatment of infectious diarrhea. Gastroenterol Clin North Am 2001;30:817-36.
92. Tribble DR, Sanders JW, Pang LW, et al. Traveler's diarrhea in Thailand: randomized, double-blind trial comparing single-dose and 3-day azithromycin-based regimens with a 3-day levofloxacin regimen. Clin Infect Dis 2007;44:338-46.
93. DuPont HL, Jiang ZD. Influence of rifaximin treatment on the susceptibility of intestinal Gram-negative flora and enterococci. Clin Microbiol Infect 2004;10:1009-11.
94. DuPont HL, Jiang ZD, Okhuysen PC, et al. Antibacterial chemoprophylaxis in the prevention of traveler's diarrhea: evaluation of poorly absorbed oral rifaximin. Clin Infect Dis 2005;41 Suppl 8:S571-6.
95. Pigrau C, Bartolome R, Almirante B, Planes AM, Gavalda J, Pahissa A. Bacteremia due to *Campylobacter* species: clinical findings and antimicrobial susceptibility patterns. Clin Infect Dis 1997;25:1414-20.
96. Snijders F, Kuijper EJ, de Wever B, van der Hoek L, Danner SA, Dankert J. Prevalence of *Campylobacter*-associated diarrhea among patients infected with human immunodeficiency virus. Clin Infect Dis 1997;24:1107-13.
97. Quinn TC, Goodell SE, Fennell C, et al. Infections with *Campylobacter jejuni* and *Campylobacter*-like organisms in homosexual men. Ann Intern Med 1984;101:187-92.
98. Molina J, Casin I, Hausfater P, et al. *Campylobacter* infections in HIV-infected patients: clinical and bacteriological features. Aids 1995;9:881-5.
99. Tam CC, Rodrigues LC, Petersen I, Islam A, Hayward A, O'Brien SJ. Incidence of Guillain-Barre syndrome among patients with *Campylobacter* infection: a general practice research database study. J Infect Dis 2006;194:95-7.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

100. Rees JH, Soudain SE, Gregson NA, Hughes RA. *Campylobacter jejuni* infection and Guillain-Barre syndrome. N Engl J Med 1995;333:1374-9.

101. Helms M, Vastrup P, Gerner-Smidt P, Molbak K. Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based study. Bmj 2003;326:357.

102. Smith GS, Blaser MJ. Fatalities associated with *Campylobacter jejuni* infections. Jama 1985;253:2873-5.

103. Lecuit M, Abachin E, Martin A, et al. Immunoproliferative small intestinal disease associated with *Campylobacter jejuni*. N Engl J Med 2004;350:239-48.

104. Al-Saleem T, Al-Mondhiry H. Immunoproliferative small intestinal disease (IPSID): a model for mature B-cell neoplasms. Blood 2005;105:2274-80.

105. Parsonnet J, Isaacson PG. Bacterial infection and MALT lymphoma. N Engl J Med 2004;350:213-5.

106. Connor BA. Sequelae of traveler's diarrhea: focus on postinfectious irritable bowel syndrome. Clin Infect Dis 2005;41 Suppl 8:S577-86.

107. Alberta Health and Wellness Health Surveillance. Health trends in Alberta 2000. (Edmonton): Alberta Health and Wellness, 2000. (http://www.health.gov.ab.ca/resources/trends_index.html).

108. McNaughton RD, Leyland R, Mueller L. Outbreak of *Campylobacter* enteritis due to consumption of raw milk. Can Med Assoc J 1982;126:657-8.

109. Outbreak of *Campylobacter jejuni* infections associated with drinking unpasteurized milk procured through a cow-leasing program--Wisconsin, 2001. MMWR Morb Mortal Wkly Rep 2002;51:548-9.

110. Lehner A, Schneck C, Feierl G, et al. Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre. Epidemiol Infect 2000;125:13-6.

111. Kalman M, Szollosi E, Czermann B, Zimanyi M, Szekeres S. Milkborne campylobacter infection in Hungary. J Food Prot 2000;63:1426-9.

112. Anonymous. Outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington County Fair-New York, 1999. MMWR Morb Mortal Wkly Rep 1999;48:803-5.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

113. Engberg J, Gerner-Smidt P, Scheutz F, Moller Nielsen E, On SL, Molbak K. Water-borne *Campylobacter jejuni* infection in a Danish town---a 6-week continuous source outbreak. Clin Microbiol Infect 1998;4:648-656.

114. Melby KK, Svendby JG, Eggebo T, et al. Outbreak of *Campylobacter* infection in a subartic community. Eur J Clin Microbiol Infect Dis 2000;19:542-4.

115. Kirk M, Waddell R, Dalton C, Creaser A, Rose N. A prolonged outbreak of *Campylobacter* infection at a training facility. Commun Dis Intell 1997;21:57-61.

116. Moore JE, Stanley T, Smithson R, O'Malley H, Murphy PG. Outbreak of *Campylobacter* food-poisoning in Northern Ireland. Clin Microbiol Infect 2000;6:399-400.

117. Gent RN, Telford DR, Syed Q. An outbreak of campylobacter food poisoning at a university campus. Commun Dis Public Health 1999;2:39-42.

118. Anonymous. From the Centers for Disease Control and Prevention. Outbreak of *Campylobacter* enteritis associated with cross-contamination of food--Oklahoma, 1996. Jama 1998;279:1341.

119. Ang CW, van Doorn PA, Endtz HP, et al. A case of Guillain-Barre syndrome following a family outbreak of *Campylobacter jejuni* enteritis. J Neuroimmunol 2000;111:229-33.

120. Harrington CS, Thomson-Carter FM, Carter PE. Molecular epidemiological investigation of an outbreak of *Campylobacter jejuni* identifies a dominant clonal line within Scottish serotype HS55 populations. Epidemiol Infect 1999;122:367-75.

121. Ronveaux O, Quoilin S, Van Loock F, Lheureux P, Struelens M, Butzler JP. A *Campylobacter coli* foodborne outbreak in Belgium. Acta Clin Belg 2000;55:307-11.

122. Raupach JC, Hundy RL. An outbreak of *Campylobacter jejuni* infection among conference delegates. Commun Dis Intell 2003;27:380-3.

123. Llovo J, Mateo E, Munoz A, Urquijo M, On SL, Fernandez-Astorga A. Molecular typing of *Campylobacter jejuni* isolates involved in a neonatal outbreak indicates nosocomial transmission. J Clin Microbiol 2003;41:3926-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

124. Ellis A, Irwin R, Hockin J, Borczyk A, Woodward D, Johnson W. Outbreak of *Campylobacter* infection among farm workers: an occupational hazard. Can Commun Dis Rep 1995;21:153-6.

125. Nadeau E, Messier S, Quessy S. Prevalence and comparison of genetic profiles of *Campylobacter* strains isolated from poultry and sporadic cases of campylobacteriosis in humans. J Food Prot 2002;65:73-8.

126. Harrison WA, Griffith CJ, Tennant D, Peters AC. Incidence of *Campylobacter* and *Salmonella* isolated from retail chicken and associated packaging in South Wales. Lett Appl Microbiol 2001;33:450-4.

127. Jorgensen F, Bailey R, Williams S, et al. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. Int J Food Microbiol 2002;76:151-64.

128. Kramer JM, Frost JA, Bolton FJ, Wareing DR. *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J Food Prot 2000;63:1654-9.

129. Dominguez C, Gomez I, Zumalacarregui J. Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. Int J Food Microbiol 2002;72:165-8.

130. Atanassova V, Ring C. Prevalence of *Campylobacter* spp. in poultry and poultry meat in Germany. Int J Food Microbiol 1999;51:187-90.

131. Uyttendaele M, De Troy P, Debevere J. Incidence of *Salmonella, Campylobacter jejuni, Campylobacter coli,* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. J Food Prot 1999;62:735-40.

132. Wedderkopp A, Gradel KO, Jorgensen JC, Madsen M. Pre-harvest surveillance of *Campylobacter* and *Salmonella* in Danish broiler flocks: a 2-year study. Int J Food Microbiol 2001;68:53-9.

133. Zhao C, Ge B, De Villena J, et al. Prevalence of *Campylobacter* spp., *Escherichia coli,* and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. Appl Environ Microbiol 2001;67:5431-6.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

134. Nielsen EM, Nielsen NL. Serotypes and typability of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry products. Int J Food Microbiol 1999;46:199-205.

135. Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M, Perin R. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. Int J Food Microbiol 2003;82:281-7.

136. Nielsen EM. Occurrence and strain diversity of thermophilic campylobacters in cattle of different age groups in dairy herds. Lett Appl Microbiol 2002;35:85-9.

137. Vanderlinde PB, Shay B, Murray J. Microbiological quality of Australian beef carcass meat and frozen bulk packed beef. J Food Prot 1998;61:437-43.

138. Jayarao BM, Henning DR. Prevalence of foodborne pathogens in bulk tank milk. J Dairy Sci 2001;84:2157-62.

139. Steele ML, McNab,W.B., Poppe,C., Griffiths,M.W., Chen,S., Degrandis,S.A, Fruhner, L.C., Larkin,C.A., Lynch,J.A., Odumeru. Survey of Ontario bulk tank raw milk for food-borne pathogens. J Food Prot 1997;60:1341-6.

140. Madden RH, Moran L, Scates P. Frequency of occurrence of *Campylobacter* spp. in red meats and poultry in Northern Ireland and their subsequent subtyping using polymerase chain reaction-restriction fragment length polymorphism and the random amplified polymorphic DNA method. J Appl Microbiol 1998;84:703-8.

141. Duffy EA, Belk KE, Sofos JN, Bellinger GR, Pape A, Smith GC. Extent of microbial contamination in United States pork retail products. J Food Prot 2001;64:172-8.

142. Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A, Wegener HC. Resistance to antimicrobial agents used for animal therapy in pathogenic-, zoonotic- and indicator bacteria isolated from different food animals in Denmark: a baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). Apmis 1998;106:745-70.

143. Van Looveren M, Chasseur-Libotte ML, Godard C, et al. Antimicrobial susceptibility of nontyphoidal *Salmonella* isolated from humans in Belgium. Acta Clin Belg 2001;56:180-6.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

144. Baker J, Barton MD, Lanser J. *Campylobacter* species in cats and dogs in South Australia. Aust Vet J 1999;77:662-6.

145. Engvall EO, Brandstrom B, Andersson L, Baverud V, Trowald-Wigh G, Englund L. Isolation and identification of thermophilic *Campylobacter* species in faecal samples from Swedish dogs. Scand J Infect Dis 2003;35:713-8.

146. Hald B, Madsen M. Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. J Clin Microbiol 1997;35:3351-2.

147. Steinhauserova I, Fojtikova K, Klimes J. The incidence and PCR detection of *Campylobacter upsaliensis* in dogs and cats. Lett Appl Microbiol 2000;31:209-12.

148. Sandberg M, Bergsjo B, Hofshagen M, Skjerve E, Kruse H. Risk factors for *Campylobacter* infection in Norwegian cats and dogs. Prev Vet Med 2002;55:241-53.

149. Lawson AJ, Logan JM, O'Neill G L, Desai M, Stanley J. Large-scale survey of *Campylobacter* species in human gastroenteritis by PCR and PCR-enzyme-linked immunosorbent assay. J Clin Microbiol 1999;37:3860-4.

150. Kapperud G, Rosef O. Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni, Yersinia* spp., and *Salmonella* spp. in Norway. Appl Environ Microbiol 1983;45:375-80.

151. Anonymous. Annual Report on Zoonoses in Denmark 2000. In: Ministry of Food AaF, ed, 2001.

152. Savill MG, Hudson JA, Ball A, et al. Enumeration of *Campylobacter* in New Zealand recreational and drinking waters. J Appl Microbiol 2001;91:38-46.

153. Rosef O, Rettedal G, Lageide L. Thermophilic campylobacters in surface water: a potential risk of campylobacteriosis. Int J Environ Health Res 2001;11:321-7.

154. Jones R, Hobbs A. Campylobacters and faecal indicators in streams and rivers subject to farm run-off. In: Newell DG, ed. Campylobacters, helicobacters, and related organisms. New York: Plenum Press, 1996.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

155. Jones K, Betaieb M, Telford DR. Thermophilic campylobacters in surface waters around Lancaster, UK: negative correlation with *Campylobacter* infections in the community. J Appl Bacteriol 1990;69:758-64.

156. Hu L, Kopecko DJ. Interactions of *Campylobacter* with eukaryotic cells: Gut luminal colonization and mucosal invasion mechanisms. In: Nachamkin I, Blaser MJ, eds. *Campylobacter*. Washington, D.C.: ASM Press, 2000.

157. Guerry P, Ewing CP, Schirm M, et al. Changes in flagellin glycosylation affect *Campylobacter* autoagglutination and virulence. Mol Microbiol 2006;60:299-311.

158. Misawa N, Blaser MJ. Detection and characterization of autoagglutination activity by *Campylobacter jejuni*. Infect Immun 2000;68:6168-75.

159. Wassenaar TM, Bleumink-Pluym NM, van der Zeijst BA. Inactivation of *Campylobacter jejuni* flagellin genes by homologous recombination demonstrates that flaA but not flaB is required for invasion. Embo J 1991;10:2055-61.

160. Konkel ME, Garvis SG, Tipton SL, Anderson DE, Jr., Cieplak W, Jr. Identification and molecular cloning of a gene encoding a fibronectin- binding protein (CadF) from *Campylobacter jejuni*. Mol Microbiol 1997;24:953-63.

161. Monteville MR, Yoon JE, Konkel ME. Maximal adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the CadF outer-membrane protein and microfilament reorganization. Microbiology 2003;149:153-65.

162. Kingsley RA, Santos RL, Keestra AM, Adams LG, Baumler AJ. *Salmonella enterica* serotype Typhimurium ShdA is an outer membrane fibronectin-binding protein that is expressed in the intestine. Mol Microbiol 2002;43:895-905.

163. Hu L, Kopecko DJ. *Campylobacter jejuni* 81-176 associates with microtubules and dynein during invasion of human intestinal cells. Infect Immun 1999;67:4171-82.

164. Konkel ME, Kim BJ, Rivera-Amill V, Garvis SG. Bacterial secreted proteins are required for the internaliztion of *Campylobacter jejuni* into cultured mammalian cells. Mol Microbiol 1999;32:691-701.

165. Rivera-Amill V, Kim BJ, Seshu J, Konkel ME. Secretion of the virulence-associated *Campylobacter* invasion antigens from *Campylobacter jejuni* requires a stimulatory signal. J Infect Dis 2001;183:1607-16.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

166. Bacon DJ, Alm RA, Burr DH, et al. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infect Immun 2000;68:4384-90.

167. Akopyants NS, Clifton SW, Kersulyte D, et al. Analyses of the cag pathogenicity island of Helicobacter pylori. Mol Microbiol 1998;28:37-53.

168. Pickett CL, Pesci EC, Cottle DL, Russell G, Erdem AN, Zeytin H. Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* sp. *cdtB* gene. Infect Immun 1996;64:2070-8.

169. Purdy D, Buswell CM, Hodgson AE, McAlpine K, Henderson I, Leach SA. Characterisation of cytolethal distending toxin (CDT) mutants of *Campylobacter jejuni*. J Med Microbiol 2000;49:473-9.

170. Hickey TE, McVeigh AL, Scott DA, et al. *Campylobacter jejuni* cytolethal distending toxin mediates release of interleukin-8 from intestinal epithelial cells. Infect Immun 2000;68:6535-41.

171. Johnson WM, Lior H. A new heat-labile cytolethal distending toxin (CLDT) produced by *Campylobacter* spp. Microb Pathog 1988;4:115-26.

172. Hickey TE, Majam G, Guerry P. Intracellular survival of *Campylobacter jejuni* in human monocytic cells and induction of apoptotic death by cytholethal distending toxin. Infect Immun 2005;73:5194-7.

173. Hu L, Hickey TE. *Campylobacter jejuni* induces secretion of proinflammatory chemokines from human intestinal epithelial cells. Infect Immun 2005;73:4437-40.

174. King DE, Malone R, Lilley SH. New classification and update on the quinolone antibiotics. Am Fam Physician 2000;61:2741-8.

175. O'Grady F, Lambert HP, Finch RG, Greenwood D. Antibiotic and chemotherapy: Anti-infectives agents and their use in therapy. New York: Churchill Livingstone, 1997.

176. Atlas RM. Principles of microbiology. Dubuque: Wm. C. Brown Publishers, 1997.

177. IMS Health Incorporated. Antibiotic prescribing trends in Canada, 2005. (http://www.ccar-ccra.com/english/ppt/CCARPerCapita04.ppt).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

178. Shaffer D, Armstrong G, Higgins K, et al. Increased US prescription trends associated with the CDC *Bacillus anthracis* antimicrobial postexposure prophylaxis campaign. Pharmacoepidemiol Drug Saf 2003;12:177-82.

179. Carrie AG, Metge CJ, Zhanel GG. Antibiotic use in a Canadian Province, 1995-1998. Ann Pharmacother 2000;34:459-64.

180. Majeed A, Moser K. Age- and sex-specific antibiotic prescribing patterns in general practice in England and Wales in 1996. Br J Gen Pract 1999;49:735-6.

181. Steinman MA, Gonzales R, Linder JA, Landefeld CS. Changing use of antibiotics in community-based outpatient practice, 1991-1999. Ann Intern Med 2003;138:525-33.

182. Rosenberg M. Pharmacoeconomics of treating uncomplicated urinary tract infections. Int J Antimicrob Agents 1999;11:247-51; discussion 261-4.

