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A Descriptive Epidemiologic Study of Campylobacteriosis in East Tennessee

Karissa Laughter
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Karissa Laughter entitled "A Descriptive Epidemiologic Study of Campylobacteriosis in East Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

Agricola Odoi, Major Professor

We have read this thesis and recommend its acceptance:

Barton Rohrbach, Arnold Saxton, Karla Matteson

Accepted for the Council:
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A Descriptive Epidemiologic Study of Campylobacteriosis in East Tennessee

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Karissa Laughter
December 2008

Dedication

This thesis is dedicated to my husband, Mark Laughter, whose love, support, advice, assistance, patience, editing skills and superior grammar have aided me greatly in my pursuit of this degree.

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I sincerely thank and acknowledge the many people who helped me complete this thesis project. I would like to thank my co-advisors Professors Agricola Odoi and Barton Rohrbach for their knowledge, guidance and encouragement throughout my time at UT. I also want to thank the other members of my committee Professors Karla Matteson and Arnold Saxton for their willingness to serve on my committee and their professional and academic support. Thanks are also due to the East Tennessee Regional and Knox County Health Departments, specifically Dr. Lorinda Sheeler and Dr. Kathleen Brown, for providing the data used in this study and working with me to help me understand the data. I would also like to thank my husband, Mark Laughter, and my parents, Don and Mary Patterson and all my family and friends for their constant support. I must also acknowledge the support and love of my Lord Jesus Christ, who makes all things possible.

Abstract

Campylobacteriosis is caused by the gram-negative bacteria *Campylobacter* and is a leading cause of gastrointestinal illness worldwide. In the United States an estimated 2.4 million cases occur annually with approximately $8.0 billion in associated costs. Due to the high cost of morbidity, understanding the epidemiology and risk factors of campylobacteriosis is important. It is unclear if the prevalence of campylobacteriosis is higher or lower in East Tennessee than other parts of the state or country or if the clinical characteristics of patients in the area are similar to the rest of the country. Therefore, the purpose of the study was to describe clinical and epidemiological characteristics of campylobacteriosis patients in East Tennessee to assist in health planning to control campylobacteriosis. Data from the Foodborne Disease Active Surveillance Network was analyzed for 2003-2006 in 16 counties in East Tennessee. The data was first assessed for its quality, then descriptive statistics were calculated and spatial and temporal patterns of reported cases and risk factors were assessed. The overall error rate in the data quality analysis was 6.5% although in the last year of the study it was only 2.6%. The mean annual prevalence of campylobacteriosis in East Tennessee was 10.4 cases per 100,000 population, which was 1.6 times higher than all of Tennessee (7.4 cases/100,000). Grainger and Jefferson Counties had higher age- and sex-adjusted prevalence estimates than the region and nation. It is yet unclear why this region has a higher prevalence of campylobacteriosis than the rest of the nation. The highest age-specific prevalence (41.6 cases/100,000) was observed in children under 5. Disease prevalence was consistently higher in the summer months compared to the other seasons. The median age of patients

was lower in the most rural counties. More patients in East Tennessee were hospitalized than the rest of the nation. The most commonly reported risk factors were animal and raw meat exposure. Improvement in data collection and entry is necessary to improve the quality and application of this surveillance data. Educational efforts on proper hygiene following animal handling, and proper well protection and disinfection should be targeted at high risk groups.

Preface

Unless otherwise specified, *Campylobacter* and *Campylobacter* spp. refer to *C. jejuni* and *C. coli*, the most common species identified in human disease. Tables and figures follow the page on which they are first referenced.

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List of Abbreviations

CCD	Census County Division
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CPR	Computer-based Patient Records
ER	Emergency Room
ETRHD	East Tennessee Regional Health Department
FoodNet	Foodborne Diseases Active Surveillance Network
GBS	Guillain-Barré Syndrome
GIS	Geographic Information Systems
GPS	Global Positioning System
GSS	Global Salmonella-Survey
HLA B27	Human Leukocyte Antigen B*27
IRB	Internal Review Board
KCHD	Knox County Health Department
LOS	Length of Stay
LPS	Lipopolysaccharide
NARMS	National Antimicrobial Resistance Monitoring System
NEDSS	National Electronic Disease Surveillance System
NGO	Non-Governmental Organization
PHLIS	Public Health Laboratory Information System
TIGER	Topologically Integrated Geographic Encoding and Referencing
US	United States
USDA	United States Department of Agriculture
WHO	World Health Organization

1.0 Introduction

Campylobacteriosis is caused by the gram-negative bacteria *Campylobacter.* Members of the *Campylobacter* species are small, non-spore forming, gram-negative bacteria that have a characteristic curved, S-shape or spiral morphology[1]. They were first observed and described in 1886 but were first cultured in 1977[2]. *Campylobacter jejuni* and *Campylobacter coli* exhibit a similar clinical course of enteric disease and are therefore often grouped together in descriptions of disease characteristics. An estimated 2.4 million people suffer from campylobacteriosis each year in the United States; 13,000 of these patients require hospitalization and 124 die[3]. The average patient misses 3.8 days of work or school due to campylobacteriosis[4], but chronic sequelae can increase this number. It is estimated that campylobacteriosis costs $8 billion a year in the United States[5], from lost wages and medical expenses related to the primary infection and any secondary complications.

Of all human *Campylobacter* infections, it is estimated that 85 to 99% are caused by *C. jejuni* and 5 to 10% by *C. coli* with *C. fetus* making up the remainder[6, 7]. Infection caused by *C. jejuni or C. coli* results in acute enteritis with clinical courses that are hard to differentiate from those of other bacterial pathogens that cause acute gastrointestinal infections such as *Salmonella* or *Escherichia coli*. Common symptoms are diarrhea (which may be bloody), nausea, fever, headache, myalgia and vomiting[8]. Most cases are self-limiting but complications can occur. These include reactive arthritis, or Guillain-Barré syndrome (observed in 1 out of 1000 campylobacteriosis patients)[9]. *Campylobacter fetus* rarely causes enteritis although it has been isolated in systemic

blood infections. It induces fever and can disseminate to numerous tissues such as the vascular endothelium, bones, and joints. Complications of *C. fetus* infection may include meningitis, endocarditis, pneumonia, thrombophlebitis, septicemia, arthritis, and peritonitis[10].

Commonly identified risk factors for human infection with *C. jejuni* and *C. coli* are chicken consumption or handling, international travel and animal exposure[11-14]. In all exposure cases, the bacteria must be ingested in some form. *Campylobacter* spp. are zoonotic and also infect household pets such as dogs and cats as well as livestock such as poultry and cattle and wild animals as well. Many animals remain asymptomatic while infected but are still able to shed the bacteria in their feces. Due to this, poor hygiene following any animal contact may result in infection. Water can become contaminated by fecal run off which is also an important source of infection[15].

Campylobacter is the most common bacterial cause of diarrhea in the United States[3] and many other developed countries[16, 17]. The overall reported incidence varies worldwide, depending on the reporting practices, healthcare systems, risk factor distributions and hygiene levels in each country. The reported incidence of campylobacteriosis ranges from 300-396 per 100,000 in New Zealand[18, 19] to 12.7 per 100,000 in the United States[20]. These incidence figures only include the patients who sought medical care for their illness and on whom laboratory tests were conducted. Due to this under-reporting, the true incidence of campylobacteriosis is most likely higher than the figures reported in the United States and other countries worldwide.

The state of Tennessee belongs to the Foodborne Active Surveillance Network (FoodNet) which conducts active surveillance for all foodborne pathogens, including

Campylobacter. Under the active surveillance, every positive laboratory report for *Campylobacter* must be reported to the health department. Additionally the health departments also conduct routine reviews of all the diagnostic laboratories in their jurisdiction to ensure all cases that are detected are reported in a timely fashion. After a case is identified, a health department representative contacts the patient to collect additional data.

The incidence of campylobacteriosis in Tennessee is 7.4 per 100,000[20], but public health officials in East Tennessee (16 counties in the eastern portion of the state) have noticed that the incidence in this region appears to be higher[21] than the rest of the state of Tennessee. It is unclear if the incidence in East Tennessee is truly higher than the rest of the state, or if there is a reason for the increased prevalence in the region. It is also unclear if the clinical characteristics of the patients in this region are similar to the rest of the country. This study is designed to determine the characteristics and risk factors among the cases of campylobacteriosis reported in East Tennessee. Understanding the disease characteristics specific to the region can help to improve disease control and prevention strategies. Demographic characteristics of reported cases will be assessed to identify high risk groups. The most commonly reported risk factors and clinical symptoms reported by the patients will be identified. Geographical and temporal patterns and any association between geographic location, risk factors, clinical symptoms and socioeconomic determinants of health will be investigated. By understanding the most common risk factors in the study area, the regional and local health departments will be able to better implement health programs to reduce future disease occurrence.

2.0 Literature Review

2.1 Etiology

The genus *Campylobacter* is a member of the division *Proteobacteria*, class *Epsilobacteria,* order *Campylobacterales,* family *Campylobacteraceae*[22]. This family is made up of gram-negative bacteria that are primarily commensals or parasites of humans, domestic animals[23], and wild mammals.

Members of the *Campylobacter* species are small, non-spore forming, gram-negative bacteria that have a characteristic curved, S-shape or spiral morphology[1] They are 0.2 to 0.9 μm wide and 0.5 to 5.0μm long with one or more spirals. Cells have a single polar unsheathed flagellum, at one or both ends, that is used for motility[24]. Some species, such as *C. gracilis* are non-motile. The flagella may be up to 2-3 times the length of the cells[22]. *Campylobacters* were first observed and described in 1886 by Theodor Escherich as non-culturable spiral-shaped bacteria that were isolated from the intestinal contents of 16 of 17 children who died of diarrheal disease[25]. Escherich observed what he described as a "vibrionen" numerous times in the feces of human neonates and kittens with diarrhea[26]. While it is not certain that what Escherich described were indeed *Campylobacters,* their morphology, failure to grow on solid media, and association with enteric illness all seem to suggest that they were indeed *Campylobacters.* In 1906, McFadyean and Stockman isolated an unknown spiral bacteria from sheep experiencing epizootic abortions [27]. Due to their curved, *Vibrio*-like appearance (similar to members of the genus *Vibrio*), the bacteria were given the name

Vibrio fetus in 1919 by Theobold Smith who was investigating infectious abortions in cattle[28]. Another species of *Vibrio* was identified in 1931 by F.S. Jones who was investigating infectious diarrhea in cattle, he named it *Vibrio jejuni* [29]. In 1963, Sebald and Véron noted that *V. fetus* and *V. jejuni* differed from the classical cholera *Vibrios*. They discovered that *V. fetus* and *V. jejuni* have a lower guanine plus cytosine (G plus C) DNA content compared to other *Vibrios*, and are unable to ferment carbohydrates[30]. Due to these differences, a new genus *Campylobacter* (meaning "a curved rod") was created.

Campylobacter species are considered microaerophillic because they grow best in atmosphere that contains only 3-15% oxygen, although some will grow under aerobic or anaerobic conditions, when necessary[22]. For energy, *Campylobacters* use amino acids or tricarboxylic acid cycle intermediates, such as α-ketoglutarate, succinate or fumarate. However all strains are not able to metabolize the same carbon sources[31]. All *Campylobacter* species will grow at 35 - 37°C but thermophillic species (*C. jejuni C. coli, C. lari* and *C. upsaliensis*) grow best at 42°C[24]. Table 2.1 shows all species of *Campylobacter* and their phenotypic characteristics[32].

Species that are important in human infections are *C. jejuni, C. coli, C. fetus,* and to a lesser degree *C. lari* and *C. upsaliensis.* The primary species identified in gastrointestinal illness are *C. jejuni* and *C. coli*; they are responsible for an estimated 90-99% and 5-10%, respectively, of human *Campylobacter* infections.

C. fetus can be broken down into two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*. *C. fetus* subsp. *fetus* primarily causes abortion in sheep and

Table 2.1: Phenotypic characteristics differentiating *Campylobacter* species

Species	Catalase	Nitrate reduction	Nitrite reduction	H_2S production (TSI)*	Hippurate hydrolysis	Indoxyl acetate hydrolysis	Growth at : 25 ℃	Growth at : 42 ℃	Growth in 1% glycine	Alkaline phosphatase	Susceptibility to: NA†	Susceptibility to: C	GC‡ content (mol %)
Campylobacter coli	+	+	-	-	-	+	-	+	+	v	S	R	30+33
Campylobacter concisus	-	+	+	+	+	-	-	+	+	v	R	R	37+41
Campylobacter curvus	-	+	+	+	-	+	-	+	+	ND	S	ND	45+46
Campylobacter fetus subsp. *fetus*	+	+	-	-	-	-	+	-	+	-	R	S	33+35
Campylobacter fetus subsp. *venerealis*	+	+	-	-	-	-	+	-	-	-	R	S	33+34
Campylobacter gracilis	-	+	+	ND	ND	ND	ND	ND	ND	ND	R	ND	44+46
Campylobacter helveticus	-	+	ND	-	-	+	-	+	+	-	S	S	34
Campylobacter hyoilei (*C. coli*)	+	+	+	+	-	ND	ND	v	+	ND	S	R	35
Campylobacter hyointestinalis subsp. *hyointestinalis*	+	+	-	+	-	-	v	+	+	v	R	S	33+36
Campylobacter jejuni subsp. *doylei*	+	-	-	-	v	+	-	-	+	+	S	S	30+31
Campylobacter jejuni subsp. *jejuni*	+	+	-	-	+	+	-	+	+	+	S	R	30+33
Campylobacter lanienae	+	+	+	-	-	-	-	+	-	+	R	R	36
Campylobacter lari	+	+	-	-	-	-	-	+	+	-	R	R	30+32
Campylobacter mucosalis	-	+	+	+	-	-	-	+	+	v	R	S	36+38
Campylobacter rectus	-	+	+	+	-	+	-	w	+	ND	S	ND	45+46
Campylobacter showae	+	+	+	+	-	+	-	+	v	-	R	S	44+46
Campylobacter sputorum bv. bubulus	-	+	+	+	-	-	-	+	+	-	R	S	29+30
Campylobacter sputorum bv. fecalis	+	+	+	+	-	-	-	+	+	v	R	S	30+32
Campylobacter sputorum bv. sputorum	-	+	+	+	-	-	-	+	+	ND	S	S	30+31
Campylobacter upsaliensis	w/-	+	-	-	-	+	-	+	v	v	S	S	32+36

*TSI: Triple Sugar Iron test, † NA: nalidixic acid; C: cephalothin, ‡GC: Guanine-Cytosine content
Test results: +, positive reaction; -, negative reaction; w, weak reaction; v, variable reaction; R, resistant; S, sensitive; ND, not determined
Table adopted from: Logan, J.M., et al., *Campylobacter lanienae sp. nov., a new species isolated from workers in an abattoir.* Int J Syst Evol Microbiol, 2000. **50 Pt 2**: p. 865-72.

sporadically in cattle, but has also been isolated from human aborted fetuses in rare occasions. In humans, *C. fetus* subsp. *fetus* causes systemic infection and occasionally gastrointestinal infections[22]. *C. fetus* subsp. *venerealis* can cause abortion and infertility in cattle. Unlike *C. fetus* subsp. *fetus, C. fetus* subsp. *venerealis* is unable to multiply in the intestinal tract of humans and other animals[33].