183. Torrance G, Walker V, Grossman R, et al. Economic evaluation of ciprofloxacin compared with usual antibacterial care for the treatment of acute exacerbations of chronic bronchitis in patients followed for 1 year. Pharmacoeconomics 1999;16:499-520.

184. Alghasham AA, Nahata MC. Clinical use of fluoroquinolones in children. Ann Pharmacother 2000;34:347-59.

185. Hampel B, Hullmann R, Schmidt H. Ciprofloxacin in pediatrics: worldwide clinical experience based on compassionate use--safety report. Pediatr Infect Dis J 1997;16:127-9; discussion 160-2.

186. Jick S. Ciprofloxacin safety in a pediatric population. Pediatr Infect Dis J 1997;16:130-3; discussion 133-4, 160-2.

187. Hentges DJ. The protective function of the indigenous intestinal flora. Pediatr Infect Dis 1986;5:S17-20.

188. Field LH, Underwood JL, Berry LJ. The role of gut flora and animal passage in the colonisation of adult mice with *Campylobacter jejuni*. J Med Microbiol 1984;17:59-66.

189. Korten V, Murray BE. Impact of the fluoroquinolones on gastrointestinal flora. Drugs 1993;45:125-33.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

190. Brumfitt W, Franklin I, Grady D, Hamilton-Miller JM, Iliffe A. Changes in the pharmacokinetics of ciprofloxacin and fecal flora during administration of a 7-day course to human volunteers. Antimicrob Agents Chemother 1984;26:757-61.

191. Veterinary Drugs Directorate. 2006: Notice of compliance for Baytril.

192. Anonymous. Notice of Compliance Listings: Health Canada, 2007. (http://www.hc-sc.gc.ca/dhp-mps/prodpharma/notices-avis/list/index_e.html#2004)

193. Anonymous. Database of Approved Animal Drug Products: FDA Center for Veterinary Medicine, 2007. (http://dil.vetmed.vt.edu/)..

194. Lopez HS, Olvera LG. Problematica del uso de enrofloxacina en la avicultura en Mexico. Vet Mex 2000;31:137-45.

195. Mitema ES, Kikuvi GM, Wegener HC, Stohr K. An assessment of antimicrobial consumption in food producing animals in Kenya. J Vet Pharmacol Ther 2001;24:385-90.

196. Okeke IN, Klugman KP, Bhutta ZA, et al. Antimicrobial resistance in developing countries. Part II: strategies for containment. Lancet Infect Dis 2005;5:568-80.

197. Marano N, Vugia D, Fiortentino T, et al. Fluoroquinolone-resistant *Campylobacter* causes longer duration of diarrhea than fluoroquinolone-susceptible *Campylobacter* strains in FoodNet sites. International Conference on Emerging Infectious Disease. Atlanta, 2000.

198. Travers K, Barza M. Morbidity of infections caused by antimicrobial-resistant bacteria. Clin Infect Dis 2002;34 Suppl 3:S131-4.

199. Maisnier-Patin S, Berg OG, Liljas L, Andersson DI. Compensatory adaptation to the deleterious effect of antibiotic resistance in *Salmonella typhimurium*. Mol Microbiol 2002;46:355-66.

200. Bjorkman J, Hughes D, Andersson DI. Virulence of antibiotic-resistant *Salmonella typhimurium*. Proc Natl Acad Sci U S A 1998;95:3949-53.

201. Blazquez R, Menasalvas A, Carpena I, Ramirez C, Guerrero C, Moreno S. Invasive disease caused by ciprofloxacin-resistant uropathogenic *Escherichia coli*. Eur J Clin Microbiol Infect Dis 1999;18:503-5.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

202. Johnson JR, van der Schee C, Kuskowski MA, Goessens W, van Belkum A. Phylogenetic background and virulence profiles of fluoroquinolone- resistant clinical *Escherichia coli* isolates from the Netherlands. J Infect Dis 2002;186:1852-6.

203. Martinez-Martinez L, Fernandez F, Perea EJ. Relationship between haemolysis production and resistance to fluoroquinolones among clinical isolates of *Escherichia coli*. J Antimicrob Chemother 1999;43:277-9.

204. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg Infect Dis 2001;7:24-34.

205. Wang Y, Huang WM, Taylor DE. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. Antimicrob Agents Chemother 1993;37:457-63.

206. Carattoli A, Dionisi A, Luzzi I. Use of a LightCycler *gyrA* mutation assay for identification of ciprofloxacin-resistant *Campylobacter coli*. FEMS Microbiol Lett 2002;214:87-93.

207. Hakanen A, Jalava J, Kotilainen P, Jousimies-Somer H, Siitonen A, Huovinen P. *gyrA* polymorphism in *Campylobacter jejuni:* detection of *gyrA* mutations in 162 C. jejuni isolates by single-strand conformation polymorphism and DNA sequencing. Antimicrob Agents Chemother 2002;46:2644-7.

208. Gibreel A, Sjogren E, Kaijser B, Wretlind B, Skold O. Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* associated with mutational changes in *gyrA* and *parC*. Antimicrob Agents Chemother 1998;42:3276-8.

209. Payot S, Cloeckaert A, Chaslus-Dancla E. Selection and characterization of fluoroquinolone-resistant mutants of *Campylobacter jejuni* using enrofloxacin. Microb Drug Resist 2002;8:335-43.

210. Piddock LJ, Ricci V, Pumbwe L, Everett MJ, Griggs DJ. Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. J Antimicrob Chemother 2003;51:19-26.

211. Zirnstein G, Li Y, Swaminathan B, Angulo F. Ciprofloxacin resistance in *Campylobacter jejuni* isolates: detection of *gyrA* resistance mutations by

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

mismatch amplification mutation assay PCR and DNA sequence analysis. J Clin Microbiol 1999;37:3276-80.

212. Bachoual R, Ouabdesselam S, Mory F, Lascols C, Soussy CJ, Tankovic J. Single or double mutational alterations of *gyrA* associated with fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli*. Microb Drug Resist 2001;7:257-61.

213. Ruiz J, Goni P, Marco F, et al. Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. Microbiol Immunol 1998;42:223-6.

214. Luo N, Sahin O, Lin J, Michel LO, Zhang Q. In vivo selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. Antimicrob Agents Chemother 2003;47:390-4.

215. Charvalos E, Tselentis Y, Hamzehpour MM, Kohler T, Pechere JC. Evidence for an efflux pump in multidrug-resistant *Campylobacter jejuni*. Antimicrob Agents Chemother 1995;39:2019-22.

216. Pumbwe L, Piddock LJ. Identification and molecular characterisation of CmeB, a *Campylobacter jejuni* multidrug efflux pump. FEMS Microbiol Lett 2002;206:185-9.

217. Lin J, Michel LO, Zhang Q. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrob Agents Chemother 2002;46:2124-31.

218. Gaudreau C, Gilbert H. Antimicrobial resistance of *Campylobacter jejuni* subsp. jejuni strains isolated from humans in 1998 to 2001 in Montreal, Canada. Antimicrob Agents Chemother 2003;47:2027-9.

219. Gupta A, Nelson JM, Barrett TJ, et al. Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. Emerg Infect Dis 2004;10:1102-9.

220. Luber P, Wagner J, Hahn H, Bartelt E. Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001-2002 from poultry and humans in Berlin, Germany. Antimicrob Agents Chemother 2003;47:3825-30.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

221. Lucey B, Cryan B, O'Halloran F, Wall PG, Buckley T, Fanning S. Trends in antimicrobial susceptibility among isolates of *Campylobacter* species in Ireland and the emergence of resistance to ciprofloxacin. Vet Rec 2002;151:317-20.

222. Bodhidatta L, Vithayasai N, Eimpokalarp B, Pitarangsi C, Serichantalergs O, Isenbarger DW. Bacterial enteric pathogens in children with acute dysentery in Thailand: increasing importance of quinolone-resistant *Campylobacter*. Southeast Asian J Trop Med Public Health 2002;33:752-7.

223. Engberg J, Neimann J, Nielsen EM, Aerestrup FM, Fussing V. Quinolone-resistant *Campylobacter* infections: risk factors and clinical consequences. Emerg Infect Dis 2004;10:1056-63.

224. Hakanen A, Jousimies-Somer H, Siitonen A, Huovinen P, Kotilainen P. Fluoroquinolone resistance in *Campylobacter jejuni* isolates in travelers returning to Finland: association of ciprofloxacin resistance to travel destination. Emerg Infect Dis 2003;9:267-70.

225. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin Infect Dis 1998;26:341-5.

226. Reina J, Borrell N, Serra A. Emergence of resistance to erythromycin and fluoroquinolones in thermotolerant *Campylobacter* strains isolated from feces 1987-1991. Eur J Clin Microbiol Infect Dis 1992;11:1163-6.

227. Gaunt PN, Piddock LJ. Ciprofloxacin resistant *Campylobacter* spp. in humans: an epidemiological and laboratory study. J Antimicrob Chemother 1996;37:747-57.

228. Kos VN, Keelan M, Taylor DE. Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from poultry from Alberta, Canada. Antimicrob Agents Chemother 2006;50:778-80.

229. Guevremont E, Nadeau E, Sirois M, Quessy S. Antimicrobial susceptibilities of thermophilic *Campylobacter* from humans, swine, and chicken broilers. Can J Vet Res 2006;70:81-6.

230. Inglis GD, Morck DW, McAllister TA, et al. Temporal prevalence of antimicrobial resistance in *Campylobacter* spp. from beef cattle in Alberta feedlots. Appl Environ Microbiol 2006;72:4088-95.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

231. Taremi M, Mehdi Soltan Dallal M, Gachkar L, MoezArdalan S, Zolfagharian K, Reza Zali M. Prevalence and antimicrobial resistance of *Campylobacter* isolated from retail raw chicken and beef meat, Tehran, Iran. Int J Food Microbiol 2006;108:401-3.

232. Kang YS, Cho YS, Yoon SK, et al. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw chicken meat and human stools in Korea. J Food Prot 2006;69:2915-23.

233. Andersen SR, Saadbye P, Shukri NM, Rosenquist H, Nielsen NL, Boel J. Antimicrobial resistance among *Campylobacter jejuni* isolated from raw poultry meat at retail level in Denmark. Int J Food Microbiol 2006;107:250-5.

234. Wilson IG. Antibiotic resistance of *Campylobacter* in raw retail chickens and imported chicken portions. Epidemiol Infect 2003;131:1181-6.

235. Ledergerber U, Regula G, Stephan R, Danuser J, Bissig B, Stark KD. Risk factors for antibiotic resistance in *Campylobacter* spp. isolated from raw poultry meat in Switzerland. BMC Public Health 2003;3:39.

236. Moore JE, Crowe M, Heaney N, Crothers E. Antibiotic resistance in *Campylobacter* spp. isolated from human faeces (1980-2000) and foods (1997-2000) in Northern Ireland: an update. J Antimicrob Chemother 2001;48:455-7.

237. Ge B, White DG, McDermott PF, et al. Antimicrobial-resistant *Campylobacter* species from retail raw meats. Appl Environ Microbiol 2003;69:3005-7.

238. Avrain L, Humbert F, L'Hospitalier R, Sanders P, Vernozy-Rozand C, Kempf I. Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. Vet Microbiol 2003;96:267-76.

239. Pedersen K, Wedderkopp A. Resistance to quinolones in *Campylobacter jejuni* and *Campylobacter coli* from Danish broilers at farm level. J Appl Microbiol 2003;94:111-9.

240. Cardinale E, Dromigny JA, Tall F, Ndiaye M, Konte M, Perrier-Gros-Claude JD. Fluoroquinolone susceptibility of *Campylobacter* strains, Senegal. Emerg Infect Dis 2003;9:1479-81.

241. Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, Taylor DE. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1999 to 2002, with special reference to *tet(O)*-mediated tetracycline resistance. Antimicrob Agents Chemother 2004;48:3442-50.

242. Kassenborg HD, Smith KE, Vugia DJ, et al. Fluoroquinolone-resistant *Campylobacter* infections: eating poultry outside of the home and foreign travel are risk factors. Clin Infect Dis 2004;38 Suppl 3:S279-84.

243. Sjogren E, Lindblom GB, Kaijser B. Norfloxacin resistance in *Campylobacter jejuni* and *Campylobacter coli* isolates from Swedish patients. J Antimicrob Chemother 1997;40:257-61.

244. Sjogren E, Kaijser B, Werner M. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* isolated in Sweden: a 10-year follow-up report. Antimicrob Agents Chemother 1992;36:2847-9.

245. Osterlund A, Hermann M, Kahlmeter G. Antibiotic resistance among *Campylobacter jejuni/coli* strains acquired in Sweden and abroad: a longitudinal study. Scand J Infect Dis 2003;35:478-81.

246. Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J Antimicrob Chemother 1991;27:199-208.

247. Hein I, Schneck C, Knogler M, et al. *Campylobacter jejuni* isolated from poultry and humans in Styria, Austria: epidemiology and ciprofloxacin resistance. Epidemiol Infect 2003;130:377-86.

248. Krausse R, Ullmann U. In vitro activities of new fluoroquinolones against *Campylobacter jejuni* and *Campylobacter coli* isolates obtained from humans in 1980 to 1982 and 1997 to 2001. Antimicrob Agents Chemother 2003;47:2946-50.

249. Prats G, Mirelis B, Llovet T, Munoz C, Miro E, Navarro F. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. Antimicrob Agents Chemother 2000;44:1140-5.

250. Unicomb L, Ferguson J, Riley TV, Collignon P. Fluoroquinolone resistance in *Campylobacter* absent from isolates, Australia. Emerg Infect Dis 2003;9:1482-3.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

251. Price LB, Johnson E, Vailes R, Silbergeld E. Fluoroquinolone-resistant *Campylobacter* isolates from conventional and antibiotic-free chicken products. Environ Health Perspect 2005;113:557-60.

252. Sanchez MX, Fluckey WM, Brashears MM, McKee SR. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion- chilled environments. J Food Prot 2002;65:948-56.

253. Fallon R, O'Sullivan N, Maher M, Carroll C. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates from broiler chickens isolated at an Irish poultry processing plant. Lett Appl Microbiol 2003;36:277-81.

254. Van Looveren M, Daube G, De Zutter L, et al. Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. J Antimicrob Chemother 2001;48:235-40.

255. Frediani-Wolf V, Stephan R. Resistance patterns of *Campylobacter* spp. strains isolated from poultry carcasses in a big Swiss poultry slaughterhouse. Int J Food Microbiol 2003;89:233-40.

256. Sackey BA, Mensah P, Collison E, Sakyi-Dawson E. *Campylobacter, Salmonella, Shigella* and *Escherichia coli* in live and dressed poultry from metropolitan Accra. Int J Food Microbiol 2001;71:21-8.

257. Poole C. Low P-values or narrow confidence intervals: which are more durable? Epidemiology 2001;12:291-4.

258. Holmberg L, Ohlander EM, Byers T, et al. A search for recall bias in a case-control study of diet and breast cancer. Int J Epidemiol 1996;25:235-44.

259. Dwyer JT, Gardner J, Halvorsen K, Krall EA, Cohen A, Valadian I. Memory of food intake in the distant past. Am J Epidemiol 1989;130:1033-46.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 3:

RISK FACTORS FOR CIPROFLOXACIN RESISTANCE IN *CAMPYLOBACTER* INFECTIONS IN SOUTHERN ALBERTA

3.1 Background

Fluoroquinolone antibiotics, such as ciprofloxacin, have been recommended by some authorities for the empirical treatment of infectious diarrhoea (1). Such recommendations often carry cautions against overuse and use when not indicated to minimize risk of the development of resistance in enteric bacteria such as *Campylobacter*. There are many reports of increasing resistance to fluoroquinolones among *Campylobacter* strains from humans, foods, and food animals (2-5). Of specific concern are reports of high levels of resistance among strains in developing parts of the world, and among strains isolated from travellers who have returned from developing countries (6-8). Case-control studies have investigated risk factors for fluoroquinolone resistance in *Campylobacter* infections in Europe and the U.S. Results vary, but primary risk factors reported include foreign travel, chicken consumption, and antibiotic use (7, 9, 10).

Within southern Alberta are the Chinook Health Region (population 152,000) and the Calgary Health Region (population 1,122,000) (11, 12). The Chinook region has a large agricultural base with many workers in the livestock industry, while the Calgary region has one of the most rapidly growing metropolitan populations in Canada (13). The incidence of *Campylobacter* infection in humans is relatively high in these regions. In 2000, in both regions there were approximately 70 infections per 100,000; higher than the province-wide incidence of 42.5 per 100,000 (14). Thus, these regions present a good location in which to investigate the relative importance of a number of risk factors for ciprofloxacin resistance among *Campylobacter* infections.

A version of this chapter has been published. Johnson *et al.* 2007. *Epidemiology and Infection.* Electronic publication ahead of print. 7:1-10.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.2 Methods

3.2.1 Study Participants

Study participants were residents of the Chinook Health Region or Calgary Health Regions, greater than 16 years of age, who submitted a stool sample that was positive for the presence of *Campylobacter,* and who were followed-up by a public health nurse or inspector between February 1, 2004 and July 29, 2005. Cases were participants infected with ciprofloxacin resistant *Campylobacter* and controls were those infected with ciprofloxacin susceptible strains. Verbal consent for study participants was recorded prior to administration of the study questionnaire. Ethics approval was granted for this study by the Health Research Ethics Board, Panel B, University of Alberta, Edmonton, the Conjoint Health Research Ethics Board, University of Calgary, Calgary, and the Chinook Health Region Regional Research Committee, Lethbridge.

Travel data and fluoroquinolone susceptibility test results were provided by the Calgary Health Region for 161 non-participants in that region. These non-participants were eligible for the study, but had declined to participate, or due to manpower shortages, were not asked to participate. No other data on these patients was made available for this study.