C. upsaliensis has frequently been isolated from canine and feline fecal specimens of both healthy and diarrheic animals[34-37]. It is classified as thermotolerant since only 80% of isolates tested have been shown to grow at 42°C[38, 39], unlike the thermophillic species where all strains can grow at 42°C. *C. upsaliensis* has been isolated from human fecal samples but infection with this species appears to cause less vomiting and nausea[39]. Unlike other *Campylobacter* species that cause enteric infections, *C. upsaliensis* has been associated with hemolytic uremic syndrome, a serious complication affecting the kidneys[40]. Since *C. upsaliensis* is sensitive to the antibiotics used in selective media for the routine culture of *C. jejuni* and *C. coli,* other techniques must be used to isolate this species. *C. upsaliensis* can be isolated by filter techniques, but these methods are not routine in most diagnostics laboratories and are usually not requested by the physician ordering the stool culture. Due to the decreased ability to isolate the bacteria, the burden of human disease from *C. upsaliensis* has been difficult to estimate and is currently unknown[41].

C. lari has been isolated from the intestines of shore birds in Sweden[42], water samples in New Zealand[43], shellfish in the Netherlands [44], and pigs in Texas[45]. It has been also been identified as a cause of bacteremia in humans[46, 47] but the true burden of disease is unknown. *C. lari* was originally described with the phenotypic

characteristic of nalidixic acid resistance, but several strains have been isolated that are nalidixic acid susceptible. This is important since the phenotypic response to nalidixic acid is often used to differentiate between species and this could lead to misidentification of species. The nalidixic acid resistance is also important because nalidixic acid is the basis for the quinolone class antibiotics and fluoroquinolones are often the antibiotic of choice for treating *Campylobacter* infections[48, 49]. Although the current burden of disease from *lari* is low, more campylobacteriosis treatment failures could occur if the burden of disease due to *C. lari* increases and the empiric treatment of campylobacteriosis with fluoroquinolones is continued.

2.2 *Campylobacter* Survival in the Environment

In order to survive long enough to be passed from one host to another, *Campylobacters* must endure stress factors such as exposure to oxygen, lower temperatures and desiccation. As such they have developed mechanisms to survive in different environments until the bacteria can find a new host to colonize.

One survival mechanism involves the ability to enter a viable but nonculturable (VBNC) state[50]. *Campylobacter* cells transform from a motile spiral form to a coccoid form when they are subjected to unfavorable conditions such as low nutrient availability or incubation at temperatures outside the optimum growth temperature[51]. During this state, *Campylobacter* does not undergo DNA replication or protein synthesis[52-54], but it is believed that proteins are synthesized when the cells initiate the VBNC state[55]. While experimental results are not always comparable (most likely due to strain

variations) VBNC *Campylobacter* cells have been resuscitated in animals in the laboratory[56-59], from aquatic environments[60], and after treatment with acid[61]. Cells can be resuscitated up to 30 days after entering the VBNC state[60].

The formation of biofilms is another technique by which *Campylobacter* spp. can survive in extreme conditions. A biofilm is composed of microcolonies of organisms, including bacteria, fungi, and protozoa, bound together by an extracellular matrix that provides a microenvironment separate from outside low nutrient and hostile conditions[62]. Biofilms are widely distributed in the environment and occur in most public water supplies and plumbing systems[63]. *C. jejuni* has been isolated from biofilms in aquatic environments and on stainless steel[63, 64]. Depending on the incubation temperature and microbial make up of the biofilm, *C. jejuni* has been documented to survive for 42 days in that environment[63]. Lower temperatures (4°C instead of 22°C) have been shown to lengthen the time that *Campylobacter* spp. can survive in aqueous environments[63].

Campylobacter spp. has been isolated from surface water sources in the USA[65], UK[66, 67] and New Zealand[68]. The presence of *Campylobacter* in these water sources may be due to fecal run off. Studies have shown that *C. jejuni* is capable of surviving in manure spread on grass for up to 63 days depending on the type of manure and animal source[69]. The longest *Campylobacter* survival time was recorded for dairy cattle slurry or liquid waste and beef cattle solid waste. Pig slurry and sheep solid waste had the shortest survival times at only 16 days, while *Campylobacter* survived in poultry solid waste was 42 days. Another study found that when the solid manure is stored in heaps the survival time is decreased to 2-4 days due to the heat in the center of the

heap[70]. The same study also showed that *Campylobacter* survived about half as long when spread on sandy soil compared to clay soil.

It has been estimated that up to 80% of retail chickens for sale in the United States are contaminated with *Campylobacter* spp.[71]. The meat most likely becomes contaminated after evisceration in the poultry processing plant due to cross contamination. *C. jejuni* have been reported to have better survival rates on poultry meat with the skin intact compared with skinless meat[72]. Under normal refrigeration conditions, all meat types contained enough bacteria for an infectious dose for humans[72].

It has also been shown that *Campylobacter* spp. can be isolated from kitchen surfaces even after cleaning with detergent and hot water[73]. Authors of one study were able to isolate viable bacterial cells from beech and polypropylene cutting boards up to 2 hours after inoculation[74]. The ability of *C. jejuni* to survive even a few hours on kitchen surfaces provides ample time for cross contamination to other foods. It has also been shown that *C. jejuni* is able to survive for 24 hours on strawberries stored at room temperature and for 72 hours on cantaloupe stored at 7°C[75].

2.3 Clinical Course

Infection with *Campylobacter* spp. can result in several clinical manifestations. Human volunteer studies have shown that asymptomatic infections occur[76]. The most common clinical manifestation is enteric, resulting in acute diarrheal disease. Enteric infections can sometimes lead to systemic infections if the bacteria invade the intestinal

cells and cross into the bloodstream. Systemic infection is characterized by fever and joint pain[10]. Post infection complications of enteric illness include Guillain-Barré syndrome (GBS) and reactive arthritis[9].

2.3.1 Enteric Infections

Campylobacter infection causes acute diarrheal disease with clinical symptoms that are similar to those of other bacterial pathogens that cause acute gastrointestinal infections. The most common presentation is acute enteritis. Symptoms caused by *C. jejuni* and *C. coli* usually start with abrupt cramping pain in the abdomen that is quickly followed by diarrhea. Approximately 30% of the patients experience a prodrome of fever, headache, myalgia, dizziness, anorexia or malaise prior to the onset of diarrhea[1, 11, 77]. The prodromal symptoms appear 12-24 hours prior to intestinal symptoms and this has been shown to indicate a more severe clinical course compared to patients who experience diarrhea first [78]. Diarrhea can vary from loose stools to stools that are profuse and watery, bloody, bile stained or slimy. In descriptive reports of outbreaks, the most frequently reported symptoms are diarrhea and abdominal pain. In Denmark, 95% of patients reported diarrhea and 86% reported abdominal pain[79]. Similarly, in two different outbreaks in Spain (2001 and 2003), diarrhea was reported by 100% and 93.6% of afflicted patients and abdominal pain was reported by 62.5% and 89.6% of patients, respectively [80, 81]. In surveys of emergency room patients, it was shown that more than 50% had 10 or more bowel movements a day[78]. Nausea is commonly reported but vomiting is reported less often than nausea[78, 82]. The duration of symptoms may last for 1-7 days or longer depending on the virulence of the species and or strain and the

immunological status of the patient[83]. On average, patients remain ill for 1-4 days but a study in Denmark found the median duration of all illness to be 10 days[11].

Using data from 17 point source outbreaks, the average incubation period was estimated to be 3.2 days, with a range of 18 hours to 8 days [8]. The authors of that study reported that the upper and lower bounds of the range may have actually been sporadic cases that were not actually part of the true outbreaks investigated. Outbreak data is usually used when calculating the incubation period since it is normally difficult to pinpoint the source of infection in sporadic cases. The Centers for Disease Control reports the average incubation period to be 2-5 days with a range of 2-10 days [84]. This slightly longer incubation period can be useful in separating *Campylobacter* from other gastrointestinal bacterial pathogens. Other common pathogens that cause diarrhea, abdominal cramps and fever are nontyphoidal *Salmonella*, enterotoxigenic *E. coli*, and *Shigella*; these bacteria have average incubations periods of 6-48 hours, 6-48 hours and 2-4 days, respectively.

A re-occurrence of symptoms is reported by 15-25% of patients whose recurring symptoms are severe enough to cause them to revisit their physician[8]. The rate of relapse may be higher since not all cases will revisit their physician for the same illness if their symptoms are mild. The clinical course of the relapse is normally characterized by abdominal pain and may vary from a relatively mild gastroenteritis to an enterocolitis with bloody diarrhea, lasting for several weeks[85].

Patients will continue to shed *Campylobacters* in the feces for several weeks after recovery. Some studies have shown that convalescent shedding period may be reduced if the antibiotics are administered early in the clinical disease course[86]. A meta analysis

of 11 double-blind studies of antibiotic use for the treatment of *Campylobacter* enteritis showed that the duration of intestinal symptoms was only shortened by 1.3 days with antibiotic use[86]. The same study showed that the duration of diarrhea was twice as long if the patient waited more than 3 days to seek medical treatment. Another study found that the average patient will wait 4 or more days before seeking medical care[87]. This delay in seeking medical care prolongs disease duration and may limit the usefulness of antibiotic treatment.

2.3.2 Systemic Infections

Campylobacters are invasive bacteria that are able to translocate and reach the blood stream; however bacteremia in human *Campylobacter* enteritis patients is rarely reported. A study in England found an average bacteremia rate of 1.5 cases of bacteremia per 1,000 intestinal infections, with a high degree of variability associated with age[88]. Elderly patients (over 65 years of age) had the highest rate of 5.9 per 1,000 intestinal infections while young children ages 1-4 had the lowest rate at 0.3 per 1000 intestinal infections[88]. *Campylobacter fetus* rarely causes enteritis and it has been isolated more frequently in systemic blood infections. It induces fever and can disseminate to numerous tissues such as the vascular endothelium, bones, and joints. Complications from *C. fetus* infections can include meningitis, endocarditis, pneumonia, thrombophlebitis, septicemia, arthritis, and peritonitis[10].

2.3.3 Post-Infection Complications

Although most cases of campylobacteriosis are self-limiting, complications such as reactive arthritis or Guillain-Barré syndrome (GBS) have been documented following

infection. However, reactive arthritis can follow infection by other intestinal bacteria such as *Salmonella*. The frequency of documented joint pain following *Campylobacter* infection ranges from <1%[77] to19.9%[11]. This percentage depends on the prevalence of the Human Leukocyte Antigen B*27 (HLA B27) tissue antigen in the population surveyed; it can range from 0-50%[89]. HLA B27 is a surface antigen on the B locus of the major histocompatibility complex and presents antigens to T cells; it has been strongly associated with certain autoimmune diseases. Patients with the antigen have a strong predisposition to reactive arthritis. In most cases the joint pain lasts a few weeks to months and almost all result in a complete recovery[8]. Other local complications such as appendicitis, peritonitis, cholecystitis, hepatitis or pancreatitis may occur but are rare.

Guillain-Barré syndrome is an autoimmune-mediated disorder of the peripheral nervous system. Affected patients will rapidly develop progressive weakness in their limbs and respiratory muscles and a loss of reflexes. While the disease is generally self-limiting, up to 20% of patients require mechanical ventilation for a portion of their recovery[90, 91] and 15-20% are left with severe neurological deficits[92-94]. *Campylobacter* enteritis is reported as the most frequent antecedent event for GBS accounting for 30-40% of all GBS cases[95, 96]. Of patients who develop GBS after campylobacteriosis, the GBS symptoms normally develop 1-3 weeks after recovery from their gastrointestinal symptoms. Some studies have suggested that patients who develop GBS after *Campylobacter* infection may have a more severe course of GBS than those who develop GBS from other causes[97]. One study found that on average, 1 out of

every 1000 patients with *C. jejuni* infection will develop GBS, but in patients with the O19 strain, the rate increases to 1 out of every 158 [9].

2.4 Immune Response/Immunity

The self-limiting nature of the clinical disease course in most campylobacteriosis patients indicates that in individuals with a healthy immune system there is an effective immune response defense mechanism to clear the infection. Further evidence of this is supported by studies involving HIV-infected patients who have a defective cellular immune response. Immuno-defficient patients experience more severe symptoms, a higher frequency of relapses and may suffer from bacteremia from *C. jejuni* because their immune system is unable to clear the bacteria when it reaches the bloodstream[98].

Black et al[76] studied experimentally infected humans, and reported that infection may provide some protection against future infections. Seventy-two adult volunteers ingested various doses of *C. jejuni* strain A3249; 75% became infected and 18% were symptomatic. One month later two volunteers, who were previously infected and experienced symptoms, were re-challenged along with five new volunteers. Neither of the re-challenged volunteers became infected while all five new volunteers did (P=0.048). Another group of 39 adult volunteers were given varying doses of *C. jejuni* strain 81-176; 100% of these volunteers became infected (all had positive stool cultures) and 46% were symptomatic. Seven members of the 81-176 strain group were re-challenged a month later along with twelve new volunteers. None of the re-challenged volunteers developed diarrheal illness while 6 of the 12 new volunteers did (P=0.034). In

both experiments patients were re-challenged with the same strain of *C. jejuni* showing that infection can provide short-term homologous immunity.

The exact mechanisms of the host recognition of the bacteria and immune response are unclear. Both an innate and adaptive immune response are involved in the response to invasion and colonization by *C. jejuni* and *C. coli*[99]. The innate immune response is also called non-specific or broad-specific immune response since the cells that are part of the response do not recognize a specific invading pathogen; rather they recognize all foreign cells and acts as soon as the foreign cells are recognized. The innate immune response also initiates the adaptive immune response which has cells that are designed to recognize a specific foreign cell but requires a lag time for antibodies to be produced.

2.4.1 Innate Immune Response

Recent studies have indicated that structural components of the bacteria and proteins synthesized by *C. jejuni* during invasion and adhesion elicit an innate immune response from the intestinal epithelial cells[100]. A study at the Naval Medical Research Center in Maryland suggested that *C. jejuni* can induce the release of interleukin-8 (IL-8) by two mechanisms. One requires adhesion to the epithelial cell wall and/or invasion into the cell and the other is activated by surface proteins on the cytolethal distending toxin (CDT)[101]. IL-8 is an inflammatory chemokine that attracts neutrophils to the site of the infection, where the responding neutrophils are able to phagocytose the invading bacteria and clear it from the cell. Another study at the University of Nottingham in the United Kingdom found that *C. jejuni* was a transcriptional activator of NF-κB (nuclear factor-kappa B)[102]. NF-κB functions as part of the innate immune response by

stimulating the transcription of the genes for cytokines and chemokines[103]. The resulting secretion of cytokines/chemokines and other mediators leads to the activation of macrophages and the recruitment of polymorphonuclear leukocytes in the inflammatory response. Macrophages ingest and phagocytose invading cells. Macrophages also play a role in the adaptive immune response by acting as antigen presenting cells in the creation of antibodies. In in-vitro experiments, when human T84 epithelial colon cells were cultured with *C. jejuni*, the epithelial cells up regulated the expression of dendritic cell and T-cell chemoattractants[104]. These chemoattractants bring T-cells to the site of the infection. The T-cell and mature dendritic cells then act as antigen presenting cells and activate the adaptive immune response[105, 106].

2.4.2 Adaptive Immune Response

The adaptive immune system produces an antibody response that is specific to *Campylobacter* infections. Antibody production is a delayed response. The initial specific antibodies, immunoglobulin M (IgM) are produced by the 8^{th} day post infection, and IgG first appear around 10 days after the first symptoms, while IgA peaks about 2 weeks post onset of symptoms[107]. Although IgM is short lived and IgA disappears after 5 weeks, IgG has been detected in the sera of infected individuals up to a year post infection[108]. A study of workers in a poultry processing facility found that long-term workers (working for > 1 month) had significantly higher anti-*Campylobacter* IgG concentrations than the short-term workers. There was no significant difference detected in the levels of IgA[109]. This indicates that the long-term workers have been exposed in the past, and have circulating IgG from past exposures that the short-term workers have

not developed yet. Current exposure is the same for both long- and short-term workers so no IgA differences are observed.

Studies of populations in hyper-exposed regions of the developing world have shown that in young children, an increase in age correlates with a decrease in the illness to infection ratio[110]. In these populations children develop a resistance to colonization between 2-5 years old. The convalescent excretion period is shortened with age due to a gradual increase in *Campylobacter*-specific circulating antibodies as the child ages[111]. A study of children in Bangladesh found that levels of IgA continued to increase throughout childhood, while IgG peaked between 2-4 years old and IgM reached a plateau at the same age[112].

Further evidence of the immunity provided by IgA was studied in a population of infants in Mexico. The attack rate for diarrhea caused by *C. jejuni* was significantly higher ($p < 0.0005$) in the group of infants that were not breast fed compared with the infants that were breast fed. The human breast milk of the infants who were fed breast milk fed infants and who developed diarrhea did not contain anti-*Campylobacter* specific IgA[113]. Babies that are breast fed receive milk that has the same antibodies as their mother. Thus in the group of infants who were breast fed but still developed diarrhea, the mother did not have the antibody to pass on to her baby.

A study of IgA in adults was carried out amongst United States military personnel participating in military exercises in Thailand. Personnel who had a pre-travel anti-*C. jeuni* antigen IgA titer less than 450 were 1.6 times as likely ($P=0.05$) to have campylobacteriosis associated diarrhea during their stay in Thailand than the personnel

who had a pre-travel titer greater than 450[114]. This indicates that high levels of IgA may provide short-term immunity against future infection.

2.5 Diagnosis

The original isolation of *Campylobacters* from stool was achieved in 1968 through the use of membrane filtration and culture. *Campylobacters* are able to pass through a filter membrane, due to their smaller size, while other bacteria that may be present in the stool sample cannot not[115]. In 1977 Martin Skirrow developed a selective media, for the culture of *Campylobacter,* containing vancomycin, polymyxin B, and trimethoprim in a blood agar base with lysed horse blood[2]. Since the development of Skirrow's culture media, several variants have been developed depending on the type of sample (fecal, environmental or water) in order to obtain more growth quicker, eliminate other competing bacteria and make the media cheaper to prepare[116-119]. Culture remains the gold standard for clinical diagnosis, although the media used may differ from laboratory to laboratory. Some laboratories have started to again use membrane filtration and a less selective media in order to culture *C. upsaliensis*[120], which cannot grow on the standard culture media.

The antimicrobial agents in the standard selective culture media (Skirrow's or similar media) are inhibitory to *C. upsaliensis* and some strains of *C. jejuni* and *C. coli.* Since their growth is normally inhibited in most diagnostic laboratories, the incidence of *C. upsaliensis,* which also causes enteric infection, is underreported. Another drawback of the standard culture method is the long incubation time (48 hours) required to grow an

isolate. Once *Campylobacter* colonies have formed on the culture plate, phenotypic tests are required to confirm the isolate and determine the species. These tests include the morphology, motility, catalase, oxidase, hippuricate hydrolysis test, indoxyl acetate hydrolysis, production of hydrogen sulfide (H_2S) and the antibiotic sensitivity to cephalothin and nalidixic acid. One problem with the phenotypic tests that are designed to differentiate between the species is that not all strains within a species behave the same for each test; 5-8% of *C. jejuni* isolates do not express hippuricase activity which has been considered a hallmark of *C. jejuni* colonies[11].

Dark-field microscopy as a method of rapid diagnosis of campylobacteriosis was proposed in Colorado in 1982[121]. Dark-field microscopy involves examination of the stool sample for the presence of organisms with the typical darting or corkscrew motion of *Campylobacter* spp., and the presence of leukocytes or erythrocytes. While this test can be performed quickly, its sensitivity is only 36% and this falls to 28% if the stool samples are viewed more than two hours post collection[121]. This decline in sensitivity could be due to a lack of motility, due to death of the organism. While the specificity was 99%, the overall positive predictive value (probability, given a positive test that a patient has the disease tested for) of this method of diagnosis was only 62%.

Due to the above indicated drawbacks with the culture method for diagnosis of *Campylobacter* enteritis there has been considerable work to look for a new diagnostic method. A Latex agglutination test has been developed to help confirm and identify the species in place of the phenotypic tests, but an evaluation of the test found it lacked the ability to differentiate between *C. jejuni/C. coli* and *C. upsaliensis* or *C. lari*[122]. The analysis of this test found that the sensitivity was 100% for *C. jejuni* or *C. coli* isolates,

but only 87% if all isolates tested are included and only 14% of *Campylobacter* isolates other than *C. jejuni* and *C. coli*. The specificity was reported at 100% of the 101 non-*Campylobacter* organisms that were tested[122].

An enzyme-linked immunosorbent assay (ProSpecT Microplate assay; Alexon-Trend, USA) is on the market for the rapid detection of *C. jejuni* and *C. coli* from fecal samples. This test has been evaluated on multiple occasions and the sensitivity has been reported at 96, 89.1 and 80%, while the specificity was reported at 99, 97.7 and 100%[123-125]. Tests using PCR to detect *Campylobacters* directly from human fecal samples have been developed, but due to the materials and labor required, are currently only used in the research setting[77].

2.6 Treatment

The majority of symptomatic *Campylobacter* infections are acute and self-limiting, as such most patients do not need antimicrobial therapy and only require oral replacement of fluids and electrolytes[82]. However, severely ill patients may need to be admitted to a hospital for parenteral fluid replacement and antimicrobial treatment in addition to oral re-hydration therapy. The antibiotics normally recommended to treat human campylobacteriosis are fluoroquinolones (ciprofloxacin and levofloxacin are the most common) and macrolides (erythromycin, azithromycin and clarithromycin). Some fluoroquinolones (enrofloxacin and sarofloxacin) have been used in the past for the prophylactic treatment of poultry.

Antibiotics may be warranted in patients that have a bloody stool, high fever, prolonged illness (defined as symptoms lasting longer than 1 week), pregnancy, and when there is coinfection with HIV or AIDS or other immunosuppressant conditions. In these situations erythromycin and azithromycin are the current drugs of choice[126-129]. While there is a small amount of resistance developing to erythromycin, it remains the drug of choice recommended by the CDC. Antibiotic therapy should only be used when necessary in order to limit further emergence of resistance to potentially life saving drugs. Since resistance rates vary by country and class of antibiotic, clinicians should consider where the infection originated when choosing antibiotic therapy[130, 131].

Erythromycin is the more cost effective macrolide when compared to azithromycin (the newer macrolide), yet both are equally as effective. *C. coli* has shown high levels of resistance to erythromycin. However, the incidence of infection by *C. coli* remains low in the United States so macrolides are still recommended for treatment of campylobacteriosis. Ciprofloxacin[132] and Norfloxacin[133] have been shown to be successful in reducing the duration of symptoms but an increasing prevalence of fluoroquinolone resistance has emerged so continued use of fluoroquinolones to treat campylobacteriosis is cautioned.

The empiric treatment of enteric infections is controversial due to the emergence of antibiotic resistance, and the possibility of treatment failure and relapse or future resistance to all antibiotics. Children who are treated empirically with antibiotics before the etiologic agent is determined, may be at a higher risk of hemolytic uremic syndrome if the agent of infection was *E. coli* O157:H7 (a more frequent cause of bloody diarrhea[134]), not *Campylobacter* spp.[135]. Clinicians, in some cases, will not

establish the etiologic agent responsible for an enteric infection before starting antibiotic treatment; therefore it is important to consider the possibility of antibiotic resistance and related complications when selecting antibiotic therapy. One study found that the usefulness of antibiotic therapy decreased dramatically if it was not administered early in the infection[86]. Although, the majority of campylobacteriosis infections are relatively mild and therefore do not require antimicrobial therapy, severe cases require antibiotic treatment, early in the clinical course, if possible. In such cases it may be important to perform antibiotic susceptibility test to ensure appropriate choice of medications.

2.7 Antibiotic Resistance

2.7.1 Prevalence

In recent years the resistance to fluoroquinolones has rapidly increased worldwide[136], and resistance to macrolides is on the rise in some areas[137]. Worldwide the prevalence of antibiotic resistance varies depending on the species of *Campylobacter* concerned and the class of antibiotic in question. In Ireland, dramatic increases were observed in the prevalence of resistance between 1996/1998 and 2000. They tested a collection of *C. jejuni* and *C. coli* and found that while resistance to erythromycin had remained constant at 2%, resistance to tetracycline increased from 14% to 31%, and resistance to ciprofloxacin had increased from 0% to 30%[138]. In separate susceptibility tests of *C. coli* isolates in Germany and Spain, 29.4%[139] and 34.5%[140], respectively, were resistant to erythromycin. None of the German isolates and only 3.2% of the Spanish *C. jejuni* isolates were resistant to erythromycin. The

observed prevalence estimates of resistance to ciprofloxacin was 45.1% in Germany[141] and 72% in Spain[140], greater than the percentages of resistance in Ireland.

In developing countries antibiotics are often sold unrestricted which can lead to improper usage in humans and animals[142]. In Thailand *Campylobacter* spp. resistance to ciprofloxacin was 84% in 1994. In a study of mixed isolates from children in Lagos, Nigeria, 79.8% were resistant to erythromycin[143].

In the United States, the prevalence of resistant strains is not as high as other parts of the world, but it is on the rise. In Minnesota, the prevalence of resistance to ciprofloxacin was only 1.3% in 1992 and but had increased to 10.2% by 1998[144]. Due to this increasing trend of antibiotic resistance, the National Antimicrobial Resistance Monitoring System for enteric bacteria (NARMS) was established in 1996. NARMS was established as a collaborative effort between the Food and Drug Administration's Center for Veterinary Medicine (FDA CVM), U.S. Department of Agriculture (USDA), and the Centers for Disease Control and Prevention (CDC)[145]. The goals of NARMS are to provide data on the extent and trends of antimicrobial drug susceptibility and resistance for enteric bacterial organisms and to inform physicians and veterinarians of the patterns they observe. A total of 297 isolates were tested in 1990 by the Centers for Disease Control (CDC), prior to the creation of NARMS, and no ciprofloxacin resistance was detected. However, the percentage of resistance had increased to 13% of 217 isolates tested by NARMS in 1997 and 19% of 384 isolates in 2001[146]. Resistance to erythromycin is variable between *C. jejuni* and *C. coli* at 1-5% and 5-9% of isolates, respectively[137].

2.7.2 Complications of Antibiotic Resistance

Several studies have shown that patients with a quinolone resistant strain of *C. jejuni* experience a longer duration of illness than those with a quinolone sensitive strain[147]. In Minnesota, patients who were treated with fluoroquinolones and were infected with a fluoroquinolone resistant strain had a median duration of diarrhea of 10 days while those who were infected with a fluoroquinolone sensitive strain had a median duration of diarrhea of only 7 days (p=0.03)[144]. FoodNet data collected in 1998-1999 showed that patients who took no antidiarrheal medication and were infected with ciprofloxacin resistant strain had a mean duration of diarrhea of 9 days while those who were infected with a ciprofloxacin sensitive strain only experienced 7 days of diarrhea (p=0.04)[148]. The same was shown by a study in Denmark in 2001-2002 where patients with a fluoroquinolone resistant strain had a mean duration of illness of 13.2 compared with 10.3 days of the sensitive strain patients(p=0.001)[147].

2.7.3 Controversy with Antibiotic Usage in the Poultry Industry

The rise of fluoroquinolone resistance appears to coincide with the widespread use of related fluoroquinolones in large scale food animal production, especially poultry production. This is important since raw and undercooked chicken is considered a major risk factor for *C. jejuni* infection. Two fluoroquinolones, sarafloxacin and enrofloxacin, were licensed for use in US poultry in 1995 and 1996 respectively, and by 1997 13% of 217 human isolates submitted to NARMS were resistant to fluoroquinolones when no fluoroquinolone resistance had been detected in prior years[146]. Due to this apparent link Abbott Laboratories withdrew sarafloxacin from use in poultry in 2000 and the FDA

withdrew enrofloxacin from the market for poultry in 2005 (after Bayer Corporation disputed the link for 4 years[149]). In the United Kingdom, a survey of retail chickens prior to the licensure of enrofloxacin for use in poultry production in the UK, found only 3% of *Campylobacter* isolates from domestically raised chickens were resistant to ciprofloxacin[150]. This study also tested chickens imported from mainland Europe, including some chickens from France where enrofloxacin was licensed for use at the time of the study. Of the imported chickens, 27% of the *Campylobacter* isolates were resistant to ciprofloxacin. More evidence supporting the theory that the increase in *Campylobacter* strains resistant to fluoroquinolones is linked with the use of fluoroquinolones in poultry production is provided by a study conducted in Denmark. Fluoroquinolones are not used in Danish poultry productions and the percentage of human *Campylobacter* isolates that are fluoroquinolone resistant is only 6.5% for infection acquired in Denmark compared to 67.4% of those infections acquired abroad (including neighboring countries where antibiotics are used in poultry production). Due to the high incidence of campylobacteriosis worldwide, it is important to limit the level of antibiotics being used in poultry production due to the ability of bacteria to become resistant through transfer from other commensal bacteria. If in the future, more strains become resistant to more and/or different classes of antibiotics, the duration of illness experienced by patients may be longer, and symptoms may be more severe.

2.8 Pathogenesis

The pathogenic mechanism of *C. jejuni* has been debated and is still not completely clear, one researcher called the mechanism "sketchy"[151]. Some of the debate may be due the high degree of genetic and phenotypic diversity amongst *C. jejuni* strains resulting in widely varying experimental results depending on the strain used. In general, *Campylobacters* enter the host intestine by passing through the stomach acid barrier and colonize the distal ileum and colon. In order to colonize and cause disease, *C. jejuni* uses a variety of mechanisms including bacterial cell motility and chemotaxis, adhesion, invasion and cytotoxin as well as enterotoxin production[152].

Campylobacters are motile through the use of their uni-polar or bi-polar flagella. The direction of their motion is driven by chemotaxis, which was first described in 1883 by Theodor Engelmann and in 1884 by Wilhelm Pfeffer[153-156]. Chemotaxis is described as the movement of bacteria toward certain stimuli and away from others. *C. jejuni* exhibits positive chemotactic behavior (movement towards) for L-fucose[157] which is the terminal sugar in mucin, but it is repelled from bile components. Further research has shown that amino acids L-aspartate, L-cysteine, L-glutamate, and L-serine, and the organic acids pyruvate, succinate, fumarate, citrate, malate, and a-ketoglutarate are all positive chemoattractants for *C. jejuni*[158]. All of the identified chemoattractants are chemicals or compounds that are found in or near the mucosa of the epithelial lining of the intestine, thus directing the bacteria towards its invasion target, the epithelial cells of the colon and closer to the wall of the intestine to avoid being cleared by fluid flow. Once a chemoattracting molecule is bound to a receptor on the bacteria,

autophosphorylation of the Che proteins conveys the signal through signal transduction[159, 160]. This same mechanism works to drive the bacteria away from chemorepellents. The signal can be to change directions and or speed. *C. jejuni* has shown the ability to move at higher velocities in more viscous environments due to its shape and flagellar activity[161]. The flagella has been shown to be necessary to overcome peristalsis and for entry into the mucous layer of the intestine[162] which allows the bacteria to cause disease.

Adhesion of the bacteria to epithelial cells on the wall of the intestine has been shown not to be essential for the colonization of the intestine, but it does facilitate cell invasion[163]. *C. jejuni* can adhere to the epithelial cells by a variety of bindings; these include polyoma enhancer binding factor 1, PEB1 (a homolog of Gram-negative ABC transport systems)[164], *Campylobacter* adhesion to Fibronectin ,CadF (a fibronectin binding protein)[165], a major outer membrane protein[166], lipooligosccharide[167] and a novel surface-exposed lipoprotein specific to *C. jejuni*[168]. The ability to adhere to the cell allows the bacteria to invade the intestinal lining.