3.2.2 Data Collection

Sample Size Calculation. The sample size calculation was based on the following assumptions: negligible correlation among covariates, prevalence of ciprofloxacin resistance among *Campylobacter* isolated from the two health regions between 20 and 40%, and an odds ratio of 1.5. To obtain a one-tailed level of significance of 5% and a power of 80%, the sample size required was between 182 and 274 (15). The combined estimated *Campylobacter* annual incidence in the Chinook Health Region and the Calgary Health Region was approximately 750, which was assumed to be adequate for this study.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Questionnaire Description and Administration. All questions for the study questionnaire required one-word answers. Potential risk exposures included travel within the previous month to destinations outside of Canada and the U.S.A., average two-week consumption of specific foods, frequency of eating in restaurants, drinking unpasteurised milk or untreated water, exposure to animal manure or faeces, administration of an antibiotic to animals or humans, and antibiotic use within the past month. The use of household products and personal hygiene items containing antibacterial agents was also included as a risk exposure. In addition, the hypothesized resistance risks associated with possessing antibiotics for future use and living more than five miles from a pharmacy (rural residence), which could increase the potential for self-medicating with antibiotics, were investigated. Other data gathered included age, sex, education, occupation, details about any antibiotic treatment, and knowledge of antibiotic susceptibility testing result.

The study questionnaire is given in Appendix 1. Public health nurses in the Chinook Health Region and public health inspectors in the Calgary Health Region were instructed on correct administration of the scripted questionnaire. Questionnaires were administered by phone during routine follow-up investigations by the public health officials.

Quinolone Susceptibility Testing. Ciprofloxacin susceptibility testing at the Chinook Health Region Laboratory used a modified Kirby Bauer method, in which colonies were streaked onto Mueller Hinton agar (BD Diagnostics, Oakville) with 5 % sheep blood (BD Diagnostics, Oakville) and incubated at 37 to 42 °C for 24 to 48 h, depending on growth characteristics. The zone of inhibition around a 5 μg ciprofloxacin disk (BD Diagnostics, Oakville) was assessed as follows: ≤15 mm, resistant; 16 to 20 mm, intermediate; ≥21 mm, susceptible. *Campylobacter* strains isolated in the Calgary Health Region by Calgary Laboratory Services were tested for nalidixic acid susceptibility using 30 μg nalidixic acid disks (Oxoid, Nepean) on blood agar plates (PML Microbiologicals, Wilsonville). Susceptibility to nalidixic acid was assessed by zone diameter ≥20 mm, and resistance was assessed by no zone of inhibition. This laboratory found that zones of

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

inhibition were never between 0 and 20 mm in diameter. Plates were incubated at 42 °C for 18 to 24 h.

3.2.3 Data Analysis

Data Handling. Data were double-entered into Statistic Package for the Social Sciences, version 13.0 (SPSS, Chicago, IL). Illnesses that started at least two days after the first day of travel outside of the USA and Canada and within three days of returning from travel were considered to be caused by foreign strains. This criterion is based on the assumption of a typical two to three day incubation period for infection (16, 17). When multiple travel destinations were given, the most likely country where an infection was acquired was determined from the individual's travel timeline and illness onset information.

Criteria for confirmed empirical treatment with an antibiotic or fluoroquinolone included any of the following conditions: a) a positive response to the question "Did you start taking the antibiotic before submitting a stool sample?"; b) the course of treatment for the diarrhoeal illness started one or more days before stool sample submission; or c) any antibiotic treatment started six or more days prior to the interview and laboratory stool sample submission date was missing. In addition, the patient must have provided the name of their medication, which had to be a recognized antibiotic. Criterion c) was created for 20 participants who had filled a prescription for an antibiotic and for whom stool sample submission data was missing. The six day cut-off was determined as the most effective predictor of empirical antibiotic treatment. When tested against data for participants for whom stool sample submission dates were available, the sensitivity of this cut-off was 95% and the specificity was 60% in predicting actual empirical antibiotic treatment. To assess the validity of criterion c) univariate logistic regression models without the aforementioned 20 participants were conducted.

Food consumption frequency data were gathered on a continuous scale, but for modelling purposes, data for most food types were classified as low, medium, or high consumption frequency based on tertiles (3 equal divisions of the study population) of the data. Cut-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

off points for food consumption categorical variables were changed for modelling domestic infections because the distribution of reported frequency of consumption for this group differed from that of the total study sample. Abbreviations used for selected variables are given in Table 3.4.1.

Logistic Regression Modelling. Univariate logistic regression models were fitted for each independent variable using the SPSS LOGISTIC REGRESSION function. Along with the crude odds ratios (OR), ORs adjusted for age, sex, health region, higher education, season, and rural residence were calculated.

One multivariate model was developed using data on all study participants. A domestic infection multivariate model (excluding travel-related infections) was not possible as most candidate variables violated the assumption of sampling adequacy (more than 20% of cell values were <5).

All variables with a Wald value significant at the 10% level in the univariate analyses were candidate variables for stepwise multivariate logistic regression model-building, using a *p*-entry value of 0.20, and *p*-removal value of 0.25. Interaction terms were selected for the final model from all potential pairs of multiplicative effect variables by testing for a difference at the 10% level in the likelihood ratios in models with and without the interaction product term. Confounders were added to the base model if, when added to the model, an exposure variable beta coefficient changed by more than 10%. When the standardized residual for any data point was outside the 1% level of significance, it was removed as an outlier (residual >2.58 or <-2.58). All final results of model analyses were assessed for significance at the 5% level.

The final model was also run with inverse probability weighting to adjust for unequal sampling across seasons. For example, sampling occurred over 2 springs, thus the weight assigned to spring infections was the inverse of 2 (0.5). Weights for each season were as follows: spring, 0.5; summer, 0.71; fall, 1.0; winter, 0.65.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Validity Assessments. To assess the effect of participation bias, travel and fluoroquinolone susceptibility data for 161 eligible non-participating patients in the Calgary Health Region from September 2004 through July 2005 was compared to participant data. To evaluate the potential impact of participation bias, the OR for foreign strain was adjusted by dividing by a selection bias factor (18), Equation 1, and compared to the unadjusted OR.

$$\frac{S_{AF} \cdot S_{BD}}{S_{AD} S_{BF}} \qquad \text{Equation 1}$$

where: S_{AF} = proportion of participants among cases with foreign strains

S_{BD} = proportion of participants among controls with domestic strains

S_{AD} = proportion of participants among cases with domestic strains

S_{BF} = proportion of participants among controls with foreign strains

The difference in susceptibility testing methods used in the two health regions was a concern; the less specific nalidixic acid disk method used in the Calgary Health Region could result in an artificially high proportion of ciprofloxacin resistance among *Campylobacter* strains in that region. Possible resistance misclassification was addressed by using the Greenland estimation (18), Equation 2, then by conducting a sensitivity analysis.

$$A = \frac{A^* - FpN}{Se + Sp - 1} \qquad \text{Equation 2}$$

where: A = adjusted fluoroquinolone resistance

A^* = observed fluoroquinolone resistance

Fp = false positive probability

N = number of affected strains

Se = probability that resistant strains were classified as resistant

Sp = probability that susceptible strains were classified as susceptible

Ten datasets were generated in which susceptibility was imputed for randomly selected resistant data points from the Calgary Health Region. The number of data points that were selected was calculated from the Greenland estimate. The foreign/domestic model

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

was re-run on the generated datasets, and the average change for the model ORs was calculated. Specificity and sensitivity estimates for the nalidixic acid disk method as a surrogate for the ciprofloxacin agar dilution method as reported by Gaudreau and Gilbert (19) was assumed and used as input for the misclassification estimate.

3.3 Results

3.3.1 Study Participants and *Campylobacter* Strains

Based on public health databases from the study area, approximately 600 cases of *Campylobacter* infections in people over the age of 16 were reported to the health regions during the study period. Manpower shortages in the Calgary Health Region precluded public health inspectors from asking all patients to participate including persons infected as part of a *Campylobacter* outbreak which occurred during the study period. All patients contacted by Chinook Health Region staff consented to participate. The number of patients who were asked to participate was 351. Two hundred and twenty-nine patients participated in the study, representing approximately 38% of all potentially eligible people and 65% of all who were asked to participate. The reason most commonly given for refusing to participate was lack of time.

The mean age of participants was 40.7 years, 62.0% had college or university education, 17.1% reported occupational handling of animals, and 19.7% were rural residents. Among the 229 *Campylobacter* isolates from study participants, 215 (93.9%) were *C. jejuni,* six (2.6%) were *C. coli,* and the species of eight strains (3.5%) was not determined. A large proportion of strains (73.8%) were from people reporting in the Calgary Health Region (Table 3.4.2), and 71 infections (31.1%) were acquired outside of the USA and Canada. The proportion of ciprofloxacin resistant strains among all strains was 28.8% (n = 229), 87.9% of which were *C. jejuni,* and 89.4% of which were from the Calgary Health Region.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.3.2 Individual Risk Exposures

Tables 3.4.3 and 3.4.4 demonstrate there were several variables associated with crude and adjusted risk for ciprofloxacin resistance. The likelihood of resistance was highly variable among the macro-regions from which infections originated, but travel to any of the three broadly classified regions outside of the U.S.A. and Canada was significantly associated with increased risk. The levels of empirical antibiotic or fluoroquinolone treatment were similar among cases and controls (Table 3.4.3). Among all participants, the percentages for the variables fluoroquinolone and antibiotic were 6.1% and 12.7%, respectively.

Among all infections and among domestically-acquired infections, cases were more likely than controls to have reported possession of non-prescribed antibiotics (Tables 3.4.3, 3.4.4), and the percentage among all participants was 6.1% (n = 229). *C. jejuni* were much less likely to be resistant than were strains that were other *Campylobacter* species. Other variables associated with resistance included season, health region, gender, and rural residence. Use of antibacterial dishwashing soap or antibacterial toothpaste was not found to be associated with ciprofloxacin resistance, nor was contact with animals or handling or dispensing of antibiotic drugs. There was a marginal risk associated with moderate levels of chicken consumption, and a marginal protective effect associated with cattle handling (Table 3.4.3).

Unexpectedly, 44 participants (19.5%, n = 226) reported that they knew the results of the antibiotic susceptibility testing conducted on their *Campylobacter* strain, which may have introduced a degree of recall bias. Resistance was more common among those who knew their results (47.7%, n = 44) than among those who did not know their results (24.7%, n = 182, Table 3.4.3).

In comparison to Table 3.4.3, there were fewer identified univariate risk factors identified among domestic infections (Table 3.4.4); only possession and knowledge were associated with likelihood of resistance.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.3.3 Multivariate Logistic Regression Analysis

Candidate variables for multivariate model building included foreign, fluoroquinolone, shellfish consumption, cat handling, cattle handling, possession, knowlege, sex, health region, rural residence, season, and *C. jejuni*. The variables selected by forward selection and backward elimination approaches were foreign, possession, sex, health region, and knowledge.

Among potential interaction terms, the term knowledge by health region was the only significant interaction term. When added to the model, the 95% confidence interval for the OR of this term was wide (OR = 30.9; 95% CI = 1.7, 545.2), suggesting the power for this estimate may be insufficient, thus, this term was not included in the final model.

There was evidence of confounding by age on the effects of knowledge, health region, and sex. Also, rural residence confounded the effect of health region. The base model, then, included foreign, possession, sex, health region, knowledge, age, and rural residence.

One outlier was identified and removed, and the significant risk factors identified by the final model and adjusted for knowledge were foreign, possession, sex, and age (Table 3.4.5). When the final model was run with weighting for season, model ORs changed by 1 to 8%.

3.3.4 Validity Assessments

Examination of a sample of eligible Calgary Health Region patients who were not asked to participate or who were asked but chose not to participate showed that the ciprofloxacin resistance rate was less among non-participants than among Calgary study participants (26.1%, 34.9%, respectively). Among the non-participants in the Calgary Health Region 19.9% acquired infection in a foreign country, and the $OR_{Foreign}$ was lower for non-participants (OR = 2.0, 95% CI: 0.9, 4.5) than for participants (OR = 29.9, 95% CI: 12.6, 70.9). The effect of this apparent participation bias was taken into consideration

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

in the bias-corrected estimate for $OR_{Foreign}$ (18). The calculated participation bias factor was 3.57, so the $OR_{Foreign}$ adjusted for participation bias was estimated as 29.9/3.57 = 8.4.

The potential effect of differential misclassification of resistance on the model caused by the use of a surrogate ciprofloxacin susceptibility testing method in the Calgary Health Region was evaluated. Gaudreau and Gilbert (19) found nalidixic acid diffusion testing had a sensitivity of 100% and specificity of 98.5%, with respect to the gold standard test for ciprofloxacin susceptibility (agar dilution). Based on these values, the estimated number of correctly classified ciprofloxacin resistant *Campylobacter* strains in the Calgary sample was 57.3 (18). This suggests approximately two of the 59 Calgary infections assumed to be ciprofloxacin resistant may have been misclassified.

The dataset was altered by imputing ciprofloxacin susceptibility for two randomly selected participants with resistant *Campylobacter* from among the Calgary region patients. The ORs calculated from ten altered data sets differed very little from the ORs calculated from the raw data; the percent difference between the average ORs for the final model variables and the original ORs ranged from 1 to 6%. All variables that were significant using the raw data were also significant with the 10 altered data sets, and those that were not significant originally remained so with the altered data.

The six day cut-off criterion for estimated empirical treatment with an antibiotic or fluoroquinolone appeared to simulate actual reported empirical treatment well. The effects of the variables for antibiotic and fluoroquinolone treatment are similar with or without the 20 participants for whom the six-day criterion was utilised. Following removal of these 20, the adjusted univariate $OR_{Fluoroquinolone}$ shifted only slightly from OR = 3.2 (95% CI = 0.8, 12.3) to 4.2 (95% CI = 0.9, 18.9). Similarly, the adjusted univariate $OR_{Antibiotic}$ changed negligibly from 1.5 (95% CI = 0.6, 4.0) to 1.5 (95% CI = 0.5, 4.3).

There was a large proportion of college and university-educated people in the study sample (62.0%) in contrast to the proportion in the province of Alberta in 2001 (54.7%) (20). This could mean the study results are not generalizable across the province, if the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

percent with higher education in the population that reports *Campylobacter* infections in the province (the study population) differed from that of the sample population. Among the study participants, higher education was not associated with travel ($\chi^2 = 0.7$; $p = 0.4$), or with possession ($\chi^2 = 1.7$; $p = 0.2$). However, if there were significant associations among the non-participants, the skewed sampling could be a source of participation bias.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.4 Tables

Table 3.4.1. Abbreviations of selected study variables

Abbreviation	Variable	Definition
Foreign	Foreign travel	Travel outside Canada and the U.S.A.; infection starting >2 days after the beginning and <3 days after the end of travel
Possession	Possession of non-prescribed antibiotics	Participant reported possession of antibiotics that were not prescribed for them that were saved for future use
Antibiotic or Fluoroquinolone	Empirical treatment with an antibiotic or fluoroquinolone	Participant reported taking an antibiotic or fluoroquinolone prior to submitting a stool sample, or this was indicated by laboratory and questionnaire data, or, in the absence of data, treatment began ≥6 days prior to interview
Knowledge	Knowledge of antibiotic susceptibility testing result	Participant was aware of the fluoroquinolone susceptibility testing result of their infective strain
Rural residence	Living more than five miles from a pharmacy	Used as an indicator for the potential for antibiotic self-medication
Higher education	College, and or, university education	Self-explanatory
(Food type)	Frequency of consumption of specific food types	Typical frequency of consumption of food types within a 2-week period
(Animal type) handling	Handling of faeces or manure	Contact with animal waste within the past month

Table 3.4.2. Characteristics of study participants with reported *Campylobacter* infections in southern Alberta, 2004 – 2005

	Number	Percent
Sex		
Male	125	54.6
Female	103	45.2
Age		
< 28	51	22.3
28 – 37	58	25.3
38 – 49	56	24.5
≥ 50	64	27.9
Education		
Elementary	5	2.6
High school	58	25.3
Trade school	22	9.6
College	52	22.7
University	90	39.3
Health region		
Chinook	60	26.2
Calgary	169	73.8

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 3.4.3. Risk factors for ciprofloxacin resistance of *Campylobacter* strains. Data collected from *Campylobacter* infections in southern Alberta, 2004 – 2005.