Host cell invasion has been observed in experimentally infected infant macaque monkeys[169] and swine primary intestinal cells[170], but no consensus has been reached as to the mechanism or pathway[171]. The damage caused by toxins released intracellularly and extracellularly can cause epithelial cell death. The cytotoxin, cytolethal distending toxin (CDT), is a tripartite toxin of the AB toxin type; two parts, CdtA and CdtC, form the binding components, while the third part, CdtB, is the active subunit[151]. Once in the cell, CdtB is transported to the nucleus and arrests the cell in the G_2 phase of the cell cycle by causing double strand breaks in the DNA[172].

Previous studies reported the cell cycle arrest was due to chromatin disruption[173]. This cell arrest leads to apoptosis. Both the toxins and dead cells stimulate inflammation and further epithelial damage leading to a loss of function of the intestinal mucosa. The toxin alters the absorptive capacity of the epithelial cells, which causes water to rush out of the cells resulting in watery diarrhea. Also as mucosal cells are damaged and die, they are no longer able to carry out their normal absorption of fluid from the intestinal tract, resulting in more fluid being passed in the stool and diarrhea[174]. In some cases, *C. jejuni* may invade the sub-mucosa. When the cells from the sub-mucosa die and fall off, a pore is opened that allows blood to enter the intestinal lumen resulting in bloody diarrhea. Mucus may also be found in the stool due to loss of adhesion of the mucosal layer from the damaged epithelium and due to the irritation of the mucin producing cells.

In response to the invasion, host cells release interleukin-8 (IL-8)[175] and leukotrine B4 (LTB4)[176] which cause the recruitment of more polymorphonuclear leukocytes and macrophages to the area. These inflammatory cytokines can cause fever in the host by acting as pyrogens and increasing the set point of the hypothalamic thermoregulatory center. The abdominal pain that is experienced by some patients may be caused by the inflammatory response as well. The inflammatory response elicited by the lipopolysaccharide interacts with signaling molecules that act on the enteric neurons to cause abdominal pain or cramps[177]. The distention and irritation caused by the inflammation of the small intestine and colon may cause vomiting as a result of the stimulation of the visceral afferent neurons[178]. Excessive distention or irritation of the duodenum provides the strongest stimulus for vomiting; impulses are transmitted by vagal and sympathetic afferents to the bilateral vomiting center of the medulla[179]. The

motor reactions are then initiated to cause vomiting. Nausea is the conscious recognition of the subconscious excitation in area of the medulla closely associated the vomiting center.

In most cases *C. jejuni* is sensitive to complement-mediated lysis and are killed rapidly in the blood stream even in the absence of specific antibodies[180]. However, there are some strains that are highly resistant to serum[181] and can colonize the blood stream or survive for up to seven days in monocytes[182]. The ability to "hide" in monocytes can result in long-term bacteremia in the host. This bacteremia can lead to further complications such as meningitis or abortion if fetal infection occurs[183].

When specific serotypes (O:19 and O:41) of *C. jejuni* are present in the blood stream, the lipooligosaccharide structures on the surface of the bacteria can molecularly mimic peripheral nerve gangliosides[184]. This mimicry results in the generation of autoreactive antibodies that cause nerve inflammation and tissue damage[185]. This damage is the start of Guillain-Barré syndrome (GBS). One theory on GBS initiation is that anti-self antibodies specific for the ganglioside to bind to the surface of Schwann cells and this starts the disease progression[186]. That binding may activate complement which forms transmembrane pores resulting in the tissue damage and loss of neural activity[187].

2.9 Pathogenicity/Virulence

Campylobacter spp. must be able to colonize the intestine of the host in order to cause disease. Therefore any mechanism that makes colonization easier may increase the

pathogenicity of the organism. Pathogenicity and virulence of *Campylobacter* are influenced by certain genes. For instance, research has evaluated how the loss of function of certain genes affects the ability of *C. jejuni* to infect the host. The spiral shape and flagellum of the individual bacteria enhance bacterial motility in viscous media such as the surface layer of the intestinal mucus. Removal of the flagella function through the creation of a mutation in the *flaA* gene resulted in decreased ability of *C. jejuni* to colonize the gut of chickens[188]. *Campylobacters* use chemotaxis to help control and direct motility. By creating a mutation in the chemotaxis regulator gene *cheY* researchers were able to reduce the number of symptomatic ferrets compared with the wild type[189]. That mutation made the bacteria unable to respond to signals from the host environment. The CadF protein is an outer membrane protein that facilitates the binding of *C. jejuni* to fibronectin. The disruption of the gene *cadF* results in the inability of that strain to colonize the cecum of day old chicks[190]. Another protein that plays a role in the adhesion of *C. jejuni* to the intestinal lining is PEB1 also known as cell binding factor 1, CFB1. Disruption of *peb1A* gene caused significant loss in invasion and adherence ability in cell culture and a significant decrease in the symptoms of mice challenged with the mutant strain[191].

The cytolethal distending toxin (CDT) causes progressive cell distention and cell death in cell culture experiments[192]. The genes *cdt A, cdtB,* and *cdtC* code for 3 proteins that combine to make a holotoxin that causes the cytolethal effect of arresting cells in the G_2 phase of their cell cycle[193]. These three genes have been found in almost all strains of *C. jejuni,* and *C. coli* as well as strains of *C. fetus* and *C. upsaliensis*[110], but the gene expression levels varies between strains of *C. jejuni* and

between species. *C. coli* has lower expression levels which could explain why the clinical course of enteritis due to *C. coli* appears to be milder[194]. Some strains have a higher expression of the *cdtABC* genes, like the well characterized *C. jejuni* 81-176, which appears to cause more severe disease. It has been hypothesized that the CDT has a negative effect on the net absorptive ability of the intestinal epithelium through inducing cell death and it affects the replication in active secretory immature enterocytes[110].

2.10 Epidemiology

2.10.1 Risk Factors

Most cases of campylobacteriosis are considered sporadic (they are not associated with an identifiable outbreak of cases) as such determining the source of infection is difficult. Therefore, there is substantial need to determine key risk factors for infection, among sporadic cases, in order to help prevent future illnesses. Several risk factors have been identified through case-control studies: poultry consumption, international travel, drinking untreated water or unpasteurized milk, contact with animals or contact with people who are similarly infected.

2.10.1.1 Consumption of Contaminated Food or Water

The most commonly identified risk factor is poultry consumption. Although each case-control study varied slightly in the questions about the specific type of poultry or where the poultry was consumed, 19 out of 24 case-control studies identified poultry consumption as a risk factor for campylobacteriosis[11]. In a 1997 Danish study, they separated poultry into 24 different categories, including popular brands and packaging

types, the only poultry exposure that was significantly associated with campylobacteriosis was undercooked poultry (all-types)[13]. Undercooked chicken was also found to be a significant risk factor for disease in the Denver and Fort Collins, Colorado area in 1981[195], in the population older than 5 in Australia in 2002[196], in the Eastern Townships of Québec in 2001[197], and in FoodNet sites in the United States in 1999[198]. The FoodNet study reported the largest population attributable fraction (24%) associated with the consumption of chicken in a restaurant. The association with the consumption of chicken in restaurants, without differentiating if the meat was undercooked, was also identified in New Zealand in 1995[199], and in Hawaii in 1998[200].

Other evidence supporting poultry as a risk factor for *Campylobacter* infection is provided by a study in Belgium in 1999 when all poultry meat and eggs were removed from the market due to contamination with dioxins. This resulted in a 40% reduction in the observed number of cases based on the expected number of cases of campylobacteriosis based on the predicted model from the previous years. The case counts returned to the predicted values after poultry products were re-introduced to the market[201]. A study in Norway found that eating poultry produced in Denmark or Sweden was strongly associated with human infection while the consumption of poultry produced in Norway was not[202]. The authors associated this with the low prevalence of *Campylobacter* infection in the poultry flocks of Norway.

Drinking untreated water has been associated with outbreaks of campylobacteriosis, and has been identified as a risk factor for infection in case-control studies. A study in Colorado in 1981 found that cases were more likely than controls to

report having drunk "raw" (untreated) water from a stream, river or lake, OR=10.74[195]. Similar associations were found in England in 1991[203] and Sweden in 2002[204]. The Swedish study also found that having a well as the household water source was significantly associated with developing campylobacteriosis. Two studies in New Zealand found non-urban household water sources [205] or rainwater as the water source [199] to be associated with increased risk of disease. In Canada, an outbreak of gastroenteritis caused by both *Campylobacter* spp. and *E. coli* O157:H7 was attributed to cattle manure entering the municipal water supply following heavy rains[206]. A study conducted in Finland in 2002 found that swimming in a natural body of water was a significant risk factor for campylobacteriosis[207].

Other risk factors that have been identified are unpasteurized milk [13, 195, 197, 204, 208], anti-secretory drugs or consumption of milk from bottles that had lids that had been attacked by birds[12], consumption of offal[196], sausages at a barbeque[202], red meat at a barbeque [13], pork[13, 208], and grapes[13].

2.10.1.2 Animal Exposure

Animal exposure is another commonly reported risk factor. The species and age of animal implicated varies from study to study. A study of infants in the United States found that having any kind of pet with diarrhea in the household was associated with increased risk of campylobacteriosis[14]. Another study of young children less than 6 years of age in Sweden in 2002 found that having a dog of any age in the household was a significant risk factor (OR=8.4)[204] as did a Norwegian study in 1992 (OR=5.04)[202]. The US FoodNet study in 2004 found an association between all ages of campylobacteriosis patients and puppies (OR=3.2)[198]. The Nottingham Health

Region study of 1995 also implicated puppies as a source of infection[12]. Similar associations with young dogs less than 6 months of age were found in an Australian study of the population older than 5[196] and in one study of children less than 35 months of age[209]. Cats or kittens were identified as risk factors in Colorado in 1981[195] and Denmark in 1997[13]. Other animals that have been implicated as risk factors for campylobacteriosis are chickens [13, 196, 208, 209], and cattle[199], [13].

Pets as a risk factor is supported by numerous cross sectional studies conducted mainly in Europe that have shown that the prevalence of *Campylobacter* infections in dogs ranges from 5% to 70%[34, 210-213] depending on the test used for diagnosis of campylobacter and the living conditions of the dogs sampled. Dogs and cats show a high prevalence of infection with *C. upsaliensis*[37], which can infect humans; however, it is not regularly identified using standard diagnostic tests in the United States due to inhibitory effects of the routine culture media[41]. When *Campylobacter* infection of pet animals is limited to just *C. jejuni*, the prevalence ranges from 3% to 22%[37, 213, 214], depending on the living conditions of the sampled animals. Although the prevalence of *C. jejuni* in pet animals may be considered low, it is an important public health concern since both healthy pets and those with diarrhea have been reported to have a similar prevalence of infection[35] with equal possibilities of shedding bacteria in feces. However, one study found a significant difference in the prevalence of infection between healthy young (less than 1 year) dogs and diarrheic dogs of the same age[215]. These results suggest that apparently healthy animals may just as likely to be infected with campylobacter as sick animals, depending on the age of the animal, and these animals can act as potential sources of infection to humans. Moreover, healthy carrier pet animals

may shed the bacteria for up to several months which could be a source of recurrent infection in a household.

Workers in the poultry industry are at a high occupational risk for *Campylobacter* infections. One study of a factory in Northern Ireland found that poultry factory workers were 3 times more likely to develop campylobacteriosis than the population of the surrounding community ($p = 0.016$)[216]. At a poultry processing plant in Sweden, an outbreak of campylobacteriosis occurred when the normal and experienced workers went on holiday and were replaced with inexperienced teenagers[217]. Only 29% of the experienced staff became sick during this outbreak, while 71% of the replacement workers did, since the replacement works did not have immunity from previous exposure. A case-control study in Michigan found that those who practiced poultry husbandry had increased odds of campylobacteriosis compared to those who did not (OR = 6.884; 95% CI = 1.438 - 32.954). The same study estimated that 18% (95% CI = 6% - 30%) of cases of human campylobacteriosis that occur in rural populations are attributable to poultry contact[218].

2.10.1.3 Other Risk Factors

Another risk factor that has consistently been identified in case-control and descriptive studies of campylobacteriosis is international travel. In the FoodNet study in 1999, 13% of the cases interviewed reported international travel 7 days prior to the development of symptoms, compared to only 1.5% of the controls, for an odds ratio (OR=10.0)[198]. Travel abroad was also identified as a risk factor in Switzerland in 1991 with an OR of 21.2[219], in Denmark in 1997[13], and the Nottingham Health Region in England in 1995[12]. *Campylobacter* spp. is frequently identified as the

etiologic agent responsible for traveler's diarrhea, adding to the implication of international travel as a risk factor for campylobacteriosis[220, 221]. Other studies have also identified antibiotic use[200], and diabetes[12] as risk factors for disease. It has been hypothesized that antibiotics may provide a selective advantage to drug-resistant bacteria by lowering the infectious dose required to produce disease, or that previous antibiotic use may result in decreased colonic bacterial flora so there is less resistance to colonization by *Campylobacter* spp[222, 223].

While person-to-person transmission is believed to occur, contact with someone with similar symptoms has not been documented as a risk factor in *Campylobacter* case-control studies. Person-to-person transmission, however, has been documented among family members [224] Childcare centers are prime locations for *Campylobacter* spp. to be spread due to large numbers of children present and the decreased hygiene practices of children in that age. In Brussels in 1991-1992 an outbreak of *C. upsaliensis* affected 44 children at 4 related child care centers[225]. Restriction fragment length polymorphism (RFLP) showed that there were two clonal variants circulating, one variant affecting one center and the other affecting the other three centers. In a case-control study of childhood diarrhea of all causes in Sao Paulo, Brazil "not sending a child to a daycare center" (childcare in the home) was found to be protective against diarrhea (OR= 0.58, P = .004)[226].

2.10.2 Incidence

In descriptive studies of enteric illnesses in developed countries, infections by *Campylobacter* spp. are among the most common. In Australia, *Campylobacter* infections are the leading causes of gastrointestinal illnesses among all the notifiable

enteric pathogens[17]. *Campylobacter* is the most common bacterial cause of diarrhea in England and Wales[16] and in the United States, it is reported more frequently than any other bacterial pathogen[3].

The incidence of disease varies widely worldwide, the highest reported incidence occurs in the South Pacific. In New Zealand, the incidence of campylobacteriosis has been reported at 300-396 per 100,000[18, 19] and in Australia the incidence was reported at 116.5 per 100,000 in 2003[17]. The reported incidence risk (per 100,000) is slightly lower in Europe at 95 in Switzerland[213], 82 in Denmark in 2001[227], and 66.65 in 2006 in Sweden[228]. The average incidence risk among sites participating in the FoodNet program in the United States was 12.71 per 100,000 in 2006 and the reported state incidence in Tennessee was 7.4 per 100,000[20]. While the reported incidence in the US is lower than other parts of the developed world, it still represents an important health issue, it has been estimated that the true incidence of campylobacteriosis in the US is 800 per 100,000 population[3]. Differences in health care systems may influence the reported incidence of campylobacteriosis. In countries where there is a higher percentage of insured individuals, either by government universal healthcare or private health insurance, these individuals will be more likely to visit the doctor when they are ill compared to individuals who don't have health insurance. In the United States it has been reported that 16% of the populations does not have any form of health insurance[229]. While countries like Canada have government health insurance plans that are designed to ensure that all residents have access to health insurance. In these countries, more cases will be reported since more individuals will visit their doctor and are more likely to have their stool cultured. Due to under-reporting, the real incidence of

campylobacteriosis may be closer to 900 cases per 100,000 people in the United States, based on an estimate in 1999[3].