Variable †	n	Ciprofloxacin resistance (%)	Crude		Adjusted ‡	
			OR	95% CI	OR	95% CI
Foreign	71	76.1	38.4 **	17.2, 85.6	41.2 **	15.7, 108.0
Infection source						
Latin America	36	75.0	36.3 **	13.9, 94.4	30.9 **	9.9, 96.3
Asia	21	90.5	114.8 **	23.9, 552.6	148.8 **	25.5, 870.0
Europe	11	45.5	10.1 **	2.7, 37.9	11.3 **	2.3, 55.0
Antibiotic	29	31.0	1.1	0.5, 2.6	1.5	0.6, 4.0
Fluoroquinlone	14	50.0	2.6 ◊	0.9, 7.9	3.2 ◊	0.8, 12.3
Chicken						
High	68	32.4	1.9	0.9, 3.9	1.5	0.6, 3.4
Moderate	78	34.6	2.1 *	1.0, 4.2	1.7	0.8, 3.8
Low	83	20.5	1.0	referent	1.0	referent
Beef						
High	60	20.0	0.5 ◊	0.2, 1.1	0.9	0.4, 2.1
Moderate	80	30.0	0.8	0.4, 1.6	0.8	0.4, 1.6
Low	89	22.7	1.0	referent	1.0	referent

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Variable †	n	Ciprofloxacin resistance (%)	Crude		Adjusted ‡	
			OR	95% CI	OR	95% CI
Pork						
High	69	27.5	0.8	0.4, 1.6	0.7	0.3, 1.5
Moderate	63	25.4	0.7	0.4, 1.5	0.7	0.3, 1.4
Low	97	32.0	1.0	referent	1.0	referent
Processed meat						
High	71	22.5	0.7	0.3, 1.3	0.9	0.4, 2.1
Moderate	74	32.4	1.1	0.6, 2.1	1.2	0.6, 2.7
Low	84	31.0	1.0	referent	1.0	referent
Shellfish	114	34.2	1.7 ◊	1.0, 3.0	1.1	0.6, 2.2
Eating outside of the home						
High	87	34.5	1.7	0.8, 3.5	1.5	0.6, 3.5
Moderate	84	26.2	1.1	0.5, 2.4	0.7	0.3, 1.7
Low	58	24.1	1.0	referent	1.0	referent
Drank unpasteurised milk	12	25.0	0.8	0.2, 3.2	0.7	0.2, 3.3
Drank untreated water	48	33.3	1.3	0.7, 2.7	2.2 ◊	1.0, 5.2
Occupational handling of animals	39	23.1	0.7	0.3, 1.6	1.1	0.4, 2.7
Bird handling	18	22.2	0.7	0.2, 2.2	1.2	0.3, 4.4
Dog handling	85	24.7	0.7	0.4, 1.3	0.9	0.4, 1.7

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Variable †	n	Ciprofloxacin resistance (%)	Crude		Adjusted ‡	
			OR	95% CI	OR	95% CI
Cat handling	49	18.4	0.5 ◊	0.2, 1.1	0.5	0.2, 1.2
Cattle handling	34	8.8	0.2 *	0.1, 0.7	0.4	0.1, 1.4
Pig handling	7	0.0	§		§	
Poultry handling	16	18.8	0.6	0.2, 2.0	0.6	0.2, 2.6
Household member taking antibiotic	27	22.2	0.8	0.3, 2.1	1.1	0.4, 3.3
Handles antibiotics for others	16	31.3	1.1	0.4, 3.4	1.3	0.4, 4.5
Previous diarrhoeal illness	41	29.3	1.0	0.5, 2.1	1.3	0.5, 3.1
Possession	14	64.3	5.0 **	1.6, 15.5	5.0 *	1.4, 18.2
Uses antibacterial dish soap	120	27.5	0.9	0.5, 1.5	0.9	0.5, 1.7
Uses toothpaste with triclosan	66	28.8	1.0	0.5, 1.9	0.8	0.4, 1.7
Knowledge	44	47.7	2.8 **	1.4, 5.5	4.5 **	2.0, 10.5
Age (quartile)						
17 – 27	51	37.3	1.0	referent	–	
28 – 37	58	20.7	0.4 ◊	0.2, 1.0	–	
38 – 49	56	33.9	0.9	0.4, 1.9	–	
≥ 50	64	25.0	0.6	0.3, 1.3	–	
Sex (female)	103	35.0	1.7 ◊	1.0, 3.0	–	
Higher education	142	32.4	1.6	0.9, 3.0	–	

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Variable †	n	Ciprofloxacin resistance (%)	Crude		Adjusted ‡	
			OR	95% CI	OR	95% CI
Health region (Calgary)	169	34.9	4.1 **	1.7, 9.5	–	
Rural residence	45	17.8	0.5 ◊	0.2, 1.1	–	
Season						
Fall	27	18.5	1.3	0.4, 3.9	–	
Winter	31	58.1	7.7 **	3.0, 19.4	–	
Spring	86	34.9	3.0 **	1.4, 6.2	–	
Summer	85	15.3	1.0	referent	–	
C. jejuni	215	27.0	0.3 *	0.1, 0.8	–	

† Refer to Table 3.4.2 for definitions of variable abbreviations

‡ Adjusted for age, sex, higher education, health region, rural residence, and season.

§ One zero cell in 2 by 2 table, therefore, odds ratio cannot be computed.

◊ Candidate variable for multivariate model, $p < 0.10$

* $p < 0.05$

** $p < 0.01$

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 3.4.4. Risk factors for ciprofloxacin resistance of *Campylobacter* strains. Data represents domestically-acquired *Campylobacter* infections in southern Alberta, 2004 – 2005.

Variable †	n	Ciprofloxacin resistance (%)	Crude		Adjusted ‡	
			OR	95% CI	OR	95% CI
Antibiotic	15	0.0	§		§	
Fluoroquinolone	4	0.0	§		§	
Chicken						
High	56	8.9	5.0	0.6, 44.3	2.6	0.3, 25.7
Moderate	49	12.2	7.1	0.8, 61.4	6.3	0.6, 61.8
Low	52	1.9	1.0	referent	1.0	referent
Beef						
High	87	5.7	0.6	0.2, 1.8	0.7	0.2, 2.6
Low	70	10.0	1.0	referent	1.0	referent
Pork						
High	50	8.0	1.2	0.3, 5.8	1.2	0.2, 7.1
Moderate	62	8.1	1.2	0.3, 5.4	1.2	0.2, 6.2
Low	45	6.7	1.0	referent	1.0	referent
Processed meat						
High	55	7.3	0.9	0.2, 4.0	1.9	0.3, 10.2
Moderate	50	8.0	1.0	0.3, 4.4	1.5	0.3, 7.8
Low	52	7.7	1.0	referent	1.0	referent

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Variable †	n	Ciprofloxacin resistance (%)	Crude		Adjusted ‡	
			OR	95% CI	OR	95% CI
Shellfish	73	12.3	3.8	1.0, 14.6	1.6	0.4, 7.5
Eating outside of the home						
High	56	7.1	0.6	0.2, 2.5	0.3	0.1, 1.8
Moderate	55	5.5	0.5	0.1, 2.1	0.2	0.0, 1.4
Low			1.0	referent	1.0	referent
Drank unpasteurised milk	7	0.0	§		§	
Drank untreated water	29	0.0	§		§	
Occupational handling of animals	31	6.5	0.8	0.2, 3.8	1.7	0.3, 11.6
Bird handling	13	7.7	1.0	0.1, 8.5	2.7	0.2, 32.7
Dog handling	62	9.7	1.6	0.5, 5.2	1.8	0.4, 7.2
Cat handling	37	5.4	0.6	0.1, 3.0	0.6	0.1, 3.3
Cattle handling	30	0.0	§		§	
Pig handling	7	0.0	§		§	
Poultry handling	14	7.1	0.9	0.1, 7.7	0.8	0.1, 8.9
Household member taking antibiotic	22	4.5	0.6	0.1, 5.3	0.6	0.0, 7.8
Handles antibiotics for others	11	0.0	§		§	
Previous diarrhoeal illness	31	9.7	1.4	0.4, 5.4	3.4	0.6, 19.5
Possession	6	50.0	15.8 **	2.8, 90.0	20.8 *	2.1, 208.4
Uses antibacterial dish soap	86	8.1	1.4	0.4, 5.0	1.7	0.4, 7.8

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Variable †	n	Ciprofloxacin resistance (%)	Crude		Adjusted ‡	
			OR	95% CI	OR	95% CI
Uses toothpaste with triclosan	47	8.5	1.2	0.3, 4.2	0.9	0.2, 3.4
Knowledge	23	17.4	3.2	0.9, 11.8	7.6 *	1.3, 43.6
Age (quartile)						
17 – 27	33	9.1	1.0	referent	–	
28 – 37	43	9.3	1.0	0.2, 4.9	–	
38 – 49	39	10.3	1.1	0.2, 5.5	–	
≥ 50	42	2.4	0.2	0.0, 2.5	–	
Sex (female)	66	12.1	3.0	0.9, 10.3	–	
Higher education	95	11.6	8.0	1.0, 63.5	–	
Health region (Calgary)	107	10.3	5.6	0.7, 44.8	–	
Rural residence	35	2.9	0.3	0.0, 2.4	–	
Season						
Winter	10	20.0	4.4	0.7, 27.8	–	
Spring	54	11.1	2.2	0.6, 8.2	–	
Summer / fall	93	4.3	1.0	referent	–	
C. jejuni	152	7.9	§		–	

† Refer to Table 3.4.2 for definitions of variable abbreviations
‡ Adjusted for age, sex, higher education, health region, rural residence, and season.
§ One zero cell in 2 by 2 table, therefore, odds ratio cannot be computed.
* $p < 0.05$
** $p < 0.01$

Table 3.4.5. Risk factors for ciprofloxacin resistance identified from multi-variable logistic regression models on all data from reported *Campylobacter* infections, southern Alberta, 2004 – 2005.

Risk factor †	OR ‡	95% CI	*p* value
Foreign	57.5	20.2, 163.8	<0.001
Possession	5.7	1.1, 29.3	0.04
Sex (female)	2.7	1.0, 7.0	0.04
Age			0.02
17 – 27	1.0	referent	
28 – 37	0.3	0.07, 1.1	
38 – 49	1.0	0.3, 3.5	
≥ 50	0.1	0.04, 0.6	

† Refer to Table 3.4.2 for definitions of variable abbreviations

‡ Adjusted for knowledge of antibiotic susceptibility testing result

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.5 Discussion

This case-control study examined a large number of hypothesized risk exposures for ciprofloxacin resistance in *Campylobacter* infections. The percentage of ciprofloxacin resistance among the *Campylobacter* strains involved in this study (28.8%) falls between levels reported within the past five years in Alberta and Quebec, which range from 2% to 47% (2, 21, 22). Similar to other reports (21, 23), the levels of resistance among *C. coli* was higher than among *C. jejuni.*

Foreign travel has been discussed in many observational studies of antibiotic resistance in *Campylobacter* infections (7-10, 24, 25). This study's data showed the effect of overseas travel dominated any other risk exposures that were examined. After controlling for possible confounders (Table 3.4.2), *Campylobacter* acquired in Asia were almost 150 times more likely to be resistant than were strains acquired domestically, while the risk of resistance for strains from European travellers was not as great. Stratification by macro-region was therefore important because it allowed a more detailed understanding of the risk associated with foreign travel. A larger study with finer geographic groupings (for example, by country) would likely have allowed us to capture a wide range of risk levels within macro-regions.

It has been shown that *Campylobacter* are able to develop high levels of resistance to fluoroquinolones soon after exposure (26, 27), and Smith et al. reported quinolone use increased risk of quinolone resistance in *Campylobacter* infections (10). In this study, the percentage of those who were empirically treated with a fluoroquinolone among cases and controls was 10.6% and 4.3%, respectively; however, early treatment with fluoroquinolone drugs did not significantly increase the likelihood of a resistant culture. This could have been due to the fact that three of the 14 who were treated empirically started treatment at least two weeks prior to stool sample submission; strains cultured from these samples may not have been exposed to selective pressure for fluoroquinolone resistance. Unfortunately, data on when antibiotic treatments for the *Campylobacter* infections stopped were not collected, so ascertainment of exposure in these cases was problematic.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Self-medicating with antibiotics has been previously reported (28-30). Among a sample of current antibiotic users, 9% admitted using a prescription that was not for their current infection and 38% of a patient population admitted they kept leftover antibiotics for future use (28, 30). While antibiotic self-medication is important and often effective in the prevention and treatment of travellers' diarrhoea (31), and while possession of leftover antibiotics may be due to oversized packaging i.e. not always due to incomplete courses of previous treatments (32), the potential for incorrect self-medication with non-prescribed antibiotics to contribute to bacterial resistance is a great concern (32, 33). I agree with McNulty et al. (32), who suggested that the use of antibiotics may not align with prescribing data, and that this could affect the results of epidemiological studies such as this one.

The potential for self-medicating was ascribed to those who answered yes to "Do you have any antibiotics that you would use for future illness?" In this study, possession of non-prescribed antibiotics contributed to the likelihood of resistance. Unfortunately, participants who reported possession of non-prescribed antibiotics were not asked if they used them. Although 78.6% of those who had non-prescribed antibiotics (n = 14) reported that they used an antibiotic for their *Campylobacter* illness, it is unclear if this was prescribed or non-prescribed use. Furthermore, it was impossible to determine if any non-prescribed antibiotics were taken prior to submitting a stool sample (empirical treatment). Future studies with a larger sample size and higher exposure numbers, as well as more detailed questions on this topic could help to clarify this issue.

In this study, food consumption data was collected in units of typical consumption frequency over a two week period, rather than positive/negative consumption prior to onset. Compared to dichotomous consumption data, trichotomized frequency data allowed for greater discrimination in risk estimates. For example, if chicken consumption was categorized as never/ever the univariate model for resistance would have a beta coefficient standard error of 0.72, while the model using the categorized frequency data had a standard error of 0.18. Nevertheless, unlike others (7, 34), the data

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

from this study did not indicate that those who eat chicken frequently were more likely to have *Campylobacter* infections with ciprofloxacin resistance than were those who eat chicken infrequently.

Compared to estimates of quinolone and fluoroquinolone resistance among *Campylobacter* isolated in retail chicken from other countries, Canadian estimates are low, ranging from 1% to 12% (3, 21, 35-38). Potentially, low prevalence of resistance in *Campylobacter* in chicken may have been a reason behind the lack of risk associated with chicken consumption here. Others have found swimming also contributed to nalidixic acid resistance risk (7), but consumption of untreated water, including unintentional consumption while swimming, was not identified as an important risk exposure among the study sample.

Work-related potential risk exposures were investigated in this study. Others have found nalidixic acid resistance to be higher among faecal bacteria of livestock workers than among those of non-agricultural workers (39). The percentage of participants in this study who handle animals as part of their occupation was higher than the percentage among the provincial workforce (2.4%) (40), but this type of work was not associated with ciprofloxacin resistance.

The need to examine the role of home cleaning and personal use antibacterial products in the development of infections from antibiotic resistant organisms has recently been put forth by several authors (41, 42). In the present study, the use of antibacterial dish soap and a brand of toothpaste known to contain triclosan, an agent that may be involved in the development of antibiotic cross-resistance, was not found to be associated with resistance. The quality of the data and the validity of these findings may be questionable, since a high proportion of participants, 54% and 29%, respectively, reported using these products. Given the number of dish soap products and toothpaste brands available, it is seems likely that the frequency of reported use is greater than that of true use. Sensing that the use of antibacterial dish soap is an indication of good hygiene, participants may have been more likely to answer yes, and the quality of recall of product details among

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

most people is probably modest. A cohort format may be a more suitable than a case-control format to examine the effects of these products on the likelihood of antibiotic resistance.

Participation bias, an increasingly formidable challenge to overcome in observational studies (43), can involve more than one subset of the population. In this study, there was data missing from a) non-participants: people who could have, but did not participate and from b) non-submitters: people who were infected, but did not seek medical help or sought medical help but were not asked to submit a stool sample.

Non-participants in non-chronic infectious disease epidemiological research such as this can be a very different sub-set of the diseased population. In this study, it was remarkable that among non-participants in the Calgary Health Region travel had no significant effect on likelihood of resistance. Even though foreign travel was still a very important risk factor for resistance among the sample population after accounting for the effect of non-participation, this effect on $OR_{Foreign}$ was considerable. The reasons for the non-participant effect are unclear. One possibility is that, in contrast to participants, non-participants, who were more commonly infected with domestically acquired and ciprofloxacin susceptible strains, were those whose infections were more easily treated, and or, shorter in duration and were, therefore, more active and had less time to answer the questionnaire. Since most participants were unaware of the resistance status of their infection at the time of interview request, it can be assumed that concern over resistance would have little influence on interest in participation.

Unfortunately, data to estimate the effect of non-submitters was not available. The lack of data on non-reported intestinal disease, which this study, along with many others, suffers from, leads to results that describe only more severe diseases (44). Tam *et al.* (44) reported that people with severe intestinal illness were much more likely to consult their general practitioner, and physicians were more likely to ask for stool samples from those who recently travelled abroad. If this applied to this study population, it can be assumed that the sample population included, relative to domestic infections, an over-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

sampling of travel-related infections. However, this over-sampling would not have biased the risk estimate for travel if the proportions of resistance among travellers and domestically-infected patients were the same among those in the study sample and those in the study population (Chinook and Calgary Health Regions).

Recall bias may have weakened the validity of this study. Some patients may have been informed that the strain that was infecting them was ciprofloxacin resistant during a follow-up visit to their physician. The data indicated that cases were much more likely to have to have known their susceptibility testing results than controls. To what degree this could have influenced responses, particularly to questions about antibiotic use, is unmeasureable, but including knowledge in the model should have controlled for some unmeasured confounding.

Nalidixic acid susceptibility testing is an effective tool for screening fluoroquinolone susceptibility in enteric pathogens (19). It appeared that the use of nalidixic acid disk testing in the Calgary Health Region as a surrogate for ciprofloxacin disk testing was not a source of differential misclassification as most OR estimates were stable in the sensitivity analysis. It must be noted, however, that this conclusion is based on the sensitivity/specificity estimates from only one study. Ciprofloxacin susceptibility testing of the study isolates should be conducted to confirm the analysis results.