2.10.2.1 Demographic Distribution

All ages can be affected, but there appears to a bimodal age distribution. The highest age-specific incidence occurs in young children[204, 230, 231]. Some reports have categorized this incidence as all children under 5 years[4, 204, 231] while others have been more specific, limiting the highest incidence to just children under 1 year[230]. In some studies, a smaller second peak has been documented, this occurs in young adults of ages 15-30 years[4, 198, 232]. It has been hypothesized that the reason infants and young children have the highest reported incidence, is that the parents of young children are more likely to take their child to the doctor than themselves[233]. With more young children seeking medical care there is a higher possibility of stool culture and documentation by the reporting system[234]. Young children may also have a higher risk of exposure due to decreased hygiene from some behavioral patterns, such as crawling on the floor and putting objects in their mouths. Skirrow studied this in 1987, he calculated the infection rate based on the total number of samples cultured; the lowest infection rate was observed in infants under one year of age, and the highest infection rate was observed in the 15-24 age group[235]. It has also been hypothesized that the reason young adults (ages 15-30) have a higher reported incidence of campylobacteriosis is that this is the age group that does the most international travel[4].

A study of the infectious disease surveillance system in Denmark, of >13000 stool samples, found that female patients were more likely than males to be cultured for *Campylobacter* infection (ratio of 1.2:1), although male patients were more likely to be

culture positive (RR 1.27, 95% CI 1.21–1.34)[236]. The same study found that the oldest age group (60 and older) were the most likely to have their stool cultured, while the 15-29 age group was the most likely to be culture positive. A study in Norway found, males were more likely to be infected overall, 57.5% of all campylobacteriosis patients were male. This percentage was even higher (60.3%) in the under 5 year old age group[4]. One theory for the higher incidence among males is that males report more unsafe food-handling practices and higher consumption of finger foods that are known to increase the risk for foodborne diseases[237, 238]. This, however, does not explain the sex difference observed in young children where the risks should be similar, due to similar hygiene and diet at this age.

A descriptive study of FoodNet sites in the United States also found that the highest incidence of campylobacteriosis was reported in infants less than 1 year (56.2 per 100.000) followed by children aged 1-4 years (41.2 per 100,000)[239]. This study also reported a smaller peak in the incidence in the population aged 20-29 (30.3 per 100,000). As seen in other countries, males had a higher average incidence of infection (24.4 per 100,000) than females (19.4 per 100,000). Other studies in the United States have also reported a higher percentage of males[195, 198]. A case-control study of the FoodNet sites found 54% of cases enrolled in the study were male[198] and a study in Colorado found 61.7% of campylobacteriosis patients were male[195]. Both case-control studies noted that there was no significant difference in the sex of the patients who participated in the study compared to those who didn't participate in the study. Overall in the United States the highest incidence is observed in the young children, and there is a slight predominance of males in most studies.

2.10.2.2 Temporal Patterns

Worldwide the reported incidence of campylobacteriosis spikes during the summer months. This trend has been noted in both the Northern and Southern hemispheres. In Denmark the highest incidence of disease occurs from July to September [236]. In a New Zealand study of recreational water isolates and human disease, the authors found the highest concentration of *Campylobacters* in water samples during the summer months, December-February, and also noted a steady increase in incidence of human disease from winter through summer[68]. Another study in New Zealand found the highest incidence of human disease during summer and noted that this seasonal variation was more amplified in the urban areas[240, 241].

In Wales, one study compared the seasonal variation of human *Campylobacter* isolates with that from commercial chicken isolates. It reported that the number of human isolates peaked around weeks 22-25, in early June while the number of chicken isolates peaked around weeks 24-26, in late June[242]. This June peak is consistent with the data from the FoodNet study in the US that found increasing infection rates during the spring and a peak in the rates in June or July[239]. The June/July peak was also demonstrated by a study in Massachusetts that compared the incidence of disease with the ambient temperature[243]. This study found that the peak in the incidence of campylobacteriosis coincided with the peak in the ambient temperature and it occurs on average around day 208, in late June. A similar trend was observed in Michigan, with more cases being reported in the summer, especially in rural areas that have a high poultry density[232].

2.10.2.3 Geographic Distribution

The geographic distributions are not as consistent worldwide; some studies have reported higher incidence in rural areas while others have reported it in the urban areas[232, 239, 244]. A study of the spatial distribution in Manitoba found that the incidence was significantly higher in populations living in rural and agricultural areas of the province[244]. This study also found that the incidence in young children under 5 was seven times higher in rural Manitoba compared to the city of Winnipeg. A study in Denmark similarly concluded that living in housing types found in rural areas and living in areas with a low population density were associated with an increased risk of infection[245].

In the Netherlands, a 2006 study found the highest incidence in the southern portion of the country (55.7 per 100,000) compared to the rest of the country (39.1 per 100,000)[236]. When the country was separated into urban and rural, the higher incidence was observed in the urban areas (41.9 per 100,000) compared with the rural (32.4 per 100,000). Similar patterns were observed in New Zealand[246]. In the United States, the highest incidence among the FoodNet sites occurs in the California[239]. The counties involved in the California site are San Francisco and Alameda counties, both of which are very urban. Contrary to that report, a study in Michigan that classified areas into low and high poultry density, found that the higher incidence of campylobacteriosis was reported in the more rural areas, which have higher densities of poultry[232].

2.11 Surveillance

The overall goal of surveillance is to collect, record, share and analyze data, and then disseminate the resulting information to relevant authorities who take action to control disease. The World Health Organization (WHO) has defined four categories of surveillance systems for foodborne pathogens: no formal surveillance system, syndromic surveillance, laboratory-based surveillance, and integrated food-chain surveillance [247].

No formal surveillance system involves investigation of large or unusual outbreaks which are performed by outside organizations, such as non-governmental organizations (NGOs). In syndromic surveillance, information is collected on syndromes without a laboratory confirmed diagnosis. Laboratory-based surveillance collects laboratory data, and differs from syndromic surveillance because it involves the identification of the etiologic agents. Integrated food-chain surveillance, involves collecting data from animals, food and humans in order to provide an overall picture. The goal is to track the specific pathogen from animal to human. The latter three forms of surveillance can be either active or passive.

The difference between passive and active surveillance is that passive surveillance programs wait for the data to be transmitted to the organization while active surveillance programs rely on information to be transmitted, but the health organizations also regularly contact health care providers or the public to solicit the information actively. Active surveillance is more costly but the information is obtained in a more timely fashion, the data may be more accurate and complete and active surveillance helps to reduce under-reporting.

In passive surveillance, reporting can be voluntary or mandatory, and the agency in charge uses routinely collected data. Enter-net is an international passive surveillance network for human gastrointestinal infections. Originally created to monitor *Salmonella* and *E. coli* in Europe, Enter-net now gathers some information on *Campylobacter* from 20 countries in Europe and around the world[248]. The modes of surveillance and information provided by each country are varied. Some countries have mandatory reporting but do not seek additional information from patients while the reporting in other countries is voluntary or only from certain sentinel sites (smaller sampling sites that are approximately representative of the population studied)[248]. These differences in surveillance programs make the comparison of the burden of disease for specific pathogens across countries and across regions within countries difficult. One of the goals of the network is to develop a consensus on standards for national participation in international surveillance. Global Salm-Surv (GSS), created by the WHO in 2000 for the worldwide surveillance of *Salmonella*, has now started new training programs for the worldwide surveillance of *Campylobacter*[249]. In the United States, passive surveillance of *Campylobacter* infections was implemented in 1982 and campylobacteriosis became a notifiable disease. During this time, isolates were reported through the Public Health Laboratory Information System (PHLIS)[7]. Under this system, reports of isolates were mailed weekly to the Centers for Disease Control and Prevention (CDC) from local health departments. These reports only included limited information since the system did not involve interviewing the patients and cases were not actively sought. Currently, *Campylobacter* is not on the list of National Notifiable

Diseases in the United States[250], but is on the list of notifiable diseases in the state of Tennessee[251].

In active surveillance, the health officials seek out information instead of waiting for it to be reported. In the United States, the Foodborne Active Surveillance Network (FoodNet) is an active surveillance system for all foodborne pathogens, including *Campylobacter*. It was created in 1995 to gather more complete information on foodborne pathogens with the specific goals of determining the burden of foodborne disease, monitoring trends in the incidence of disease, attributing the burden of disease to specific foods or settings, and to develop and assess interventions designed to reduce the burden of disease[252]. Seven states and parts of three other states, participate in this network which requires the mandatory reporting of all laboratory confirmed cases. In 1999, 11 counties in Tennessee joined FoodNet[253] and the rest followed suit in 2003[254]. All laboratories in the participating areas, must report any positive laboratory results for foodborne diseases to their local health departments. Depending on the state and laboratory, some cultures are sent to the state laboratory for speciation. The local heath department conducts an interview with each patient to collect demographic and risk factor information. The major differences between FoodNet and the previous PHLIS system are the follow up on each patient by the health department to collect additional information and the requirement that health officials must routinely audit the laboratories to ensure that all positive results were indeed forwarded to the health department[255]. Compiled data is electronically submitted to the state health department and CDC daily. The CDC then monitors nationwide spatial and temporal patterns.

One of the major problems with all surveillance schemes, especially passive systems, is under-reporting. In order for a case to be reported the person must go through approximately 7 steps (see Figure 2.1)[252]. At each step the percentage of infected individuals who continue onto the next steps is diminished. At the bottom of the pyramid are all the people who are exposed to *Campylobacter,* but then the patient must develop symptoms, see their doctor, provide a stool sample and have the organism isolated and reported. At each of these steps there are number of factors that might influence whether a patient continues onto the next step: health insurance, perceived severity of the symptoms, distance to the nearest diagnostic laboratory, fulfillment of stool sample request and thoroughness of the laboratory. In countries where health care is not universal, the second step may be the biggest detriment to a case being reported, the symptoms must be severe enough for the individual to seek medical care. The factors that influence reporting may also explain some of the distributions observed, since parents of young children are more likely to take them to the doctor, so children will be most represented in the database. One goal of FoodNet is to reduce the number of patients that are lost at each step by actively seeking reports from the laboratories and monitoring the reporting process (each event that occurs along the pyramid). This allows for a more accurate and precise estimate of the burden of foodborne disease. In *Salmonella* it has been estimated that for every case that is reported, 38 go unreported[256]. Using some of assumptions used to estimate the true incidence of *Salmonella* and applying similar mathmatical equations to the data for *Campylobacter*, it has been estimated that 1,400,000 to 2,453,926 cases of campylobacteriosis occur annually in the US[3, 239].

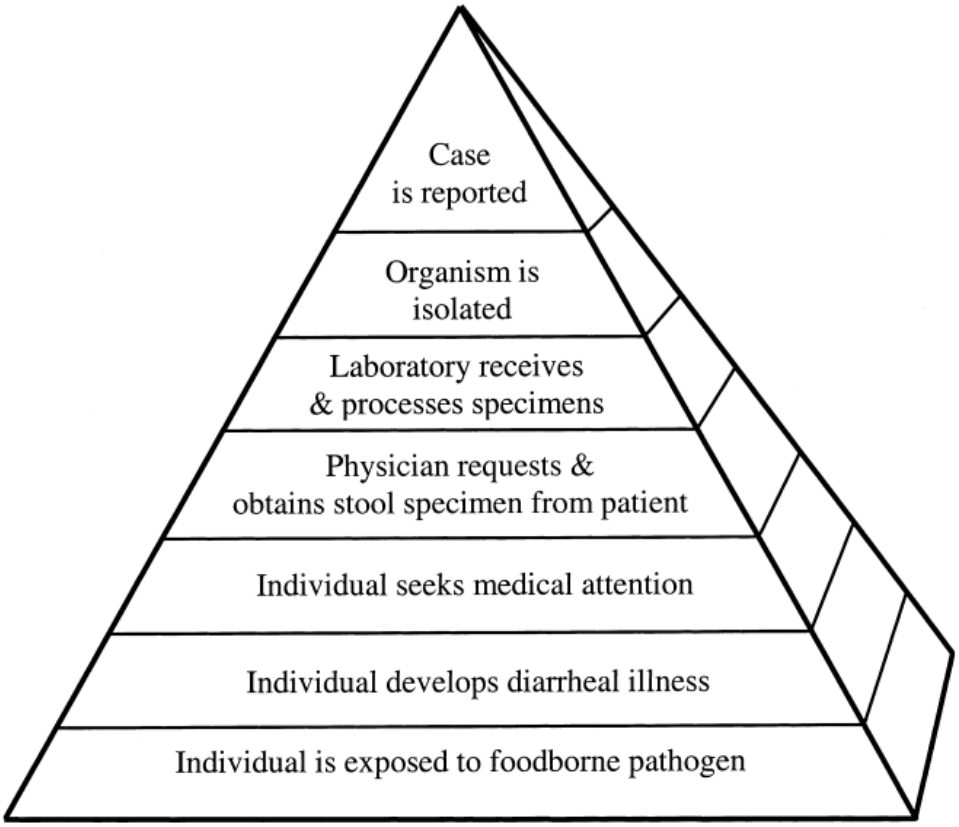


Figure 2.1: Burden of disease pyramid to describe the steps a campylobacteriosis patient must go through before the case is reported to the health department (Adopted from: Hardnett, F.P., 2004)

3.0 Materials and Methods

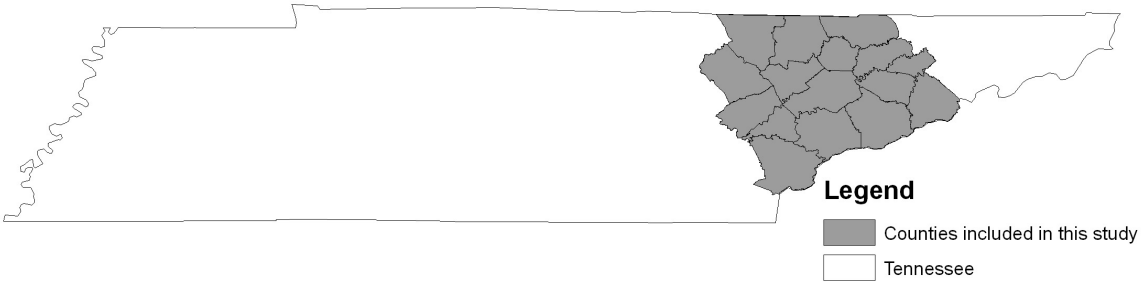
3.1 Study Area

This study was carried out in sixteen counties in the Eastern portion of the state of Tennessee (Figure 3.1, Map A). The selected counties are served by two health departments: the East Tennessee Regional Health Department serves fifteen counties, while Knox County maintains it own health department (Figure 3.1, Map B). The major cities in the study region are Knoxville, La Follette, Maryville, Morristown, Oak Ridge, Pigeon Forge, and Sevierville. The Tennessee portion of the Great Smokey Mountains National Park and the Big South Fork National River and Recreation Area are also located within the boundaries of this area. Many lakes and rivers are spread throughout the area including Watts Bar, Fort Loudon, Chilhowee, Tellico, Douglass, Cherokee and Norris Lakes, the Tennessee and Big South Fork Rivers and the Melton Hill Reservoir. Numerous recreational activities are available on these bodies of water, which may have implications for the transmission of campylobacteriosis.