In conclusion, results from this case-control study demonstrate the overwhelming influence of foreign travel on the likelihood of fluoroquinolone resistance among *Campylobacter* infections in southern Alberta, and the possibility that individuals who have personal reserves of antibiotics may, through self-medication, be more likely to be infected with a resistant strain. Increasing frequency of chicken consumption was not a significant risk exposure for resistance. A larger study is required to more conclusively test the effect of exposures such as fluoroquinolone use and possession of non-prescribed antibiotics, which are uncommon among the population.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.5 References

1. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001; 32:331-51.
2. Gaudreau C, Gilbert H. Antimicrobial resistance of *Campylobacter jejuni* subsp. jejuni strains isolated from humans in 1998 to 2001 in Montreal, Canada. Antimicrob Agents Chemother 2003; 47:2027-9.
3. Gupta A, Nelson JM, Barrett TJ, et al. Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. Emerg Infect Dis 2004; 10:1102-9.
4. Lucey B, Cryan B, O'Halloran F, Wall PG, Buckley T, Fanning S. Trends in antimicrobial susceptibility among isolates of *Campylobacter* species in Ireland and the emergence of resistance to ciprofloxacin. Vet Rec 2002; 151:317-20.
5. Nachamkin I, Ung H, Li M. Increasing Fluoroquinolone Resistance in *Campylobacter jejuni,* Pennsylvania, USA,1982-2001. Emerg Infect Dis 2002; 8:1501-3.
6. Bodhidatta L, Vithayasai N, Eimpokalarp B, Pitarangsi C, Serichantalergs O, Isenbarger DW. Bacterial enteric pathogens in children with acute dysentery in Thailand: increasing importance of quinolone-resistant *Campylobacter*. Southeast Asian J Trop Med Public Health 2002; 33:752-7.
7. Engberg J, Neimann J, Nielsen EM, Aerestrup FM, Fussing V. Quinolone-resistant *Campylobacter* infections: risk factors and clinical consequences. Emerg Infect Dis 2004; 10:1056-63.
8. Hakanen A, Jousimies-Somer H, Siitonen A, Huovinen P, Kotilainen P. Fluoroquinolone resistance in *Campylobacter jejuni* isolates in travelers returning to Finland: association of ciprofloxacin resistance to travel destination. Emerg Infect Dis 2003; 9:267-70.
9. Kassenborg HD, Smith KE, Vugia DJ, et al. Fluoroquinolone-resistant *Campylobacter* infections: eating poultry outside of the home and foreign travel are risk factors. Clin Infect Dis 2004; 38:S279-84.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

10. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N Engl J Med 1999; 340:1525-32.
11. Alberta Health and Wellness. Chinook Regional Health Authority. In: Regional Health Authorities: Government of Alberta, 2005.
12. Alberta Health and Wellness. Calgary Health Region. In: Regional Health Authorities: Government of Alberta, 2005.
13. Statistics Canada. Population of census metropolitan areas (2001 Census boundaries). In: Summary Tables, 2006.
14. Alberta Health and Wellness Health Surveillance. Health trends in Alberta 2000. In. Edmonton: Alberta Health and Wellness, 2000.
15. Hsieh FY. Sample size tables for logistic regression. Stat Med 1989;8:795-802
16. Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ. Experimental *Campylobacter jejuni* infection in humans. J Infect Dis 1988; 157:472-9.
17. Blaser MJ. Epidemiologic and clinical features of *Campylobacter jejuni* infections. J Infect Dis 1997; 176:S103-5.
18. Greenland S. Basic methods for sensitivity analysis and external adjustment. In: Modern Epidemiology. Philadelphia: Lippincott-Raven, 1998:343-55.
19. Gaudreau C, Gilbert H. Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. J Antimicrob Chemother 1997; 39:707-12.
20. Statistics Canada. Population 15 years and over by highest level of schooling, by province and territory (2001 Census)(Alberta, British Columbia, Yukon Territory), 2004. (http://www40.statcan.ca/l01/cst01/educ43c.htm)
21. Guevremont E, Nadeau E, Sirois M, Quessy S. Antimicrobial susceptibilities of thermophilic *Campylobacter* from humans, swine, and chicken broilers. Can J Vet Res 2006; 70:81-6.
22. Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, Taylor DE. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance. Antimicrob Agents Chemother 2004; 48:3442-50.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

23. Thwaites RT, Frost JA. Drug resistance in *Campylobacter jejuni, C coli*, and *C lari* isolated from humans in north west England and Wales, 1997. J Clin Pathol 1999; 52:812-4.
24. Campylobacter Sentinel Surveillance Scheme Collaborators. Ciprofloxacin resistance in *Campylobacter jejuni*: case-case analysis as a tool for elucidating risks at home and abroad. J Antimicrob Chemother 2002; 50:561-8.
25. Gaunt PN, Piddock LJ. Ciprofloxacin resistant *Campylobacter* spp. in humans: an epidemiological and laboratory study. J Antimicrob Chemother 1996; 37:747-57.
26. Griggs DJ, Johnson MM, Frost JA, Humphrey T, Jorgensen F, Piddock LJ. Incidence and mechanism of ciprofloxacin resistance in *Campylobacter* spp. isolated from commercial poultry flocks in the United Kingdom before, during, and after fluoroquinolone treatment. Antimicrob Agents Chemother 2005; 49:699-707.
27. McDermott PF, Bodeis SM, English LL, et al. Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. J Infect Dis 2002; 185:837-40.
28. Carey B, Cryan B. Antibiotic misuse in the community--a contributor to resistance? Ir Med J 2003; 96:43-4, 6.
29. Pechere JC. Patients' interviews and misuse of antibiotics. Clin Infect Dis 2001; 33:S170-3.
30. Vanden Eng J, Marcus R, Hadler JL, et al. Consumer attitudes and use of antibiotics. Emerg Infect Dis 2003; 9:1128-35.
31. DuPont HL. Travellers' diarrhoea: Contemporary approaches to therapy and prevention. Drugs 2006; 66: 303-14.
32. McNulty CA, Boyle P, Nichols T, et al. Antimicrobial drugs in the home, United Kingdom. Emerg Infect Dis; 12: 1523-6
33. Reeves DS, Finch RG, Bax RP, et al. Self-medication of antibacterials without prescription (also called 'over-the-counter'use). J Antimicrob Chemother 1999; 44: 163-77.
34. Kassenborg H, Smith K, Vugia D, et al. Eating chicken or turkey outside the home associated with domestically acquired fluoroquinolone-resistant *Campylobacter*

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

infections: A FoodNet case-control study. In: International Conference on Emerging Infectious Diseases. Atlanta: CDC, 2000.

35. Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M, Perin R. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. Int J Food Microbiol 2003; 82:281-7.
36. Price LB, Johnson E, Vailes R, Silbergeld E. Fluoroquinolone-resistant *Campylobacter* isolates from conventional and antibiotic-free chicken products. Environ Health Perspect 2005; 113:557-60.
37. Kos VN, Keelan M, Taylor DE. Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from poultry from Alberta, Canada. Antimicrob Agents Chemother 2006; 50:778-80.
38. VanderKop M, McFall M, Sorensen O. *Campylobacter* spp. in chickens - prevalence and antibiotic resistance. In: Poultry Service Industry Workshop. Banff, 2002.
39. Aubry-Damon H, Grenet K, Sall-Ndiaye P, et al. Antimicrobial resistance in commensal flora of pig farmers. Emerg Infect Dis 2004; 10:873-9.
40. Statistics and Data Development Unit. Employment in Alberta Agri-Food Industries, 2004: Alberta Agriculture, Food, and Rural Development; 2005 April, 7. Report No.: 88.
41. Aiello AE, Larson E. Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. Lancet Infect Dis 2003; 3:501-6.
42. Levy SB. Antibacterial household products: cause for concern. Emerg Infect Dis 2001; 7:512-5..
43. Hartge P. Participation in population studies. Epidemiology 2006; 17:252-4.
44. Tam CC, Rodrigues LC, O'Brien SJ. The study of infectious intestinal disease in England: what risk factors for presentation to general practice tell us about potential for selection bias in case-control studies of reported cases of diarrhoea. Int J Epidemiol 2003; 32:99-105.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 4:
THE EFFECTS OF TRAVEL AND ANTIBIOTIC TREATMENT ON THE DURATION OF DIARRHOEA IN *CAMPYLOBACTER JEJUNI* INFECTIONS

4.1 Background

Campylobacter are common agents of foodborne diarrhoeal illness in Canada (1, 2). The range of reported mean (and median) duration of diarrhoea in *Campylobacter* illness in research conducted in developed countries is between 7 and 15 days (8 and 11 days) (3, 4), and reports of typical duration in traveller's diarrhoea, which is commonly caused by *Campylobacter* infection, varies from <3 days (5) to >14 days (6) . In the present study, I was interested in the possibility that infections caused by *C. jejuni* from countries other than Canada and the U.S.A. could be responsible for longer illnesses than those caused by indigenous strains. The question regarding the relative influence that foreign travel and ciprofloxacin resistance have on the duration of *Campylobacter* illness has been debated recently (4, 7). While a Foodborne Diseases Active Surveillance Network project found that ciprofloxacin resistance was the key determinant for the duration of diarrhoea (4), others challenged the methods used to reach this conclusion (7).

In bacterial gastroenteritis, invasion can play a role in prolonged diarrhoea (8). The pVir plasmid identified in the highly pathogenic strain *C. jejuni* 81-176 was initially perceived as a potentially important contributor to the invasion process (9), however, there is conflicting evidence of the relationship between the presence of pVir in *C. jejuni* strains isolated from humans and specific symptoms of gastroenteritis (10, 11). In this study, the effect of pVir presence on diarrhoea duration was examined.

This study also assessed the effect of antibiotic treatment in an observational setting. *Campylobacter* infections are commonly self-limiting, but are often treated with ciprofloxacin, erythromycin, or azithromycin, particularly when illness is associated with travel, severe illness or bloody diarrhoea (12-14). In a clinical trial, duration of community-acquired gastroenteritis, predominantly caused by *Campylobacter* infection

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

was much shorter among those treated with ciprofloxacin, than among those who received placebo (15).

4.2 Methods

4.2.1 Epidemiological Data Collection

Epidemiological data used in this study were gathered concurrently with data from the case-control study on ciprofloxacin resistance of *Campylobacter* infections in southern Alberta (Chapter 3, this thesis). Study participants were among those in the Chinook Health Region and Calgary Health Region, greater than 16 years of age, who submitted stool samples that were positive for the presence of *Campylobacter* and were later contacted by health region staff between February 1, 2004 and July 29, 2005. The study questionnaire was administered by public health inspectors and nurses by phone during routine follow-up enteric investigations. Data gathered included age, sex, education, travel completed within the past month, duration of diarrhoea, and antibiotic treatment (duration of treatment, name of drug or brand, if antibiotic for diarrhoea was prescribed before stool sample submission). Verbal consent was recorded prior to administration of the study questionnaire. Ethics approval was granted for this work by the Health Research Ethics Board, Panel B, University of Alberta, Edmonton, Conjoint Health Research Ethics Board, University of Calgary, Calgary, and Chinook Health Region Regional Research Committee, Lethbridge.

4.2.2 Detection of pVir Plasmid

A sub-sample of the *C. jejuni* strains isolated from patients in the Calgary Health Region from September 2004 through July 2005 was tested for the presence of the pVir plasmid. Frozen cultures were maintained at -80 °C, and prior to polymerase chain reaction (PCR) amplification, bacteria were grown on charcoal cefoperazone deoxycholate agar (Oxoid, Basingstoke) and incubated at 42°C for 48 hours. Single colonies were inoculated into Bolton broth (Oxoid, Basingstoke) and incubated at 42°C for 24 to 48 hours. Supernatant samples were removed following centrifugation. A 2.5 µl of bacterial extract was used for each PCR. Plasmid DNA isolation methods and the *virB11* plasmid primer set used

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

followed those used by Bacon *et al.* (9) with adjustments to PCR conditions as follows: initial melting temperature of 95°C for 1 min; 35 cycles of 95°C for 1 min, 55°C for 30 sec, and 72°C for 1 min; final extension at 72°C for 5 min.

4.2.3 Ciprofloxacin Susceptibility Testing

Fluoroquinolone susceptibility of *C. jejuni* strains was tested at two laboratories. The Chinook Health Region Laboratory used a modified Kirby Bauer method, in which colonies were streaked onto Mueller Hinton agar (BD Diagnostics, Oakville) with 5% sheep blood (BD Diagnostics, Oakville) and incubated at 37 to 42°C for 24 to 48 h., depending on growth characteristics. The zone of inhibition around a 5 μg ciprofloxacin disk (BD Diagnostics, Oakville) was assessed as follows: ≤15 mm, resistant; 16 to 20 mm, intermediate; ≥21 mm, susceptible. *Campylobacter* strains isolated in the Calgary Health Region by Calgary Laboratory Services were tested for nalidixic acid susceptibility using 30 μg nalidixic acid disks (Oxoid, Nepean) on blood agar plates (PML Microbiologicals, Wilsonville). Susceptibility to nalidixic acid was assessed by zone diameter ≥20 mm, and resistance was assessed by no zone of inhibition. This laboratory found that zones of inhibition were never between 0 and 20 mm in diameter. Plates were incubated at 42°C for 18 to 24 h. Nalidixic acid susceptibility was assumed to be an acceptable surrogate for ciprofloxacin susceptibility (16).

4.2.4 Data Handling and Analyses

Data were double-entered into the Statistical Package for the Social Sciences, Base version 13.0 (SPSS, Chicago, IL). The definition of chronic diarrhoea varies widely from 14 days to 3 months (17, 18). The 14-day criterion, accepted by some (17), and a longer, more inclusive 30-day criterion were used in this study. The effects of independent variables on duration within 14 and 30-day data sets and within a shorter period, 7-day, data set were examined. Diarrhoeal illnesses that started at least two days after the first day of travel outside of the US and Canada and within three days of returning from travel were considered to be caused by foreign strains.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

For cases in which diarrhoea had ceased at the time of interview, the effect of variables on mean diarrhoea duration was assessed with two-independent sample *t*-tests, while Pearson correlations were computed between age and duration. When skew and kurtosis were >2 and <-2, duration data were logarithmically transformed to adjust for non-normality. Levene's test was used to assess homogeneity of variance. Multivariate ANOVA was used to test the effect of travel on duration, while controlling for other variables. Marginal mean plots and stepwise model-building were used to identify interaction terms for the multivariate ANOVA model.

Cox proportional hazard regression univariate and multivariate models were used to assess the effects of variables on duration of all cases of non-chronic diarrhoea, including cases in which diarrhoea was on-going at the time of interview. Also, Fisher's Exact test was used to assess the relationship between foreign travel and the presence of the pVir plasmid.

4.3 Results

There were 209 *C. jejuni* cases available to participate in this study, but 8 cases had diarrhoea that lasted >30 days and were excluded due to prolonged, and possibly chronic, diarrhoea. Eighty of the remaining 201 participants had on-going diarrhoea at the time of interview. Approximately 60% (n = 351) of eligible patients participated; lack of time appeared to be the most common reason for non-participation. Among the 201 participants, the mean age of participants was 40.2 years, 56.0% were male, and 61.7% had college or university education. Among the 201 *C. jejuni* strains, 28.9% were resistant to ciprofloxacin and 28.9% originated from locations outside of the U.S.A. and Canada. Among the sub-sample of 73 *C. jejuni* strains tested, 13.7% were positive for the presence of the pVir plasmid.

The median duration of diarrhoea among the 201 participants with ceased and on-going diarrhoea was 9 days (inter-quartile range (IQR) 5.5 days), mean duration was 9.5 days (SD 5.0 days) and the range of duration was 2 to 27 days. Among those whose diarrhoea had ceased at the time of interview, the median duration was 7 days (IQR five days) and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the mean was 8.5 days (SD 4.6 days). Among the 8 excluded cases with prolonged diarrhoea, the range was 32 to 85 days.

4.3.1 Univariate Analyses

Duration data for all cases of non-chronic, ceased diarrhoea had a non-normal distribution (skew = 1.6, kurtosis = 3.3), but data sets for diarrhoea up to 14 days and 7 days followed normal distributions. Univariate *t*-tests and multivariate ANOVA using the 30 day data set were run on log-transformed duration data, but the remaining analyses, including Cox regression models were run on raw data.

Univariate model results based on data from cases in which diarrhoea had ceased at the time of interview are given in Table 4.4.1. Duration within the 30 day and 14 day data sets was significantly longer among travel-related infections than among domestically-acquired infections, but there was no such difference within the 7 day data (Table 4.4.1). Duration was longer for those infected with ciprofloxacin resistant strains, but only within the 30 day data set (Table 4.4.1).

Table 4.4.2 gives results from Cox regression analyses based on on-going cases as well as ceased cases of diarrhoea, and these results differ from those in Table 4.4.1. Within the 30 day data set, travel had a marginally protective effect on duration of diarrhoea (Table 4.4.2).

Age was not correlated to log-transformed duration (Pearson correlation $p = 0.99$). pVir presence was not associated with duration; however, pVir presence was more common among travel-related strains (33.3%, n = 28) than among domestic strain (7.3%, n = 41; Fisher's Exact 1-sided $p = 0.045$)

4.3.2 Multivariate Analysis

The primary variable of interest in multivariate testing was travel-related infection. After controlling for the potential confounding effects of ciprofloxacin resistance and fluoroquinolone treatment, foreign travel was an independent significant predictor of

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

duration in the multivariate ANOVA model with the 30 day data set, but not with the 14 day data set (Table 4.4.3). No significant interaction terms were identified. Travel was not significant in Cox regression models adjusting for fluoroquinolone treatment, ciprofloxacin resistance (Table 4.4.3)

4.3.3 Antibiotic Treatment

One-hundred-thirty-eight participants (69.0%, n = 201) reported filling out a prescription for their diarrhoeal illness, and 127 (92.0%, n = 138) were able to name the drug prescribed. Among those who named their antibiotic treatment, ciprofloxacin was the most commonly prescribed first course of antibiotic (44.1%, n = 127). Among all antibiotic treatments, including second and third course treatments (n = 146), 44.5% were fluoroquinolone drugs, 29.5% were erythromycin, and 11.6% were azithromycin. Remarkably, 19 participants (9.5%, n = 201) reported starting a course of antibiotics after diarrhoea had stopped. In following analyses of effectiveness of antibiotic and fluoroquinolone treatment these participants were excluded.