3.1.1 Climate

The area has a humid subtropical climate that is characterized by hot and humid summers and chilly to mild winters. In Knoxville, the average high/low temperatures are 88°F/69°F in the summer and 46°F/29°F in the winter, the record high temperature is 103°F, while the record low is -24°F. In the higher altitudes of the Smokey Mountain National Park, average temperatures range from -20°F to 50°F in the winter with snow

Map A



Map B

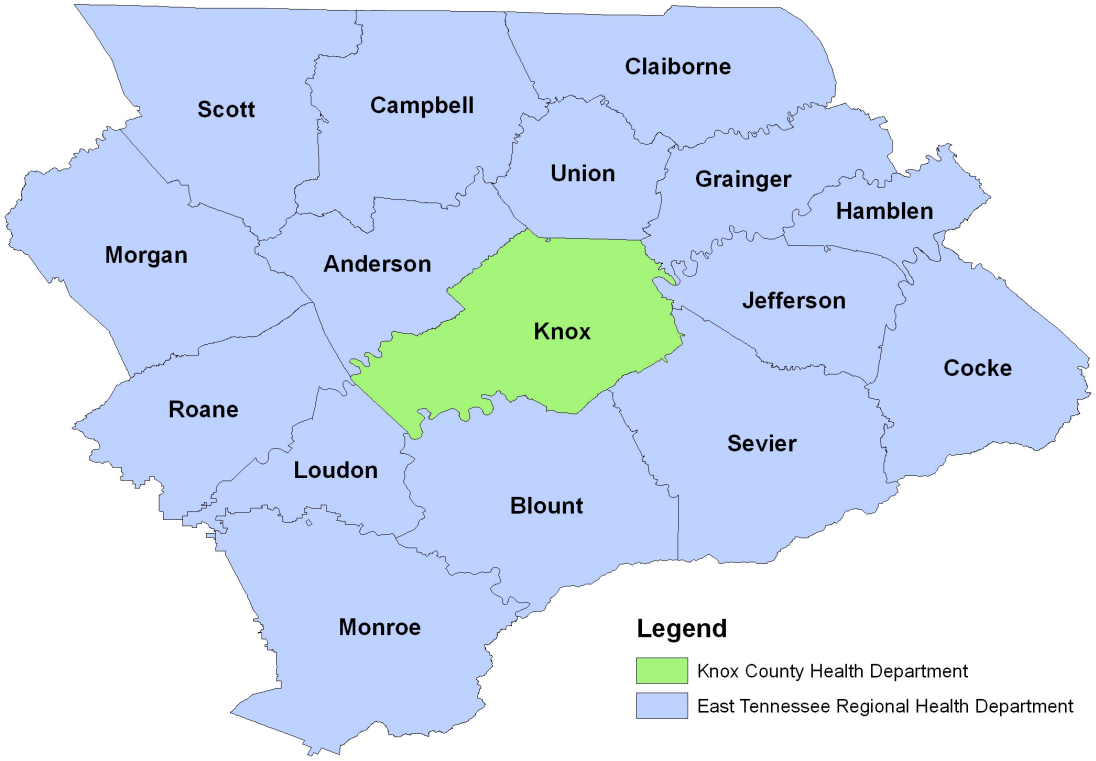


Figure 3.1: The 16 counties involved in the study of campylobacteriosis from 2003-2006 (map A) and the two participating health departments serving the counties (map B)

accumulation, and summer temperatures do not rise above 80°F. The average precipitation in Knoxville ranges from less than 3" per month during autumn to more than 5" per month during spring. In the winter, the higher elevations of the Smokey Mountains average 8" of rainfall and 20" of snow per month, while autumn months average 5" of precipitation per month. The climate in Knoxville is similar for all 16 counties in the study area.

3.1.2 Population

The population in the study region was approximately 1,045,400 as of the 2000 US census. The region has a mixture of urban and rural areas. The US Census defines an urban area as all territory, population and housing units located within areas designated as urbanized areas (population greater than 50,000) or urban clusters (population less than 50,000)[257]. Urbanized areas and urban clusters consist of the census blocks where the population density is at least 1,000 people per square mile, the surrounding census blocks where the population density is at least 500 people per square mile and any census blocks that may connect these clusters. The most populous county is Knox County which had about 382,000 residents in the 2000 census while Union county is the smallest county with only 17,800 residents in 2000. Using United States census definitions for urban and rural, there are some counties (Grainger and Union) where none of the population lives in an urban area, while in Knox County 87% of the residents are considered to live in an urban area. At the census county division (CCD) and census tract level, there are some census tracts and CCDs where 100% of the population is considered to live in an urban area. These are found in Anderson, Blount, Hamblen and Knox Counties. Census tracts are small, relatively permanent statistical subdivisions of a county that are delineated by

the U.S. Census Bureau; the optimum size of each tract is 4,000 people but the population may be between 1,500 and 8,000 people [258]. A CCD is a county subdivision that has been delineated by the U.S. Census Bureau in cooperation with state and local officials for the purposes of presenting statistical data that is generally larger than a census tract but smaller than a county.

The population density in the study region ranges from 38 persons per square mile in Morgan County to 751 persons per square mile in Knox County. Examining population density at the census tract level, the highest densities are 9173 and 7832 persons per square mile in Knox County in two census tracts located near the University of Tennessee. The lowest population densities occur in Claiborne County in a census tract with 14 persons per square mile and one in Cocke County with a density of 16 persons per square mile.

3.2 Data Sources

3.2.1 Campylobacter Case Data

Campylobacteriosis data collected from January 2003 through December 2006 by the East Tennessee Regional and Knox County Health Departments were obtained for this study. Campylobacteriosis has been a notifiable disease in the state of Tennessee since 2003 as a result of the state's participation in FoodNet. Participation in the FoodNet active surveillance system requires that any positive laboratory diagnosis/identification of *Campylobacter* spp. be reported to the local health department[259]. Tennessee is one of 10 states participating in this network and as such

laboratories and physicians are required by Tennessee law (Tennessee Code Annotated 68-10-101) to report any *Campylobacter* positive laboratory results[251] (either stool culture or serologic tests). The campylobacteriosis case definition for this study was adapted from the FoodNet definition. Thus, a case of campylobacteriosis was defined as someone who resides in one of the 16 counties in the study area who was ill, and had a positive laboratory test result for *Campylobacter* spp. from either stool culture or serologic tests.

Once a case is reported, a representative from the health department contacts the patient to follow up and administer a standardized questionnaire to obtain information on patient demographics, clinical symptoms and possible exposures, (see Appendices A1-A2 for list of information collected by health departments). Most of the information collected is entered into an electronic database and electronically submitted, via the National Electronic Disease Surveillance System, to the Tennessee State Health Department and CDC in real time. The datasets used in this study were requested and obtained from the Tennessee State Health Department by each participating health department that then provided to the study investigators. Additional information not included in the original electronic database, but present on the health department paper case report forms was added to the dataset by the investigators (see Appendix A3).

The investigators signed data-user agreements with both participating health departments to keep the identification information for each patient in the dataset secure and confidential. Identifiers (name, address and phone number) were removed from the dataset prior to analysis. This study was approved for research involving human subjects by the University of Tennessee Internal Review Board (approval # 7634B). In order to

limit the possibility of patient identification from the plots of the geographic distribution of cases, geographic analysis was limited to data aggregated at the census tract, CCD, county and region levels.

3.2.2 Demographic and Socio-economic Data

The population data for each county, CCD and census tract were obtained from the 2000 census to serve as the denominators for the calculation of the prevalence of campylobacteriosis. Age- and sex-specific populations were also obtained from the census in order to calculate age- and sex-specific prevalence proportions. The same population was used as the denominator for all 4 years of the study since the population increases proposed by the postcensus estimates were small. The percentage of the population living in an urban area (urbancity) was also obtained from the 2000 census at the county, CCD and census tract level. Additionally the total population of the United States, grouped by age and sex, was downloaded from the 1990 census to use as the standard population for standardization of the prevalences in this study. The 1990 population is used to standardize the prevalence in the population because the 2000 census population was already part of the calculation of the crude prevalence.

3.2.3 Geographic Data

In order to perform geographic analyses, shape files (also called cartographic boundary files) were obtained from the Topologically Integrated Geographic Encoding and Referencing (TIGER) files on the U.S. Census Bureau website (http://www.census.gov/geo/www/cob/bdy_files.html). TIGER files are the automated format that is used by the U.S. Census Bureau to describe land attributes and areas.

These shape files were obtained at different geographic scales: state, county, CCD and census tract. The map data were merged (based on the county, CCD or census tract) with the demographic census data in order to analyze spatial patterns and associations between the geographic location and the prevalence of campylobacteriosis. The area of each polygon (county, CCD and census tract) was also obtained from the TIGER files. Area on the census website is provided in square meters and was converted to square miles. This area information was used to calculate human population densities which were investigated for the potential association with the prevalence of campylobacteriosis.

3.3 Evaluation of *Campylobacter* Data Quality

The quality of the electronic datasets provided by each health department was assessed by evaluating a random sample of 20% of the cases in each dataset. The random number generator function "RANDBETWEEN" of Microsoft Excel version 2003 (Microsoft, Redmond, WA) was used to randomly select the cases for analysis. For each health department and each year of the study, the data quality evaluation involved the assessment of the completeness and accuracy of data entry. When comparing each patient's paper case report form with their record in the computer dataset, the paper case report form was considered the gold standard. If the variable field in the computer record was blank, but information was included on the case report form, this lack of data was considered an incomplete data entry or missing information error. For the purpose of evaluating the dataset, all omissions were counted as errors, since it is impossible to know which omissions are due to inadvertent oversight, data loss during conversion, or

purposeful exclusion. A data accuracy error was defined as a discrepancy between the paper case report and the computer record. For both classes of errors, after the error was noted, the computer record was corrected before further analyses were performed. After the data quality assessment was complete, the other 80% of the computer records were corrected for errors as well. Due to variations in the computer databases used by the state of Tennessee during the study, and a change in the case report form, the number of variables analyzed varied by year, (see Appendix A4 for variables included in the dataset each year).

3.4 Data Manipulations

New variables were created based on the information obtained in the case report forms. These variables (see Appendix A5) were used to investigate potential associations with the prevalence of campylobacteriosis. The patients were divided into 6 groups based on their age: under 5, 5-14, 15-29, 30-49, 50-64, and 65 and over. This classification was selected to correspond with the available groupings in the 1990 and 2000 censuses. Patient age groups were also selected based on other factors such as grade school attendance, and the normal work and retirement age and the epidemiology of campylobacteriosis (highest prevalences reported in children under 5 [239]). To assess differences in care seeking behaviors of different population groups (age group and geographic location), the variable "time waited before seeking medical care" was created. This variable was calculated based on the difference between the date the patient reported the onset of their symptoms and the date of the stool culture submission (considered by

the health department to be the diagnosis date.) Similarly, to determine if patients in East Tennessee differed in the length of stay in the hospital, the number of days in the hospital was calculated from the hospital admission and discharge dates.

To compare the severity of disease among different groups (age or geographic location) of campylobacteriosis patients in this study, a severity of disease score was calculated. The scale used to establish the severity of disease is shown in Table 3.1, which was adopted from a similar scale developed by investigators in a Canadian study[260]. The possible severity scores can range from 0-16 and were grouped into the following categories: mild 1-7, moderate 8-11 and severe ≥12. Additionally, the 16 counties in this study were divided into 4 groups based on the urbanicity (percentage of the population living in an urban area) in each county. Counties were grouped based on the urbanicity of the county in order to assess if the prevalence of campylobacteriosis, the symptoms experienced or the potential risk factors reported were associated with urban or rural areas. The groupings were determined by dividing the total range of urbanicity values (0-87%) by 4, and using this value (22%) as the break point, so the same range of urbanicity values are present in each group. The county groupings are shown in Appendix B1.

Table 3.1: Scale used to assess the severity of campylobacteriosis in patients from a 16 county region in East Tennessee, 2003-2006

Variable	Response	Points Assigned
Duration	<2 days	1
	3-5 days	2
	6-10 days	3
	11-14	4
	>15 days	5
Bloody Stool	No	0
	Yes	3
Fever	No	0
	Yes	2
Cramps	No	0
	Yes	1
Nausea	No	0
	Yes	1
Vomitting	No	0
	Yes	1
Muscle Aches	No	0
	Yes	1
Fatigue	No	0
	Yes	1
Headache	No	0
	Yes	1

3.5 Statistical Analysis

3.5.1 Summary Statistics

Demographic and clinical characteristics together with risk factor variables were summarized to describe the overall characteristics of the study population. Frequency distributions were computed for categorical variables (Table 3.2) and reported as the percent of patients with the characteristic of interest. Binomial 95% confidence intervals around the percentages were also computed. Missing or unknown values were excluded from these computations. The treatments reported were categorized by the type of treatment received, and class of antibiotic, if applicable. Proportions were also calculated for treatment types. Patients were included in more than one category if they were prescribed multiple classes of antibiotics or classes of treatments. The normality of continuous variables (duration of disease, age, severity of disease, time hospitalized, and time waited to seek medical care) was assessed using the Shapiro-Wilk test statistic. Median and range were computed for variables that did not conform to a normal distribution (duration, age, time hospitalized, and time waited). The mean and standard deviation were used to summarize variables with a normal distribution (severity of disease). Each summary statistic was calculated for the entire study region, each county, each year, each age group, and each urbanicity grouping. Differences in medians were tested using the Kruskal-Wallis and Mann-Whitney U tests. All above calculations were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Table 3.2: Categorical variables summarized with patient proportions for campylobacteriosis in a 16 county region of East Tennessee, 2003-2006

Categorical variables analyzed
Age group
Animal exposure
Contact with other sick individuals
Drink untreated water
Handled raw meat
Hike, camp or swim
Household member in daycare
Patient died
Patient hospitalized
Prevalence group
Race
Severity of illness
Serotype
Specimen source
Symptom variables
Test ordered
Travel
Treatment information
Urbanicity group
Water source
Where treatment sought

3.5.2 Assessment of Statistical Associations

To assess differences in the distribution of clinical characteristics, potential risk factors, or treatments among different patient groups, the percentages calculated for each variable above, were compared across groups. The groups included in these analyses were age category, sex, hospitalization status, season, location of where medical care was sought, if an antibiotic was prescribed, urbanicity group, and prevalence group. Possible associations between these variables and clinical and potential risk factor variable were compared across the different strata of each variable. Additionally when comparing the age groups, the under 5 age group was also compared to the rest of the study population over 5 years old. Clinical symptom variables were compared for statistical associations in order to identify combinations of symptoms that were often reported together. Categorical variables were compared using Fisher's Exact test, (for 2x2 comparisons) and Pearson χ^2 test (for larger tables) in SAS. Overall test p value ≤ 0.05 was considered significant. Associations were presented as odds ratios with a 95% confidence interval. When a variable had multiple categories, one was selected as the reference group which was compared with all other groups of that variable. The reference group selected for comparisons was either the first or last group of the variable and the one with the larger proportion of patients in the group.

3.5.3 Prevalence Calculation and Standardization

The crude average annual prevalence of campylobacteriosis was calculated for each county by dividing the average case count over the four year study period by the total population for each county. The average case count was calculated taking the total

number of cases reported in each county over the four year study period and dividing by 4. The 2000 census was used as the population for the denominator in all four years of the study. The resulting prevalence was then multiplied by 100,000 to express the prevalence as cases per 100,000 persons.

The prevalence proportions for each county and CCD were age- and sex-standardized using the direct method and the 1990 total United States population was used as the reference population. Standardization was performed using the "dstdize" command in Stata version 10.0 (Stata Corp., College Station, TX). The standardized prevalence proportion was calculated to remove the influence of age and sex in different populations. This allows for the comparison of the prevalence of campylobacteriosis between counties in the study region and the comparison with the reported prevalence of other states that have been similarly standardized.