Among the 182 participants (including on-going cases of diarrhoea) who were not excluded were 117 who were treated with an antibiotic. The median treatment lag time, the period between the start of diarrhoea and the start of treatment, was 6 days (IQR, five days), and the median duration of diarrhoea following initiation of treatment was three days (IQR, four days). Using a Cox regression model on these data, treatment lag time was not a predictor of duration of diarrhoea following initiation of treatment (hazard ratio, HR = 1.0 (95% CI = 1.0, 1.1; $p = 0.7$). Among the 54 cases treated with a fluoroquinolone, treatment lag time, with or without controlling for ciprofloxacin resistance did not predict duration [with adjustment: HR = 1.0; 95% CI(HR) = 0.9, 1.1; $p = 0.5$; without adjustment: HR = 1.0; 95% CI(HR) = 0.9, 1.1, $p = 0.4$].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

4.4 Tables

Table 4.4.1. Univariate unadjusted associations between dichotomous characteristics and duration of diarrhoea in *C. jejuni* infection, southern Alberta, 2004–2005. Includes only patients who no longer had diarrhoea at time of interview.

Variable (contrast group)	Duration ≤ 30 days (n = 121) †		Duration ≤ 14 days (n = 109)		Duration ≤ 7 days (n = 64)	
	Mean difference (SEM)	*p* ‡	Mean difference (SEM)	*p* ‡	Mean difference (SEM)	*p* ‡
Gender (female)	0.2 (0.8)	1.0	-0.4 (0.5)	0.4	-0.2 (0.36)	0.6
Higher education § (yes)	-1.1 (0.9)	0.4	-0.3 (0.6)	0.6	0.2 (0.4)	0.5
Travel-related infection (yes)	-3.6 (0.9)	<0.001	-1.4 (0.6)	0.03	0.2 (0.5)	0.7
Ciprofloxacin resistance (yes)	-2.8 (0.9)	0.004	-1.1 (0.6)	0.07	0.1 (0.5)	0.8
pVir presence (yes)	2.0 (2.5)	0.4	0.5 (1.3)	0.7	NA	—

Mean difference: mean for reference group minus mean for contrast group
SEM: standard error of the mean
† *t*-tests performed on log-transformed data where duration ≤ 30 days
‡ Two-tailed significance of *t*-statistic
§ Completed college or university
NA: Not available, inadequate sample size

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 4.4.2. Univariate unadjusted associations between dichotomous characteristics and duration of diarrhoea in *C. jejuni* infection, southern Alberta, 2004–2005. Includes patients who no longer had diarrhoea at time of interview and those with on-going diarrhoea.

Variable	Duration ≤ 30 days (n = 201)		Duration ≤ 14 days (n = 176)		Duration ≤ 7 days (n = 82)	
	HR † (95% CI)	*p* ‡	HR † (95% CI)	*p* ‡	HR † (95% CI)	*p* ‡
Gender	1.0 (0.7, 1.4)	1.0	1.2 (0.8, 1.7)	0.4	1.1 (0.7, 1.8)	0.7
Higher education	0.8 (0.5, 1.1)	0.2	0.9 (0.6, 1.3)	0.6	1.2 (0.7, 2.0)	0.4
Travel-related infection	0.6 (0.4, 1.0)	0.04	0.9 (0.6, 1.4)	0.7	1.0 (0.5, 2.0)	1.0
Ciprofloxacin resistant strain	0.8 (0.5, 1.2)	0.3	0.9 (0.6, 1.4)	0.6	1.1 (0.6, 2.0)	0.9
pVir-positive strain	0.7 (0.2, 1.9)	0.5	0.9 (0.3, 2.6)	0.9	2.1 (0.4, 9.5)	0.4

CI: confidence interval of the hazard ratio, HR

† Unadjusted Cox proportional hazard regression hazard ratio

‡ Wald statistic

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 4.4.3. Multivariate analyses of diarrhoea duration in *C. jejuni* infections, southern Alberta, 2004-2005.

	Duration ≤ 30 days		Duration ≤ 14 days	
	ANOVA *F*-statistic (*p*)	Cox regression HR (95% CI)	ANOVA *F*-statistic (*p*)	Cox regression HR (95% CI)
Travel-related infection	5.0 (0.03)	0.6 (0.4, 1.1)	1.6 (0.2)	1.0 (0.6, 1.7)
Ciprofloxacin resistant strain	0.1 (0.8)	1.1 (0.6, 1.8)	0.1 (0.8)	0.9 (0.5, 1.5)
Fluoroquinolone treatment	0.7 (0.2)	0.8 (0.6, 1.2)	0.01 (0.9)	0.9 (0.6, 1.4)

4.5 Discussion

In this study, the effects of foreign travel, ciprofloxacin resistance, antibiotic and fluoroquinolone treatment, and the presence of the putative virulence plasmid, pVir, on the duration of diarrhoea caused by *C. jejuni* infection were examined. Travellers from developed countries visiting other countries with less developed economies are at risk of acquiring *C. jejuni* infection (19, 20), and others have previously investigated the interplay between travel, antibiotic treatment and resistance, and duration of diarrhoea associated with these infections (4). This work showed that travel had an effect on duration among those who had diarrhoea that lasted up to 30 days.

Contrary to the report by Nelson *et al*, (4) which documented the work conducted by the Foodborne Diseases Active Surveillance Network, the data from this study indicated that ciprofloxacin resistance was not independently associated with duration in *C. jejuni* infections. Nelson *et al.* suggested that the effect of anti-diarrhoeal medication on the duration of *Campylobacter* infections is important and that control of this effect could dramatically decrease the dominant influence of travel on duration. Unfortunately, participants were not questioned on their use of anti-diarrhoeal medications. It is reasonable to hypothesize that ciprofloxacin resistance could cause a slower resolution of symptoms among those who were treated with a fluoroquinolone, however, this work and those of others (21) has shown that this has not been the case.

The univariate analyses here suggested there may be some differences in the effect of travel on duration depending on the range of duration considered. When the analysis was restricted to only those whose diarrhoea had resolved within 7 days, travel had no influence on the duration of diarrhoea. The mean duration of diarrhoea associated with *Campylobacter* infection reported by others ranges between 5 to 10 days (3, 4, 21). If this is the case, these results suggest that among most cases of *Campylobacter*-associated diarrhoea, the duration would likely not differ between travel-related and domestic infections.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Cases of diarrhea lasting more than one week may involve coinfection, malabsorption, or post-infectious irritable bowel syndrome (22). However, prolonged diarrhoea can also be due to persistent bacterial infection. Laboratory-confirmed cases of persistent *Campylobacter* infection have been reported, in which the mean interval between onset of illness and last positive specimen was 37.6 days (3). The data analysis in this study was structured to capture similar cases of longer duration infection. It appeared that, when a broader sample population that included people with diarrhoea up to 30 days was used, those who acquired their infection abroad suffered for more days than did people who acquired infection at home. There are several possible reasons for this. One possible reason is that travellers might not have timely access to medical assistance and this could result in longer illness. Another possibility is that the genomes of travel-associated strains of *C. jejuni* are more likely to possess genes encoding resistance to host immune responses than are domestic strains, allowing them to persist longer once they have colonized the intestine.

Unlike many other identified virulence determinants of *C. jejuni,* there is some variability in the prevalence of the pVir plasmid (23); this fact presented an opportunity to test the hypothesis that variation in the illness caused by *C. jejuni* could be, in part, due to its presence. Similar to other reports of the clinical effects of pVir, (11) this study indicated that duration of diarrhoea was unaffected by its presence, but larger studies are required to generate more reliable conclusions on this issue. There was an interesting contrast between the report by Tracz *et al.* (11) that none of the patients who had pVir-positive *C. jejuni* strains they examined had recently travelled and this study's observation of a positive association between the presence of pVir and recent travel. Travel was not common among the sample of patients used by Tracz *et al.* (10 of 104, 9.6%); it may have been difficult to observe the effects of travel from data with a low level of exposure.

To make a valid assessment of antibiotic treatment in this observational study of diarrhoea duration in *C. jejuni* infections, I required comparable duration data for the non-treated participants. These data are essentially counterfactual in nature: it is impossible to know when antibiotic treatment would have commenced among those who

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

were not treated if they were treated. I did, however, examine the effects of timing of antibiotic treatment on time to recovery. Delays in treatment with a fluoroquinolone or other antibiotic did not lead to prolonged diarrhoea. This suggests that early treatment may not improve outcome, and supports those who caution against the empirical treatment of *Campylobacter* infections due to the potential for increasing levels of fluoroquinolone resistance (24). Supplemental to this finding is the observation that resistance was not a factor in the time it took for fluoroquinolone treated infections to resolve. The sub-sample analysis, though, was limited to a small sample, thus it remains unclear if the benefits associated with empirical treatment outweigh the costs.

Further research is required to confirm the findings of this study. Future surveys should enquire about the use of antidiarrheals, history of gastrointestinal disorders and other underlying medical conditions, such as HIV (25) and diabetes (26), which are associated with chronic diarrhoea. With information on these factors, coupled with a larger sample population, investigators could exclude the affected cases and filter out the effects these factors have on diarrhoea duration. Although the data here and elsewhere (21) suggests ciprofloxacin resistance does not hinder the effectiveness of fluoroquinolone treatment of *Campylobacter* infections, the statistical results on which these claims are based would have greater power if the sample population was larger. Also, future surveys should enquire about symptom details, to ensure participants' illnesses meet consistent definitions of diarrhoea.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

4.6 References

1. Bowman C, Flint J, Pollari F. Canadian integrated surveillance report: *Salmonella, Campylobacter*, pathogenic *E. coli* and *Shigella*, from 1996 to 1999. Can Commun Dis Rep 2003;29 Suppl 1:i-vi, 1-32 (eng); i-vi, 1-34 (fre).
2. Division of Foodborne and Enteric Diseases. Canadian integrated surveillance report for 1995 on *Salmonella, Campylobacter* and pathogenic *E. coli*. Winnipeg: Laboratory Centre for Disease Control, Health Canada, 1998.
3. Kapperud G, Lassen J, Ostroff SM, Aasen S. Clinical features of sporadic *Campylobacter* infections in Norway. Scand J Infect Dis 1992;24:741-9.
4. Nelson JM, Smith KE, Vugia DJ, et al. Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. J Infect Dis 2004;190:1150-7.
5. Hill DR. Occurrence and self-treatment of diarrhea in a large cohort of Americans traveling to developing countries. Am J Trop Med Hyg 2000;62:585-9.
6. Gallardo F, Gascon J, Ruiz J, Corachan M, Jimenez de Anta M, Vila J. *Campylobacter jejuni* as a cause of traveler's diarrhea: clinical features and antimicrobial susceptibility. J Travel Med 1998;5:23-6.
7. Cox LA, Jr., Copeland D, Vaughn M. Ciprofloxacin resistance does not affect duration of domestically acquired campylobacteriosis. J Infect Dis 2005;191:1565-6; author reply 1566-7.
8. Janda JM, Abbott SL, Woodward D, Khashe S. Invasion of HEp-2 and other eukaryotic cell lines by Providenciae: further evidence supporting the role of *Providencia alcalifaciens* in bacterial gastroenteritis. Curr Microbiol 1998;37:159-65.
9. Bacon DJ, Alm RA, Burr DH, et al. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infect Immun 2000;68:4384-90.
10. Louwen RP, van Belkum A, Wagenaar JA, Doorduyn Y, Achterberg R, Endtz HP. Lack of association between the presence of the pVir plasmid and bloody diarrhea in *Campylobacter jejuni* enteritis. J Clin Microbiol 2006;44:1867-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

11. Tracz DM, Keelan M, Ahmed-Bentley J, Gibreel A, Kowalewska-Grochowska K, Taylor DE. pVir and bloody diarrhea in Campylobacter jejuni enteritis. Emerg Infect Dis 2005;11:838-43.
12. DuPont HL. Treatment of travelers' diarrhea. J Travel Med 2001;8:S31-3.
13. Blaser MJ. Epidemiologic and clinical features of *Campylobacter jejuni* infections. J Infect Dis 1997;176 Suppl 2:S103-5.
14. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32:331-51.
15. Dryden MS, Gabb RJ, Wright SK. Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin. Clin Infect Dis 1996;22:1019-25.
16. Gaudreau C, Gilbert H. Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. J Antimicrob Chemother 1997;39:707-12.
17. Holt PR. Gastrointestinal diseases in the elderly. Curr Opin Clin Nutr Metab Care 2003;6:41-8.
18. Lysy J, Israeli E, Goldin E. The prevalence of chronic diarrhea among diabetic patients. Am J Gastroenterol 1999;94:2165-70.
19. Campylobacter Sentinel Surveillance Scheme Collaborators. Foreign and domestic travel and the risk of *Campylobacter* infection: results from a population-based sentinel surveillance scheme. J Travel Med 2003;10:136-8.
20. Rodrigues LC, Cowden JM, Wheeler JG, et al. The study of infectious intestinal disease in England: risk factors for cases of infectious intestinal disease with *Campylobacter jejuni* infection. Epidemiol Infect 2001;127:185-93.
21. Tabibian, N, Clarridge, JE, Smith, L, et al. Clinical impact of stool cultures for *Campylobacter* in adults with acute or chronic diarrhea. South Med J 1987; 80:709-11.
22. Connor, BA. Sequelae of traveler's diarrhea: Focus on postinfectious irritable bowel syndrome. Clin Infect Dis 2005; 41:S577-86.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

23. Datta S, Niwa H, Itoh K. Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. J Med Microbiol 2003;52:345-8.
24. Oldfield EC, 3rd, Wallace MR. The role of antibiotics in the treatment of infectious diarrhea. Gastroenterol Clin North Am 2001;30:817-36.
25. Snijders F, Kuijper EJ, de Wever B, van der Hoek L, Danner SA, Dankert J. Prevalence of *Campylobacter*-associated diarrhea among patients infected with human immunodeficiency virus. Clin Infect Dis 1997;24:1107-13.
26. Lysy J, Israeli E, Goldin E. The prevalence of chronic diarrhea among diabetic patients. Am J Gastroenterol 1999;94:2165-70

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 5:

KNOWLEDGE OF THE PREVENTION AND TREATMENT OF TRAVELLERS' DIARRHOEA

5.1 Background

Travellers from developed countries who visit regions such as southeast Asia, Latin America, and Africa are frequently afflicted with travellers' diarrhoea (TD) caused by *Campylobacter* spp., enterotoxigenic *Escherichia coli, Salmonella* spp., and *Shigella* (1-6). Studies of diarrhoea episodes among American college and university students in Mexico reported attack rates of 29% to 55% (4, 7) The case-control study suggested that more than half of the individuals within the Calgary Health Region who have been infected with *Campylobacter* during the spring and summer of 2004 travelled outside of Canada and the U.S.A.; a large proportion of those travelled to Mexico (Chapter 3, this thesis). Previous work has demonstrated that Canadian travellers are aware of the risk of foodborne bacterial infections associated with travel abroad (8). The objective of this study was to assess the level of knowledge about TD among travellers to Mexico, and to determine if travellers who sought travel-related health advice prior to travel have a greater understanding of TD prevention and treatment than do those who do not obtain advice.

5.2 Methods

5.2.1 Questionnaire Development

A self-administered questionnaire developed for this study included TD knowledge test items and questions regarding travel health advice acquired prior to travel, perception of disease risk, and possession of antimicrobials. Travel advice sources listed in the questionnaire included the internet, friends and colleagues, physicians, travel agents, and travel clinics. Sixteen TD knowledge test items included multiple-choice and yes/no items that addressed prevention and food risks (8 questions), agents and symptoms (6 questions) and treatment (2 questions). The information used for the content of these

A version of this chapter has been published. Johnson *et al.* 2006. *Journal of Travel Medicine.* 13:351-355.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

items came from health agencies at national and regional levels (9, 10). Scientific literature and a statement from the Committee to Advise on Tropical Medicine and Travel (CATMAT) were reviewed to determine the symptoms of TD, current guidelines and recommendations regarding treatment of TD, and risk factors for acquiring TD abroad (9, 11). The web page on TD posted by the Calgary Health Region was also used as a source of information (10). Personal data collected included age, gender and education. Yes/no and multiple-choice formats were used for knowledge questions, and Likert-scaled format was used to estimate respondents' concern about their risk of TD. The order of questions was determined by coin toss. The questionnaire is presented as Appendix 2.

A previously validated patient knowledge study was used as a model for the structure of the multiple choice question section (12). A previously published food-safety knowledge study in which internal reliability was assessed was used as a guide for the construction of some knowledge test items (9, 13). Content validity was evaluated through a review by travel health advice-providers. Ease-of-use and wording of the questionnaire items and the introductory letter/consent form was pilot-tested among travellers at the Calgary International Airport.