After the standardized prevalence for each county was calculated, the counties were divided into 4 groups based on this prevalence. The groupings used were 0-6 cases, 6-12 cases, 12-18 cases and 18-24 cases per 100,000 persons. The groupings by county are shown in Appendices B2 and B3. These groupings were determined by dividing the maximum prevalence in the region (23 cases/100,000) by 4, so 6 cases per 100,000 was used as the cut point in creating the groups. These groups were created to assess differences in the reported potential risk factors, and clinical characteristics based on the standardized prevalence.

Mean annual age-specific, sex-specific and age- and sex-specific prevalence proportions were also calculated. Exact confidence intervals for the prevalence proportions were calculated in Stata. Comparisons of the prevalence proportions across

age groups, sex, or sex and age group were performed using the "prtesti" command in Stata for comparing two proportions. Simes method was used to adjust for multiple comparisons. Overall test p value ≤0.05 was considered significant.

3.5.4 Geographic Analysis

GPS (Global Positioning System) coordinates were obtained for each patient using the home address provided on the case report form. These addresses were geocoded using GPS Visualizer (www.gpsvisualizer.com). The geocoding was conducted at the health department before the data were released to the investigator to ensure that potentially identifying patient information did not leave the health department premises. Point-in-polygon technique of GIS (Geographic Information System) was used to join the GPS coordinates of the address of each case to the county, CCD and census tract map layers, using ArcView GIS 9.2 (ESRI Redlands, CA). This procedure geographically plotted each case in the appropriate county, CCD or census tract based on the geographic location. After plotting, the sum of the total number of cases reported in each county or CCD was calculated. This sum was used to calculate the prevalence for each county. These area specific prevalences were displayed in ArcView GIS, in order to identify areas of high risk. The CCDs were also grouped into 11 groups by urbanicity (0%, 10%, 20%…100%). The group specific prevalence proportions were calculated, as described above (section 3.5.3). Exact 95% confidence intervals were calculated in Stata around each prevalence proportion so as to compare the prevalence proportions for each urbanicity grouping of the CCDs.

3.5.5 Temporal Analysis

The annual prevalence estimates of campylobacteriosis were calculated for each year, as described in section 3.5.3. In order to compare the prevalence of campylobacteriosis in East Tennessee region with the state of Tennessee and all of the FoodNet sites, the annual case counts of reported cases of campylobacteriosis in Tennessee and all of FoodNet were obtained from FoodNet reports[20, 261-265]. The annual prevalence for each year, 2003-2006, was also calculated for the state of Tennessee and all the sites of the FoodNet surveillance program using postcensus estimates for the denominator. These were the populations described in the published reports that were used the denominator for prevalence calculation. The monthly prevalence of campylobacteriosis in East Tennessee was calculated for each of the 48 months in the study. Moving averages were calculated over a 3 month period to smooth out any short-term fluctuations in the prevalence, and highlight the overall trends or cycles. Additionally, the average number of cases occurring in East Tennessee in each calendar month was calculated and the average monthly prevalence determined. In order to assess broader seasonal trends, the months were aggregated into seasons (Spring: March, April, May; Summer: June, July, August; Fall: September, October, November; Winter: December, January, February). The average case count was calculated for each season and used as the numerator to calculate the average seasonal prevalence. The 95% confidence intervals for each of the prevalence proportions described above were computed. Comparisons of these prevalence proportions between years and season was also performed as described under section 2.5.3.

4.0 Results

4.1 Data Quality

Among the 20% subset of cases (79 cases) that were randomly selected, the overall error rate (data accuracy and missing information errors combined) was 6.5% (128/1960). Data accuracy errors comprised 46.9% (60/128) of the total errors, while incomplete data entry or missing information errors comprised the other 53.1% (68/128) of the errors identified. The inaccuracy error rate was 3.0% (60/1960) while the missing information error rate was 3.4% (68/1960). There were some common errors between the health departments. Inaccuracy errors identified in both health departments' datasets were found in the following variables: diagnosis date, zip code and ethnicity. Missing information errors common to both health departments' datasets were observed in: address, zip code, ethnicity and race.

The overall error rate for the East Tennessee Regional Health Department (ETRHD) was 4.7% (51/1090); 70.6% (36/51) of the errors were in data accuracy and 29.4% (15/51) were missing information errors. For the Knox County Health Department (KCHD), the overall error rate was 8.8% (77/870), with 31.2% (24/77) of the errors in data accuracy and 68.8% (53/77) were missing information errors. The overall KCHD error rate was significantly (p=0.0002) higher than that of the ETRHD. Table 4.1 shows the error rates for each health department and year.

The most common variables with errors for the ETRHD were "ethnicity" (13 inaccuracies and 2 cases with missing information), and "source of the specimen tested"

Table 4.1: Data quality evaluation of 20% sample of 8 campylobacteriosis datasets from East Tennessee Regional and Knox County Health Departments, 2003-2006

Health Department	Year	Number of variables in dataset	Cases with errors/Cases analyzed (%)	Total # of fields analyzed	Overall error percentage (total # errors)	Inaccuracy error percentage (# of inaccuracies)	Percentage of missing information errors (# of incomplete entries)
East Tennessee	2003	22	6/16 (37.5)	352	4.26 (15)	3.41 (12)	0.85 (3)
	2004	24	10/13 (76.9)	312	8.33 (26)	4.83 (17)	2.88 (9)
	2005	28	7/12 (58.3)	336	2.98 (10)	2.08 (7)	0.89 (3)
	2006	28	0/4 (0)	112	0	0	0
Knox County	2003	24	8/11 (72.7)	264	5.68 (15)	3.03 (8)	2.65 (7)
	2004	24	8/8 (100)	192	18.2 (35)	6.25 (12)	12.0 (23)
	2005	28	7/7(100)	196	9.18 (18)	0.51 (1)	8.67 (17)
	2006	28	6/8 (75)	224	4.02 (9)	1.34 (3)	2.68 (6)

(10 inaccuracies and 1 case with missing information). The most common inaccuracy errors in the ETRHD dataset included listing an ethnicity when none was specified on the case report form, or listing the blood as the specimen when stool was specified on the case report form. Other variables with inaccuracy errors identified only in the ETRHD datasets, included: city, county, zip code, race, diagnosis date, source of report, whether the patient was hospitalized, hospital name, the date of discharge from the hospital and the status of the patient (hospitalized or outpatient). Variables with missing information errors in the ETRHD datasets were: serotype, race, address, zip code, and city. The 2006 analysis was performed partway through the year so only 4 cases were evaluated for data quality, and no inaccuracies or missing information were observed (Table 4.1).

For the KCHD, the most common variables with errors were ethnicity (8 errors in data accuracy and 6 missing information errors) and travel (3 errors in data accuracy and 8 missing information errors). Other variables with errors in accuracy identified only in the KCHD datasets were: diagnosis date, zip code, street address, the hospital discharge date and patients outcome (alive or dead). Variables that had missing information in the computer dataset were: home phone, physician name, street address, zip code, race, travel dates, and middle name.

Table 4.2 shows the percentage of missing or unknown values for each variable in the corrected dataset. Certain variables have a higher percentage of unknowns due to a change in the data collection form. For example, the "household drinking water source" and the "location where the patient sought medical care" fields were removed from the new case report form introduced in 2006. Additionally, the question about treatment was changed from "any treatment received" to "any antibiotic given". The wording about

Table 4.2: Percentage of patients with missing or unknown information for each variable in the corrected campylobacteriosis dataset from a 16 county region in East Tennessee, 2003-2006 (n=436)

Variable	Number of missing or unknowns values(%)
Race	22 (5.0)
Specimen source	7 (1.6)
Serotype	214 (49.1) *
Test ordered	28 (6.4)
Patient hospitalized	4 (0.9)
Patient died	111 (25.5)
Symptom variables	39 (8.9)
Where treatment sought	228 (52.3) †
Travel	37 (8.5)
Animal exposure	36 (8.3)
Handled raw meat	58 (13.3)
Household member in daycare	40 (9.2)
Contact with other sick	48 (11.0)
Hike, camp or swim	40 (9.2)
Drink bad water	51 (11.7)
Water source	172 (39.4) ‡
Treatment information	126 (28.9)

* Serotype not identified, listed as *Campylobacter* spp.
† 81 unknown, 147 not on case report form
‡ 5 unknown, 167 not on case report form

drinking untreated water was changed as well from "Drink from a spring, stream, or lake?" to "Did the patient drink untreated water in the 7 days prior to onset of illness?" These changes led to a larger percentage of unknowns in the corrected 2006 dataset compared to other years.

4.2 Descriptive Statistics

4.2.1 Demographic Characteristics of Patients

A total of 436 cases of campylobacteriosis were reported in the 16 counties over the 4 year study period. The median age of all cases was 26 (range 1 month to 89 years). There was no significant (p=0.08) difference in the median age of the cases across all years of the study. The majority of cases (53.7%, 234/436) were male; the median age for males was 23.5 while the median age for females was 30.5, but this difference was not statistically significant (p=0.155). Most cases (95.6%, 396/414) were classified as white. Only 3.9% occurred in children aged 6 months or younger, but 15.4% occurred in children aged 1 year or younger. Males accounted for 59% of the under 5 age group, 60.7% of the cases in the 5-14 age group but only 42.9% of the cases in the 65 and over age group.

There was a significant (p=0.0004) difference in the median age of patients when the counties were grouped based on urbanicity (Table 4.3). There was also a significant (p=0.0210) difference in the median age of the patients when the counties were grouped based on disease prevalence. Although the median age is lower in the rural and higher prevalence counties, the percentage of the population that is under 5 years old is not

Table 4.3: Distribution of campylobacteriosis cases based on the prevalence of disease or the percentage of the county that is considered urban in a 16 county region in East Tennessee, 2003-2006 (n=436)

Grouping Variable	No of cases (%)	Percentage of population under 5 years of age	Median age	Crude Group Specific Prevalence
Urbanicity Group				
66% or more	184 (42.2)	6.2	31.5	10.4
44-66%	116 (26.6)	5.7	31.0	10.8
22-44%	102 (23.4)	6.0	16.5**	8.9
22% or less	34 (7.8)	6.4	5.5**	10.7
Prevalence Group				
0 – 6 *	25 (5.73)	6.3	24.0	-
6 – 12	268 (61.5)	6.1	29.0	-
12 – 18	90 (20.6)	5.9	26.5	-
18 – 24	53 (12.2)	6.1	8.0‡	-

*Listed as cases per 100,000 population
- Not applicable
**Significantly different (Simes corrected p=0.025)
‡Group 4 (18-24) is significantly different from group 2 (6-12) (Simes corrected p=0.0083)

significantly different among the counties. The median age also varied greatly among the 16 counties in the study. The county with the lowest median age (3) was Cocke County, while Hamblen County had the highest median age (39 years) (Table 4.4).

4.2.2 Care Seeking and Diagnostic Characteristics

The median delay in seeking a physician's care from the onset of symptoms reported by patients was 4 days (range = 1 – 181 days). Most patients either sought care at a doctor's office (46.6%, 97/208) or an emergency room (ER) (47.6%, 99/208), (Table 4.5). Most (99.5%, 427/429) patients submitted a stool sample (Table 4.5) and stool culture was the predominant test ordered (93.4%, 381/408), but additional laboratory tests were ordered in some cases. The species of *Campylobacter* was identified in 50.9% (222/436) of cases. The most commonly identified species was *Campylobacter jejuni* (98.7%), followed by 2 cases of *C. coli* (0.9%) and the co-infection of *C. jejuni* and *C. coli* in 1 case (0.45%). Twenty-five percent of cases had missing information regarding the disposition (alive or deceased) of the patient after infection with campylobacteriosis, but of those with known information, 1 patient died.

4.2.3 Clinical Description of Cases

The most common clinical symptoms and signs reported were: diarrhea (97.5%), fever (62.5%), cramps (56.2%) and nausea (47.4%), (Table 4.6). Specific combinations of symptoms tended to occur in some patients. Those patients who reported nausea (47.4%) were also likely to report vomiting (38.5%); 25.9% reported both (χ^2 =39.8026, p=<0.0001). Patients who reported suffering from headaches were also likely to report

Table 4.4: Median age of cases reported in each county in a 16 county region in East Tennessee, 2003-2006

County	Median age of cases reported	Range of ages reported
Anderson	38*	1 – 65 years
Blount	30.5	7 months – 74 years
Campbell	33	10 months – 70 years
Claiborne	23	1 month – 87 years
Cocke	3	1 month – 75 years
Grainger	6	2 months – 58 years
Hamblen	39	6 – 75 years
Jefferson	10	1 month – 84 years
Knox	30	1 month – 89 years
Loudon	30	2 months – 62 years
Monroe	9	7 months – 69 years
Morgan	13.5	1 – 55 years
Roane	15.4	2 months – 39 years
Scott	13.5	1 – 66 years
Sevier	24.0	8 months – 71 years
Union	4.0	1 – 75 years

*No significant differences were identified between all counties due to the number of comparisons (120)

Table 4.5: Physician visit location, laboratory and hospitalization information for reported cases of campylobacteriosis in a 16 county region in East Tennessee, 2003-2006

Variable	Number of cases (%)
Location patient sought treatment (n=208)	
Doctor's Office	97 (46.6)
Emergency Room	99 (47.6)
Emergency Room and Doctor's Office	10 (4.8)
Urgent Care Clinic	2 (1.0)
Source of specimen used to identify *Campylobacter* (n=429)	
Stool	427 (99.5)
Blood	2 (0.5)
Test ordered by physician (n=408)	
Culture	381 (93.4)
Culture and Serological	19 (4.6)
Culture and Ova & Parasite	4 (1.0)
Culture, Ova & Parasite and Serological	1 (0.2)
Other	1 (0.2)
Ova & Parasite	1 (0.2)
Serological	1 (0.2)
Campylobacter serotype identified (n=222)	
Jejuni	219 (98.6)
Coli	2 (0.9)
Coli and *Jejuni*	1 (0.4)

Table 4.6: Self reported clinical signs among patients with campylobacteriosis in a 16 county region in East Tennessee, 2003-2006

Variable	No cases (%)
Symptom (n=397)	
Diarrhea	387 (97.5)
Fever	248 (62.5)
Cramps	223 (56.2)
Nausea	188 (47.4)
Vomiting	152 (38.5)
Bloody Stool	150 (37.8)
Fatigue	122 (30.7)
Muscle Aches	122 (30.7)
Headache	106 (26.7)
Chills*	32 (8.1)
Severity (n=374)	
Mild (1-7)	179 (47.9)
Moderate (8-11)	158 (42.2)
Severe (≥12)	37 (9.9)

*Chills as a symptom was not introduced as a check box on the case reports until 2006, but could be added under "Other" prior to 2006

fatigue; 15.4% reported experiencing both (χ^2 =48.8542, p=<0.0001). Other associated symptoms were cramps and nausea (χ^2 =48.5601, p=<0.0001) and headache and muscle aches (χ^2 =42.2214, p=<0.0001). Fever was a commonly reported symptom with 62.5% (248/397) of patients reporting experiencing a fever. Not all cases who reported fever gave the maximum temperature. Only 70.6% (175/248) reported the temperature reading of the fever. The median high temperature was 102.0°F (range: 99.0 - 105.0°F).

Certain symptoms of campylobacteriosis were significantly associated with the age of the patient (Table 4.7). The youngest age group (under 5) was used as the reference group in all comparisons. Bloody stool was the only symptom for which all older age groups had lower odds of reporting the symptom when compared with the under 5 age group. For the other symptoms listed above the older age groups were more likely to report experiencing the symptom. Symptoms were also statistically associated with the urbanicity grouping of the county where the patient resided (Table 4.8). When, the most urban category (66-88% urban) was used as the reference group, the more rural counties had higher odds of reporting bloody stool, nausea, vomiting, cramps, and fatigue.