5.2.2 Data Collection and Analyses

Questionnaires were given to, and collected from, travellers over the age of 16, at departure gates 20 to 30 minutes prior to boarding flights destined for Mexico. Research personnel distributed questionnaires to every third person seated in the departure gate area. Travellers were awaiting charter flights to Cancun and Puerto Vallarta, Mexico, between March 10, 2005 and April 25, 2005. Typically, one or two flights per week departed from Calgary during business hours to these destinations during the sampling period. Data collection from all flights was attempted; however, two flights out of eight were missed due to reasons not related to destination or date. Study approval was obtained from the Calgary International Airport Authority and the Health Research Ethics Board, Panel B, University of Alberta, Edmonton.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Data were entered into a database (SPSS for Windows Release 10.0.5, SPSS Inc., Chicago IL), and checked by double entry. Knowledge questions were scored one for the correct answer and 0 for incorrect or don't know answers. In cases where none of the options, among yes, no, and "don't know", were checked, the data was considered missing. Subjects who entered yes in response to "Are you carrying an antibiotic or a prescription for an antibiotic with you on this trip?", but entered a name of a non-antibiotic drug for "What is the name of the antibiotic you are carrying?", were entered into the database as not carrying an antibiotic. Two-tailed Student's *t*-tests, χ^2 tests, Fisher's exact tests, and general linear model ANOVA tests were performed using SPSS.

5.3 Results

Of the 111 people who were approached and were eligible to participate in the study, 104 (93.7%) completed the survey. Although the questionnaires were distributed to every third person among travellers in departure gates, more women than men completed the questionnaire (Table 5.41). Information on travel-related disease was sought by almost half of the study participants, and more than one source of information was reported by 22.1%. The most common sources of information, friends/family and nurse/physician/pharmacist, provided information to 23.1% and 21.2% of participants, respectively. Internet, travel agents, and travel clinics were used among 11.5%, 10.6% and 5.8% of participants, respectively.

5.3.1 Knowledge Assessment

The mean total TD knowledge score among all participants was 65.6% (SD 14.4). The ability to identify the correct response varied among questions, but, in general, the scores for food safety questions were high (Table 5.4.2). Food safety questions required participants to identify if partially cooked beef and chicken, salad, beer, and pop, when consumed in a developing country, posed a "moderate to high" or "low to none" risk. Less than ½ knew that chlorinated water is not always safe to drink, while 81.7% to 92.3% correctly answered other food risk questions. Almost all participants responded

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

that TD can be acquired from bacteria, but only 43.7% knew viruses can cause TD, and 25% incorrectly identified fungi as sources of TD.

The question regarding antibiotic treatment of TD was apparently difficult, as 56.7% answered don't know; however, among those who felt they had an answer, 48.9% answered correctly. This question required participants to correctly identify the statement "The duration of travellers' diarrhoea will likely be reduced if you take ciprofloxacin" as true. Three participants (2.9%) identified "You should stop taking antibiotics if your diarrhoea stops after one day" or "All travellers, including you, should take an antibiotic to prevent travellers' diarrhoea" as true. The remaining option "You should avoid using a product like Imodium while on antibiotics" was chosen by 16.3% of participants.

5.3.2 Information and Knowledge

The mean overall score among those who reported receiving information on travel-related disease was not significantly higher than the mean score of those who did not receive this type of information (Table 5.4.3). A mean score difference of 5.3% was observed between those who received information specifically about TD and those who did not; however, the difference not quite large enough for statistical significance. Eleven of the 16 knowledge questions were answered correctly more often among those who had TD information than among those who did not; however, the number who answered correctly was significantly higher for only two questions. These questions focused on the risk of TD associated with ice cubes ($t = 2.28, p = 0.025$) and pop ($t = 2.44, p = 0.016$). Individual sources of information, including medical sources, did not significantly impact on knowledge scores (Table 5.4.3).

The mean overall score of people with college or university education was higher than that of people without this level of education (Table 5.4.3). In this study, people without college or university education were more likely to obtain TD information (46.7% of 45) than were those with college or university education (35.1% of 57), although the difference was not significant ($\chi^2 = 1.40, p = 0.24$). Furthermore, the effect of TD information was somewhat greater among the former. With TD information, the mean

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

score for people without college or university increased by 12.3% from 58.6% to 65.8% ($t = 1.64, p = 0.11$), while mean score for college or university-educated people increased by 4.8% from 68.9% to 72.2% ($t = 1.42, p = 0.16$). Independent of education, TD information had a significant effect on overall score ($F = 4.76, p = 0.032$). No interaction was evident between TD information and education ($F = 0.11, p = 0.74$).

Those who reported carrying an antibiotic were more likely to have answered the antibiotic treatment question correctly (72.7% of 11) than were those who did not have an antibiotic (15.2% of 92; Fisher's exact $p < 0.001$). However, those who received information from a medical professional correctly answered the antibiotic treatment question only slightly more often than those who did not get medical advice (Fisher's exact $p = 0.78$). Increased risk of diarrhoea during travel to a developing country was consistently identified by participants (82.5%). Risk perception was not, however, associated with access of travel-related disease information ($\chi^2 = 0.15, p = 0.70$), possession of antibiotics (Fisher's exact $p = 0.68$), or TD knowledge level (Table 5.4.3).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.4 Tables

Table 5.4.1. Characteristics of study sample population among travellers departing from Calgary, Alberta to Mexico, during March and April 2005.

Characteristic	%
Gender (n=104)	
Male	43.3
Female	56.7
Age (n=100)	
17-29	20.0
30-49	47.0
≥ 50	33.0
Education (n=103)	
High school, trade school	43.7
College, university	56.4
Received information on:	
Travel-related disease (n=104)	48.1
Travellers' diarrhoea (n=102)	40.2
Carrying an antibiotic (n=103)	10.7

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 5.4.2. Percent of correct answers among study participants (n = 104).

Question (correct answer)	**Answered correctly** (%)
Food risk	
Salad (yes)	85.6
Beer (no)	82.7
Beef, partially cooked (yes)	83.7
Chicken, partially cooked (yes)	92.3
Pop (no)	88.5
Ice cubes (yes)	81.7
Chlorinated water (yes)	47.1
Travellers' diarrhoea sources	
Bacteria (yes)	96.1
Viruses (yes)	43.7
Symptoms	
Abdominal cramps (yes)	92.3
Dizziness (no)	6.7
Headache (no)	8.7
Nausea (yes)	76.9
Treatment & Hygiene	
Consult MD if diarrhoea is bloody (yes)	88.5
Antibiotic treatment (ciprofloxacin)	21.2
Hand washing effective (yes)	55.8

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 5.4.3. Mean overall knowledge score and characteristics of study participants.

Characteristic	Score, % correct (95%CI)	p [a]
Gender		0.77
Male	65.1 (63.2,67.1)	
Female	66.0 (64.0,68.0)	
Age group		0.53 [b]
17-29	65.9 (62.1, 69.8)	
30-49	64.8 (62.8, 66.7)	
≥ 50	68.4 (66.1, 70.6)	
Education		0.013
High school/ trade school	61.7 (59.5,63.9)	
College / university	68.8 (67.0, 70.5)	
Received travel-related disease information		0.20
Yes	67.3 (65.9,69.4)	
No	63.9 (61.7,66.1)	
Received travellers' diarrhoea information		0.056
Yes	68.9 (67.1,70.7)	
No	63.6 (61.6,65.7)	
Information from family/friends		0.42
Yes	67.1 (64.9, 70.5)	
No	65.0 (63.4, 66.6)	
Information from medical professional		0.35
Yes	68.2 (65.2, 71.2)	
No	64.9 (63.3, 66.5)	
Carrying an antibiotic		0.064
Yes	73.3 (70.6, 76.0)	
No	64.7 (63.2, 66.3)	
Perceived increased TD risk with travel		0.58
Yes	66.0 (64.4, 67.6)	
No	63.9 (60.3, 67.4)	

[a] p-value based on t-test unless otherwise specified
[b] p-value from ANOVA test

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.5 Discussion

Various guidelines and review articles have pointed out the importance of informing people prior to travel about the prevention and treatment of travel-related diseases including TD; some stress the critical role of health professionals in this process (14-16). The percentage of Canadian and American travellers that receive travel health information prior to travel has been estimated to be between 15% and 81% (8, 17, 18). Among this study's sample, almost one half of people travelling from Calgary to Mexico sought some information, although less than one quarter consulted health professionals. This level is similar to the proportion of Australian travellers who sought advice from a family doctor (23% of 2101) (19). Presumably, advice from medical professionals should convey high quality information to travellers; however, those who reported seeking advice from a medical source did not score significantly higher than those who had not obtained information from this type of source.

It appears that, when travellers are stratified into groups who have or do not have college/university education, receiving information that specifically addresses TD prevention and treatment is associated with higher levels of TD knowledge. This may be an indication of the effectiveness of TD information, but it could also be due to a higher level of awareness of the topic among those who seek information. University and college-educated travellers appear to have a better understanding of TD issues, but the reason for this is not explained by the study data. This group did not seek information more commonly than did other travellers; moreover, TD information did not have an effect on their overall scores. Previous work supported the concept that business travellers are better prepared for the health risks associated with foreign travel (17). Although the purpose of travel was not asked in this survey, it appeared that few of the travellers surveyed were business travellers.

One of the limitations of this study was the lack of detailed questioning within individual topics. Departure gate surveys present logistical difficulties. A surveyor hoping to capture a large and unbiased sample waits until a high proportion of travellers have

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

arrived at the departure area. By that time, there is only 10-15 minutes until the call for boarding; thus, the questionnaire must be sufficiently short so that travellers are not inconvenienced or are unable to complete it before boarding. The questionnaire design for this study sacrificed depth of investigation for coverage of a broad range of question topics. Another questionnaire design weakness of the study relates to the column format of the questions related to symptoms. Almost half of the participants (47.1%) checked the yes boxes for all four symptoms; possibly indicative of yea-saying responders (20).

The study sample size was small and sampling was limited to a small geographical area. For these reasons, the results reported here may not be generalizable to a wider population. Additional studies with greater sample sizes and with pre- and post-education testing would produce a more reliable assessment of the influence of travel health information on knowledge levels. Finally, potential participation bias due to a very small gender imbalance may have been caused by instances in which a woman in a male-female couple completed questionnaire forms given to a man.

In summary, a high proportion of participants understood they were at a higher risk of diarrhoea while travelling to a developing country, and that the level of knowledge about food risks was high. More advanced and effective education to a wider audience of pre-travellers may be required to increase awareness of TD prevention and the less commonly known facts regarding antibiotic treatment of TD.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.6 References

1. Slavin MA, Jennens I, Tee W. Infection with ciprofloxacin-resistant *Campylobacter jejuni* in travellers returning from Asia. Eur J Clin Microbiol Infect Dis 1996;15:348-50.
2. Hakanen A, Kotilainen P, Huovinen P, Helenius H, Siitonen A. Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from Southeast Asia. Emerg Infect Dis 2001;7:996-1003.
3. Steffen R, Collard F, Tornieporth N, et al. Epidemiology, etiology, and impact of traveler's diarrhea in Jamaica. JAMA 1999;281:811-7.
4. Bouckenooghe AR, Jiang ZD, De La Cabada FJ, Ericsson CD, DuPont HL. Enterotoxigenic *Escherichia coli* as cause of diarrhea among Mexican adults and US travelers in Mexico. J Travel Med 2002;9:137-40.
5. Steffen R, Tornieporth N, Clemens SA, et al. Epidemiology of travelers' diarrhea: details of a global survey. J Travel Med 2004;11:231-7.
6. McKendrick M. Infectious diseases and the returning traveller--experience from a regional infectious diseases unit over 20 years. J Appl Microbiol 2003;94 Suppl:25S-30S.
7. Ericsson CD, DuPont HL, Mathewson IJ. Epidemiologic Observations on Diarrhea Developing in U.S. and Mexican Students Living in Guadalajara, Mexico. J Travel Med 1995;2:6-10.
8. Provost S, Soto JC. Perception and knowledge about some infectious diseases among travelers from Quebec, Canada. J Travel Med 2002;9:184-9.
9. An Advisory Committee Statement (ACS). Statement on travellers' diarrhea. Can Commun Dis Rep 2001;27:1-12.
10. Calgary Health Region. Traveller's diarrhea: http://yourhealth.calgaryhealthregion.ca/Categories.jsp, 2005.
11. Thomas T. Travelers' diarrhea. Top Emerg Med 2003;25:49-58.
12. Hennell SL, Brownsell C, Dawson JK. Development, validation and use of a patient knowledge questionnaire (PKQ) for patients with early rheumatoid arthritis. Rheumatology (Oxford) 2004;43:467-71.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

13. Haapala I, Probart C. Food safety knowledge, perceptions, and behaviors among middle school students. J Nutr Educ Behav 2004;36:71-6.
14. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32:331-51.
15. Juckett G. Prevention and treatment of traveler's diarrhea. Am Fam Physician 1999;60:119-24, 135-6.
16. Steffen R, Kollaritsch H, Fleischer K. Travelers' diarrhea in the new millennium: consensus among experts from German-speaking countries. J Travel Med 2003;10:38-45.
17. Duval B, De Serre G, Shadmani R, et al. A population-based comparison between travelers who consulted travel clinics and those who did not. J Travel Med 2003;10:4-10.
18. Hamer DH, Connor BA. Travel health knowledge, attitudes and practices among United States travelers. J Travel Med 2004;11:23-6.
19. Wilder-Smith A, Khairullah NS, Song JH, Chen CY, Torresi J. Travel health knowledge, attitudes and practices among Australasian travelers. J Travel Med 2004;11:9-15.
20. Choi BC, Pak AW. A catalog of biases in questionnaires. Prev Chronic Dis 2005;2:A13.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 6:

DISCUSSION AND CONCLUSION

Presently, there is a huge body of published scientific research on *Campylobacter* and the epidemiology of the infections these organisms cause, yet a vast number of questions remain unanswered and some unasked. This thesis touched on only a few aspects of *Campylobacter* infection and it succeeded in extending the body of research in a number of directions. There was a considerable amount of careful planning required in the development of the case-control study and the departure gate survey, and the use of the collected data to generate valid results was controlled and focused. Nevertheless, some of the new knowledge was not planned in advance of data collection, i.e. the new information did not emerge from the testing of specific hypotheses proposed during the development of the projects. Therefore, the scientific value of this thesis resulted from careful planning, thorough analytical investigations, and a measure of serendipity.

6.1 Novel Approaches to Risk Factor Analysis of Ciprofloxacin Resistance in *Campylobacter* Infections

As initially surmised prior to the development of this project, the measured level of ciprofloxacin resistance in southern Alberta, 28.8%, was relatively high, much higher than the level previously reported by other researchers in Alberta (1). Although this is unfortunate from a public health perspective, it provided us a sufficiently large case population in which to conduct risk factor analyses.

There are several case-control studies that have been published examining risk factors for fluoroquinolone resistance in *Campylobacter* infections (2-4), but the study in this thesis was the first to be conducted in Canada and the first to include potential risk exposures related to antibacterial product use and possession of antibiotics not prescribed for a current illness. Antibacterial use was one of the main hypothesized risk exposures for this project; however, this study did not show that the use of products such as toothpaste and dish soap with antibacterials increased the likelihood of ciprofloxacin resistance

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

among the *Campylobacter* strains of study participants. In retrospect, the quality of the antibacterial use data could be questionable. These are commonly purchased items and there is a plethora of toothpaste and dish soap products available to the consumer, so recall of brand details may not be accurate. Although public health inspectors reported some participants went to check on the items in their house during the interview, it cannot be assumed that level of dedication to accuracy among all participants, especially since they would have been on the phone for at least 10 minutes by the time they were asked this question. More specific questions regarding brand specifics could have generated data of higher quality (5).

I was surprised to find that after adjusting for the effect of foreign travel, possession of antibiotics for future use was a significant risk factor for resistance. This finding should generate more questions about this behaviour – how common is it in the wider population, and what motivates people to follow or ignore advice to complete all prescribed doses of antibiotics and to properly dispose of any unused doses? In the case-control study, possession of antibiotics was used as an indicator for the potential for self-medicating. Among those who have been directed by a physician to self-medicate and are fully compliant, self-medication may not increase risk of the development of resistance. However, self-directed misuse of antibiotics could, in theory, lead to the generation of resistant mutants in the host. In this study, participants were not directly ask about self-medication, and there is no research to suggest that these two behaviours are related, although there are reports of both occurring in sample populations (6-8). It would be interesting, although problematic on many fronts, to conduct further research on self-medication and the outcomes related to the reported use of stockpiled antibiotics.

It was not surprising to find foreign travel was the dominant risk factor for ciprofloxacin resistance; its effect has been reported consistently by others (2-4). The magnitude of the risk was impressive (adjusted odds ratio, 41.2), as was the difference between macro-regions. The risk associated with travel to Asia was much stronger than the risk associated with travel to Europe (adjusted odds ratios, 170.2 and 9.0, respectively), demonstrating wide geographic variability in risks. Among travel destinations, the trend

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

in risk appears to parallel the trend in fluoroquinolone resistance in *Campylobacter* isolated from humans and foods. Recent reports of resistance in strains isolated in Asia ranges from 84% to 97% in strains from humans (9, 10) and 88% in strains from foods (11). In Europe the range starts much lower, 4% to 82%, in strains from humans (12, 13), and 5% to 74% in strains from foods (14, 15), with decidedly higher levels reported from Spain.

Validation of analytical conclusions in published epidemiological work is often precluded by the absence of appropriate data. Furthermore, limitations to text length set by journals may lead authors to omit any validation efforts in manuscripts. Sensitivity analyses can provide readers with confidence regarding the stability of conclusions and disclosure of diagnostic test results give similar confidence that assumptions behind statistical methods have been checked by the authors.

Following the case-control study analysis, I was fortunate to have access to a partial database that permitted the examination of the effect of non-participation. This data provided evidence of a bias in the estimate of the effect of foreign travel on the risk of ciprofloxacin resistance among our sample that was directed away from null. The magnitude of the effect of participation bias was stronger than imagined prior to the analysis, and it should give other researchers reason to be cautious when presenting conclusions that could be similarly affected. Were it not for the strength of the estimated risk associated with travel and that of the calculated OR adjusted for the bias the conclusion with respect to the effect of travel would be questionable.

A sensitivity analysis was conducted to assess the potential for misclassification bias arising from the use of surrogate ciprofloxacin susceptibility testing in the Calgary Health Region, and the results indicated that misclassification had little effect and could not have biased the results substantially. Finally, results from diagnostic tests and sensitivity analyses of the effect of unbalanced sampling with respect to season were reported.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

6.2 Multi-factorial Causes of Prolonged Diarrhoea in *Campylobacter jejuni* Illness

Within the case-control study questionnaire were questions about the duration of diarrhoea and the antibiotic treatment received for the *Campylobacter* illness. The data generated led to questions about predictors of duration. Aside from background immune status, which the questionnaire did not address, strain virulence and disease treatments could play an important role in the duration of illness. One potential measure of virulence, the presence of the putative virulence plasmid, pVir, was considered in this study. The role of pVir in disease has been debated recently (16, 17), and the plasmid presence-absence test results of *C. jejuni* strains from Calgary participants obtained from the Public Health Agency of Canada Lethbridge Laboratory contributed to those arguments against a role for the plasmid in clinical disease. The observation that the presence of pVir was more common among travel-related strains than among domestic strains was unplanned, but interesting, and should be followed up by tests of greater sample size to look for more detailed geographic variability.

Without the controls of a clinical trial setting, an observational study to address the question of the effectiveness of antibiotic treatment on the duration of diarrhoea caused by bacterial infection is plagued by confounding variables. For many of the case-control study participants, confirmation of *Campylobacter* infection took place days, weeks, or months after the onset of symptoms, so it was unclear if early stages of all illness had a bacterial aetiology. In addition, treatment may have started early in the infection, during periods of peak antibody response, or when the immune system had largely controlled the infection.

Without an appropriate study design, there was no valid control population to the antibiotic-treated group. I did, however, test the hypothesis that the lag time to antibiotic treatment might be predictive of time to cessation of diarrhoea; that delayed treatment might lead to longer illness. Although there was considerable variation in lag time to treat (range from 0 to >20 days), most diarrhoea stopped within five days. There appears, then, to be no advantage to empirical treatment of *Campylobacter*-associated diarrhoea, and this study's findings support warnings against the empirical treatment of bacterial

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

infection designed to control levels of antibiotic resistance among bacteria in the community (18).

A questionnaire designed to test predictors of duration of diarrhoea should include questions on the use of anti-diarrhoea medications. Unfortunately, this secondary analysis was not anticipated during the case-control study design stage; thus, data on this topic was not collected. A group of researchers primarily from the CDC found ciprofloxacin resistance was predictive of duration of diarrhoea when their multi-variable model adjusted for travel and anti-diarrhoea medication use (19). In my sample population, ciprofloxacin resistance was not predictive, but duration was longer among those who recently travelled in the sample population, which is in agreement with the view of Cox *et al* (20). The use of anti-diarrhoea medication is probably more common among travellers than among those at home, since the value of travel time is high. It seems reasonable, then, to assume that the effect on diarrhoea duration is greater among travellers than among domestically-infected, and if this effect was controlled, the duration of travel-related infections would be even longer than domestic infections.

Unexpectedly, shorter term illnesses, those less than one week in duration, were not affected by the geographic source of infective strain. It is likely that this is partly due to the low proportion of travel-related infections in this sub-sample (15.6%, n = 64). Other variables such as immune response factors and anti-diarrhoea medication use could also be responsible. As with the case-control study, the results here proffer as many questions as answers. This line of questioning should be further pursued with research involving detailed strain assays to incorporate strain variation into the puzzle, particularly in very prolonged cases of diarrhoea.

6.3 The Education of Travellers about Travellers' Diarrhoea

It was apparent from preliminary analyses of the case-control data that foreign travel was likely to present considerable influence on the characteristics of *Campylobacter* strains from study participants and the outcomes of their infections. From a public health perspective, the question that arose from this observation was "What can be done to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reduce these effects?" Short of restricting travel to outside of Canada and the U.S.A, a very reasonable approach is the reduction of infection through education of travellers on the risks of infection. Much of the focus of travel health information available from various travel-related internet sources is on vaccination requirements and dangers of toxic fauna found in common travel destinations. It seems that the topic of travellers' diarrhoea may be slightly offensive to potential travel clients and it is, therefore, not drawn to the attention of those planning a vacation to a "sun destination". The expectation, during the development of the departure gate survey was, then, that not many travellers would be armed with information about the prevention of travellers' diarrhoea and the level of knowledge on this topic would be poor. I was happy to report that, from my brief cross-sectional sampling of the study population, these expectations were exceeded, although some gaps in knowledge remained.

This study, it should be noted, was not a detailed examination of the effectiveness of education sources, which would require pre and post-testing; nevertheless, some points were worth reporting. Overall knowledge of food risks among travellers was good, and the proportion of people who reported seeking information on travellers' diarrhoea was higher than expected. It was also satisfying to find in this small study that it appeared that information-seekers were more knowledgeable than their counterparts. In retrospect, it would have been helpful to include more questions on preventive measures, such as the prophylactic use of bismuth subsalicylate, and then to provide answers to the travellers, informing them of less-commonly known measures.

Word-of-mouth and the internet were two important sources of information for our sample population. This raises concerns about the quality of information gained; both sources are prone to inconsistency and superficiality. Ideally, travel clients should be given brochures or information about how to find online information from reputable sources (health regions, Health Canada, CDC, and the World Health Organization) by sellers of travel packages. This would ensure a broad dissemination of comprehensive information to those who will be immediately at risk.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

6.4 Last Thoughts

Overall, these thesis projects were both challenging and revealing. Many design hypotheses were tested, some developed into more detailed questions or questions from different perspectives, and some observations emerged independent of initial hypotheses. During the many iterations of the analyses not reported here, were revealed the importance of ensuring that the analyses were focused on the important questions at hand and that the data used truly answered the specific questions. Once the appropriateness of the analyses and data were ensured, the results demonstrated the complex, and sometimes, hidden stories behind the likelihood of ciprofloxacin resistance in *Campylobacter* infections, the prolongation of diarrhoea caused by these infections, and the measures that can prevent them.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

6.5 References

1. Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, Taylor DE. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance. Antimicrob Agents Chemother 2004;48:3442-50.
2. Campylobacter Sentinel Surveillance Scheme Collaborators. Ciprofloxacin resistance in *Campylobacter jejuni*: case-case analysis as a tool for elucidating risks at home and abroad. J Antimicrob Chemother 2002;50:561-8.
3. Kassenborg HD, Smith KE, Vugia DJ, et al. Fluoroquinolone-resistant *Campylobacter* infections: eating poultry outside of the home and foreign travel are risk factors. Clin Infect Dis 2004;38 Suppl 3:S279-84.
4. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N Engl J Med 1999;340:1525-32.
5. Kimmel SE, Lewis JD, Jaskowiak J, Kishel L, Hennessy S. Enhancement of medication recall using medication pictures and lists in telephone interviews. Pharmacoepidemiol Drug Saf 2003;12:1-8.
6. Pechere JC. Patients' interviews and misuse of antibiotics. Clin Infect Dis 2001;33 Suppl 3:S170-3.
7. Richman PB, Garra G, Eskin B, Nashed AH, Cody R. Oral antibiotic use without consulting a physician: a survey of ED patients. Am J Emerg Med 2001;19:57-60.
8. Erramouspe J, Adamcik BA, Carlson RK. Veterinarian perception of the intentional misuse of veterinary medications in humans: a preliminary survey of Idaho-licensed practitioners. J Rural Health 2002;18:311-8.
9. Bodhidatta L, Vithayasai N, Eimpokalarp B, Pitarangsi C, Serichantalergs O, Isenbarger DW. Bacterial enteric pathogens in children with acute dysentery in Thailand: increasing importance of quinolone-resistant *Campylobacter*. Southeast Asian J Trop Med Public Health 2002;33:752-7.
10. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin Infect Dis 1998;26:341-5.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

11. Kang YS, Cho YS, Yoon SK, et al. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw chicken meat and human stools in Korea. J Food Prot 2006;69:2915-23.
12. Osterlund A, Hermann M, Kahlmeter G. Antibiotic resistance among *Campylobacter jejuni/coli* strains acquired in Sweden and abroad: a longitudinal study. Scand J Infect Dis 2003;35:478-81.
13. Prats G, Mirelis B, Llovet T, Munoz C, Miro E, Navarro F. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. Antimicrob Agents Chemother 2000;44:1140-5.
14. Moore JE, Crowe M, Heaney N, Crothers E. Antibiotic resistance in *Campylobacter* spp. isolated from human faeces (1980-2000) and foods (1997-2000) in Northern Ireland: an update. J Antimicrob Chemother 2001;48:455-7.
15. Saenz Y, Zarazaga M, Lantero M, Gastanares MJ, Baquero F, Torres C. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. Antimicrob Agents Chemother 2000;44:267-71.
16. Tracz DM, Keelan M, Ahmed-Bentley J, Gibreel A, Kowalewska-Grochowska K, Taylor DE. pVir and bloody diarrhea in *Campylobacter jejuni* enteritis. Emerg Infect Dis 2005;11:838-43.
17. Louwen RP, van Belkum A, Wagenaar JA, Doorduyn Y, Achterberg R, Endtz HP. Lack of association between the presence of the pVir plasmid and bloody diarrhea in *Campylobacter jejuni* enteritis. J Clin Microbiol 2006;44:1867-8.
18. DuPont HL. Travellers' diarrhoea: contemporary approaches to therapy and prevention. Drugs 2006;66:303-14.
19. Nelson JM, Smith KE, Vugia DJ, et al. Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. J Infect Dis 2004;190:1150-7.
20. Cox LA, Jr., Copeland D, Vaughn M. Ciprofloxacin resistance does not affect duration of domestically acquired campylobacteriosis. J Infect Dis 2005;191:1565-6; author reply 1566-7.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

APPENDIX 1:

QUESTIONNAIRE FOR CASE-CONTROL STUDY OF CIPROFLOXACIN RESISTANCE IN *CAMPYLOBACTER* INFECTIONS

Date:

1) Have you completed this questionnaire before for a previous illness? *If the respondent answered yes, do not continue the questionnaire with this person.*

2) What is your age? *If the respondent is less than 16 years of age, do not continue the questionnaire with this person.*

3) Are you answering this questionnaire on behalf of someone else? *If the respondent answered yes, all further questions will refer to the patient, not the respondent.*

4) Are you male or female?

5) Are you fluent in English?

6) What is your highest education level?

7) What is your current occupation/job(s)?

8) Does your current occupation/job(s) require you to handle animals?

In the following section on food consumption think about the meals you usually have for breakfast, lunch, dinner, and snacks. Keep in mind that if you have 3 or 4 meals each day, that's approximately 40 to 60 meals over a 2-week period. On average, how many meals do you have during a typical 2-week period that contain:

9) Chicken

10) Turkey

11) Beef

12) Pork

13) Luncheon meat, deli meat, or sausage

14) Shellfish (For example: crab, shrimp, clams, oysters)

15) On average, how many times in a 2-week period do you eat at restaurants or eat take-out foods?

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

16) In the past month did you eat at a place where meals are provided free of charge to members of the public? (For example: group luncheons, meetings, weddings, soup kitchens)

17) In the past month did you drink unpasteurized milk?

18) In the past month did you drink water that was not treated? (For example: well or dugout water, stream, lake, or ocean water)

19) Do you drink municipal water or bottled water?

In the following section, think about the pets and livestock with which you come in contact.

In the past month have you come in contact with animal manure or cleaned up animal faeces of:

20) Pet birds

21) Dogs

22) Cats

23) Cows or cattle

24) Pigs

25) Chickens or turkey

26) In the past month have you given an antibiotic, directly or through feed or water, to an animal? *If the patient answered yes to question 26 ask him/her* :

26.1) In what form was the antibiotic(s) given? (For example, injection, feed, water)

26.2) What is/are the name(s) of the antibiotic(s)?

26.3) What type of animal did you give it to?

26.4) Over what time period was it given?

In the next section, think about your recent diarrhoeal illness that you've just seen your doctor for.

27) How many days ago did you first notice symptoms of your recent illness?

28) Have the symptoms of your recent illness stopped? *If the patient answered yes to question 28 ask him/her*:

28.1) How many days ago did the symptoms stop?

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

29) Has your doctor prescribed an antibiotic for your recent illness? *If the patient answered yes to question 29 ask him/her*:

29.1) How many days ago did you start taking it?

29.2) What is the name of the antibiotic?

29.3) Did you start taking the antibiotic before submitting a stool sample?

29.4) Was the antibiotic prescribed by a doctor in Canada?

In this final section I'll ask you some questions about your recent past.

30) In the past month did you travel outside of Canada or the United States? *If the patient answered yes to question 30, read questions 30.1 through 30.3 for each country visited.*

30.1) What country did you travel to?

30.2) What day did you arrive?

30.3) What day did you depart?

31) Other than during your recent illness, have you had diarrhoea in the past 2 months?

32) Not including any antibiotics for your recent illness, have you taken any antibiotics within the past month? *If the patient answered yes to question 32, read questions 32.1 through 32.8 for each antibiotic taken.*

32.1) If the antibiotic was taken to treat a disease or condition, what was it?

32.2) Was the antibiotic taken to prevent infection following surgery?

32.3) Was the antibiotic taken to prevent illness during travel?

32.4) What was the name of the antibiotic?

32.5) How many days did you take it?

32.6) How many days ago did you stop taking it?

32.7) Was this prescribed by your doctor?

32.8) Was it prescribed for you?

33) Among the people in your family or household, have any of them been taking antibiotics in the past month?

34) In the past month have you handled antibiotics for someone other than yourself?

35) Do you have any antibiotics that you would use for future illness?

36) In the past month have you used an antacid?

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

37) In the past month have you or someone in your household used dishwashing liquid that is labelled as antibacterial?

38) In the past month have you used mouthwash that is labelled as antibacterial?

39) In the past month have you used one of the following toothpastes: Crest Tartar Protection ®, Colgate Total ®, or Colgate Cavity Protection ®? *If the patient answered yes to question 39 ask him/her:*

39.1) Which brand of toothpaste did you use?

40) Do you live more than 8 kilometres or 5 miles (a 10-minute drive) from the nearest pharmacy?

41) Were you given the results of the antibiotic-resistance testing done on the stool sample you submitted for your recent illness?

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

APPENDIX 2:

QUESTIONNAIRE FOR DEPARTURE GATE SURVEY OF TRAVELLERS' DIARRHOEA KNOWLEDGE

Please check √ the boxes below, then return this questionnaire to survey staff.

Section A: General information

Did you receive information on how to prevent travel-related disease before your trip?

- ☐ Yes
- ☐ No Skip to Q4
- ☐ Don't know Skip to Q4

From which of the following sources did you receive information?

- ☐ Friends, family
- ☐ Nurse, physician, pharmacist
- ☐ Internet
- ☐ Travel agent
- ☐ Travel clinic
- ☐ Other (specify)

Did the information you received include ways to prevent and treat diarrhea?

- ☐ Yes
- ☐ No
- ☐ Don't know

Are you carrying an antibiotic or a prescription for an antibiotic with you on this trip?

- ☐ Yes
- ☐ No Skip to Q6
- ☐ Don't know Skip to Q6

What is the name of the antibiotic you are carrying?

Section B: Travelers' diarrhea

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Is washing your hands with a hand sanitizer or soap and water effective in preventing travelers' diarrhea?

- ☐ Yes
- ☐ No
- ☐ Don't know

Are ice cubes safe to have in a beverage?

- ☐ Yes
- ☐ No
- ☐ Don't know

Do chlorine tablets in water make it safe to drink?

- ☐ Yes
- ☐ No
- ☐ Don't know

Is it true that if you have travelers' diarrhea with bloody stools you should visit a doctor as soon as possible?

- ☐ Yes
- ☐ No
- ☐ Don't know

Which of the following are symptoms of traveler's diarrhea illness?

	Yes	No	Don't know
Abdominal cramps			
Dizziness			
Headache			
Nausea			

Which of the following can cause travelers' diarrhea? (Check as many boxes as you feel apply)

- ☐ Virus
- ☐ Bacteria
- ☐ Fungus

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

For people traveling to developing countries, how high is the risk of getting diarrhea from eating or drinking the following?

Risk	Salad	Beer	Beef, partially cooked	Chicken, partially cooked	Bottled pop
Moderate to high					
Low to none					
Don't know					

If you had to take an antibiotic to treat travelers' diarrhea, which one of the following is true?

- ☐ You can stop taking antibiotics if your diarrhea stops after one day.
- ☐ You should avoid using a product like Imodium while on antibiotics.
- ☐ The duration of your travelers' diarrhea will likely be reduced if you take ciprofloxacin.
- ☐ All travelers, including you, should take an antibiotic to prevent travelers' diarrhea.
- ☐ Don't know

Some people believe that Canadians have a high risk of getting travelers' diarrhea during travel to developing countries. Others believe this risk is very low. Do you agree or disagree that you have a greater risk of getting diarrhea while on your trip than when not traveling?

- ☐ Strongly agree
- ☐ Moderately agree
- ☐ Neutral
- ☐ Moderately disagree
- ☐ Strongly disagree

Section C: Information about yourself

Year of birth

Gender:

Highest level of education:

- ☐ Elementary school

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

- ☐ High school
- ☐ Technical school
- ☐ College
- ☐ University

Thank you for your time

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.