The median duration of disease was 7 days (range: 1-60, 180 days); this was the same for both males and females. There was no significant difference in the duration of symptoms when the counties were grouped by urbanicity or by the prevalence of disease. The mean severity of disease score was 7.6 ±3.1 (severity score was normally distributed) and this was the same for males and females. Patients with a score of 1-7 were considered mild (47.9%), 8-11 moderate (42.2%) and a score of 12 or more was

Table 4.7: Odds ratios of statistical associations between disease characteristics and the age group of the patient in a 16 county region in East Tennessee, 2003-2006

Comparison Variable	Age Group*	Odds Ratio (Confidence Interval)	Exact p value
Was the patient hospitalized? (yes)	5-14 years old	2.9 (1.2-6.7)	0.015
	15-29 years old	1.0 (0.40-2.7)	1
	30-49 years old	2.1 (0.97-4.4)	0.068
	50-64 years old	4.4 (1.9-9.7)	0.0004
	65 years and older	11.5 (4.6-28.4)	0.0004
	5 years and older†	2.8 (1.5-5.5)	0.0008
Bloody Stool (yes)	5-14 years old	0.60 (0.30-1.2)	0.21
	15-29 years old	0.72 (0.37-1.4)	0.33
	30-49 years old	0.37 (0.21-0.66)	0.0009
	50-64 years old	0.24 (0.11-0.51)	0.0001
	65 years and older	0.21 (0.084-0.53)	0.0006
	5 years and older	0.41 (0.26-0.66)	0.0002
Headache	5-14 years old	17.1 (3.7-79.6)	<0.0001
	15-29 years old	36.5 (8.2-162.0)	<0.0001
	30-49 years old	29.5 (6.9-126.2)	<0.0001
	50-64 years old	21.1 (4.6-96.0)	<0.0001
	65 years and older	9.5 (1.8-49.7)	0.005
	5 years and older	24.0 (5.8-99.5)	<0.0001
Nausea	5-14 years old	3.2 (1.4-7.0)	0.004
	15-29 years old	9.3 (4.4-19.7)	<0.0001
	30-49 years old	8.2 (4.2-16.1)	<0.0001
	50-64 years old	6.1 (4.6-96.0)	<0.0001
	65 years and older	4.6 (2.0-10.8)	0.0005
	5 years and older	6.4 (3.6-11.5)	<0.0001
Cramps	5-14 years old	3.2 (1.5-6.6)	0.0019
	15-29 years old	7.6 (3.6-15.8)	<0.0001
	30-49 years old	6.8 (3.7-12.7)	<0.0001
	50-64 years old	3.3 (1.7-6.7)	0.0008
	65 years and older	1.9 (0.83-4.2)	0.14
	5 years and older	4.5 (2.7-7.5)	<0.0001
Muscle Aches	5-14 years old	1.8 (0.62-5.4)	0.26
	15-29 years old	6.1 (2.5-14.8)	<0.0001
	30-49 years old	9.0 (4.0-20.6)	<0.0001
	50-64 years old	10.8 (4.4-26.4)	<0.0001
	65 years and older	4.3 (1.5-12.0)	0.0078
	5 years and older	6.4 (3.0-13.8)	<0.0001
Fatigue	5-14 years old	2.2 (0.89-5.6)	0.09
	15-29 years old	6.4 (2.9-14.3)	<0.0001
	30-49 years old	3.6 (1.7-7.6)	0.0006
	50-64 years old	7.0 (3.1-16.0)	<0.0001
	65 years and older	3.9 (1.5-10.1)	0.008
	5 years and older	13.0 (6.7-25.50	<0.0001

*Reference Group: Youngest age group (under 5 years old)
†Under 5 age group compared to all groups over 5 years old

Table 4.8: Associations between symptoms of campylobacteriosis and the urbanicity of the county of residence for a 16 county region in East Tennessee, 2003-2006

Variable	Urbanicity Group*	Odds Ratio (Confidence Interval)	Exact p value
Bloody Stool	44-66% urban	1.8 (1.1-3.1)	0.025
	22-44% urban	2.2 (1.3-3.7)	0.0042
	0-22% urban	3.2 (1.5-7.1)	0.0053
Fever	44-66% urban	2.1 (1.2-3.5)	0.0054
	22-44% urban	1.9 (1.1-3.3)	0.018
	0-22% urban	1.2 (0.55-2.6)	0.7
Headache	44-66% urban	2.8 (1.6-4.8)	0.0004
	22-44% urban	1.5 (0.83-2.8)	0.2
	0-22% urban	1.8 (0.75-4.3)	0.2
Nausea	44-66% urban	3.6 (2.2-6.0)	<0.0001
	22-44% urban	1.4 (0.81-2.3)	0.3
	0-22% urban	2.6 (1.2-5.6)	0.025
Vomiting	44-66% urban	1.5 (0.90-2.5)	0.15
	22-44% urban	2.4 (1.4-4.0)	0.0014
	0-22% urban	2.6 (1.2-5.6)	0.02
Cramps	44-66% urban	3.6 (2.2-6.2)	<0.0001
	22-44% urban	1.2 (0.72-2.0)	0.5
	0-22% urban	4.4 (1.8-10.7)	0.0007
Muscle Aches	44-66% urban	2.5 (1.5-4.4)	0.0009
	22-44% urban	2.6 (1.5-4.6)	0.0013
	0-22% urban	1.9 (0.83-4.5)	0.15
Fatigue	44-66% urban	6.4 (3.5-12.0)	<0.0001
	22-44% urban	5.2 (2.7-9.8)	<0.0001
	0-22% urban	6.4 (2.7-15.0)	<0.0001

*Reference Group: most urban (66-88% urban)

considered severe (9.9%) as shown in Table 4.6. The majority of the 37 cases in the severe category were in the 30-49 age group (15/37, 40.5%). The mean severity score was highest in the 15-29 age group at 8.6 (95% CI: 7.7 - 9.4) and the lowest in the 65 and over age group at 6.5 (95% CI: 5.3 - 7.6).

4.2.4 Treatment

A wide rage of therapeutic agents were prescribed, namely: broad spectrum antibiotics, antiprotoazoals and antivirals (Table 4.9). Most (73.6%) patients were prescribed only 1 therapeutic agent, 15.5% were prescribed 2, 3.2% were prescribed 3 and 7.7% had no therapeutic agent prescribed. Antibiotics were the most commonly prescribed therapeutic agents with 84.5% of patients reporting receiving at least 1 antibiotic. Of the patients who knew the class of antibiotics prescribed, fluoroquinolones and macrolides were the two most common.

4.2.5 Hospitalization

Of the 432 cases with known hospitalization information, 101 (23.4%) were hospitalized. The median length of stay in the hospital was 2 days (range: <1 to 11). During the 4 years of the study, 2004 had the highest percentage of annual hospitalizations (26.4%). The annual, seasonal, and monthly hospitalization percentages are shown in Table 4.10 along with the 95% confidence interval. Among the four seasons, the highest percentage of hospitalizations was observed in summer 28.4% (46/162) but this was not significantly different from other seasons. September had the highest percentage of cases hospitalized at 38.9% (14/36); September and July (30.9%, 21/68) each had a significantly higher monthly percentage hospitalizations than the

Table 4.9: Treatments reported by campylobacteriosis patients in a 16 county region of East Tennessee, 2003-2006 (n=310)

Treatment type	No cases reporting treatment(%)*
Antibiotic	262 (84.5)
B-Lactam	4 (1.5)
Cephalosporin	5 (1.9)
Fluoroquinolone	95 (36.3)
Macrolide	84 (32.1)
Sulfonamide	5 (1.9)
Tetracycline	5 (1.9)
Unknown Antibiotic	73 (27.9)
Intravenous fluids	31 (10.0)
No treatment	18 (5.8)
Antidiarrheal/AntiNausea	14 (4.5)
Antiprotazoal	14 (4.5)
Oral rehydration	6 (1.9)
Other†	11 (3.6)
Number of therapeutic agents prescribed	
1 therapeutic agent	228 (73.6)
2 therapeutic agents	48 (15.5)
3 therapeutic agents	10 (3.2)

* Percentages sum to over 100 since some therapeutic agents were prescribed in combination

† "Other" includes: pain drugs (3), proton pump inhibitors (2), steroids (2), appendectomy (2), antiviral (1), and H_2 receptor antagonist (1) (blocks histamine receptor- 2 to decrease stomach acid production)

Table 4.10: Temporal and seasonal proportions of patients hospitalized due to campylobacteriosis in a 16 county region in East Tennessee, 2003-2006 (n=436)

Time frame	Total number of cases (%)	Number of cases hospitalized (%)	Confidence interval for percentage of cases hospitalized
Year			
2003	140 (32.1)	36 (25.7)	18.7-33.8
2004	110 (25.2)	29 (26.4)	18.4-35.6
2005	97 (22.2)	21 (21.6)	13.9-31.2
2006	89 (20.4)	17 (19.1)	11.5-28.8
Season*			
Summer	162 (37.2)	46 (28.4)	21.6-36.0
Fall	103 (23.6)	21 (20.4)	13.1-29.5
Winter	91 (20.9)	18 (19.8)	12.2-29.4
Spring	80 (18.4)	18 (22.5)	13.9-33.2
Month			
January	26 (6.0)	2 (7.6)	9.4-25.1
February	29 (6.6)	5 (17.2)	5.8-35.8
March	27 (6.2)	7 (25.9)	11.1-46.3
April	24 (5.5)	6 (25.0)	9.7-46.7
May	29 (6.6)	5 (17.2)	5.8-35.8
June	48 (11.0)	14 (29.2)	17.0-44.1
July	68 (15.6)	21 (30.9)**	20.2-43.2
August	46 (10.6)	11 (23.9)	12.5-38.8
September	36 (8.3)	14 (38.9)**	23.1-56.5
October	29 (6.6)	5 (17.2)	5.8-35.8
November	38 (8.7)	2 (5.3)**	0.6-17.7
December	36 (8.3)	11 (30.6)	16.3-48.1

*Seasons were defined as, Summer: June, July, August, Fall: September, October, November, Winter: December, January, February, Spring: March, April, May

**June and September significantly differed from November

month of November (5.3, 2/38), which had the lowest percentage of hospitalized campylobacteriosis patients.

The oldest age group (65 and above) experienced the highest percentage of hospitalization (60.0%), followed by age groups 50-64 (36.2%) and 5-14 (27.3%). The lowest percentages of hospitalizations were observed in the under 5 age group (11.5%), followed by 15-29 (11.9%) and 30-49 (21.2%). Hospitalization proportions were significantly associated with age (p=<0.0001). Using the under 5 age group as the reference group, these associations are shown in Table 4.7. All groups except the 15-29 age group had significantly higher odds of being hospitalized than the under 5 age group. Patients over 5 years old had much higher odds of hospitalization than those under 5 (OR: 2.8, CI: 1.5-5.5). The most rural counties had the lowest percentage of cases hospitalized at 12.0% (4/34; CI 3.3 – 27.4), while the counties that were 44-66% urban had the highest percentage of cases hospitalized at 29.6% (34/115; CI 21.4 – 38.8%). This difference was statistically significant (p=0.036).

4.2.6 Risk Factors for Campylobacteriosis

Exposure to animals was the most commonly reported risk factor with 74.2% of cases reporting being exposed to one or more animals in the week (up to 10 days) prior to the onset of symptoms (Table 4.11). Of those exposed to animals, the most common animal was dogs (79.5%) and the least common was turkeys (0.7%). Animal exposure was significantly associated (p=0.0041) with the age group of the patient, with patients in the 65 and older age group having significantly lower odds of reporting animal exposure than the under 5 year old patients (Table 4.12). None of the other age groups had

Table 4.11: Exposure to suspected risk factors for acquiring campylobacteriosis in the 10 days prior to symptom onset as reported by patients in a 16 county region in East Tennessee, 2003-2006

Risk Factor	No of cases (%)
Any animal exposure (n=400)	297 (74.2)
Specific animal exposure (n=297)	
Cats	98 (33.0)
Cattle	17 (5.7)
Chickens	29 (9.8)
Dogs	236 (79.5)
Goats	11 (3.7)
Horses	4 (1.4)
Lizards	4 (1.4)
Rodents	13 (4.4)
Turkeys	2 (0.7)
Turtles	4 (1.4)
Handle raw meat/poultry (n=378)	80 (21.2)
Have a household member in daycare (n=394)	33 (8.4)
Have contact with someone with similar symptoms (n=388)	57 (14.7)
Hike, camp, fish or swim (n=394)	75 (19.0)
Drink from a spring, stream, or untreated water (n=385)	40 (10.4)
Travel (n=399)	86 (21.6)
Destination –International	45 (11.3)
Destination – Domestic	41 (10.3)
Water source (n=262)	
City	172 (65.7)
Well	82 (31.3)
Spring	8 (3.1)

Table 4.12: Odds ratios of statistical associations between risk factors for campylobacteriosis and the age group of the patient, in a 16 county region in East Tennessee, 2003-2006

Risk Factor	Age Group*	Odds Ratio (95% Confidence Interval)	Exact p value
Animal Exposure (yes)	5-14 years old	1.9 (0.72-5.1)	0.26
	15-29 years old	0.99 (0.45-2.2)	1
	30-49 years old	0.75 (0.39-1.4)	0.42
	50-64 years old	0.54 (0.26-1.1)	0.12
	65 years and older	0.29 (0.13-0.66)	0.004
Handle Raw Meat	5-14 years old	1.0 (0.72-5.1)	1
	15-29 years old	10.0 (3.2-31.6)	<0.0001
	30-49 years old	12.8 (4.3-37.9)	<0.0001
	50-64 years old	9.4 (2.9-30.0)	<0.0001
	65 years and older	2.8 (0.66-11.9)	0.22
Household Member in Daycare	5-14 years old	0.59 (0.18-1.9)	0.58
	15-29 years old	0.22 (0.048-1.0)	0.049
	30-49 years old	0.94 (0.41-2.2)	1
	50-64 years old	0.056 (0.0032-0.96)	0.002
	65 years and older	0.19 (0.024-1.5)	0.11

*Reference group: Youngest age group (under 5 years old)

significant associations with animal exposure when compared with the reference age group.

International travel was reported by 52.3% of patients reporting travel, while the other 47.7% reported domestic travel. Travel outside the area of residence was significantly associated with the age of the patient (p=0.0055), the urbanicity of the county (p<0.0001), and the prevalence grouping of the county (p=0.0007) (Table 4.13). All age groups except the 5-14 year olds had higher odds of reporting travel outside the area when compared with the under 5 years age group. Similarly, patients in the more rural counties had lower odds of reporting travel than those in the most urban counties and patients from counties of lower prevalence had increased odds of travel outside the area of residence when compared with patients who reside in counties of higher prevalence.

Having a private well as the household drinking water source was reported by 31.3% of all cases. Drinking untreated water (not limited to the household water source) was significantly associated with the urbanicity of the county of residence (p=0.0002) Patients in the middle urbanicity groups (22-44% and 44-66% urban) had lower odds of reporting drinking untreated water at 0.37 (95% CI: 0.15-0.95) and 0.25 (95% CI: 0.094-0.69) respectively, when compared with cases who lived in the most urban counties. The most rural counties were not significantly different in the percentage of patients who reported drinking untreated water when compared to the reference group – the most urban counties.

Hiking, camping, fishing or swimming in the 10 days prior to the onset of symptoms was reported by 19% of the cases. Engaging in one of the four activities was

Table 4.13: Associations between travel outside the area and age group, urbanicity group and the prevalence group for a 16 county region in East Tennessee, 2003-2006

Grouping Variable	Group*	Odds Ratio (95% Confidence Interval)	Exact p value
Age Group	5-14 years old	2.3 (0.84-6.2)	0.11
	15-29 years old	5.1 (2.1-12.0)	0.0001
	30-49 years old	3.3 (1.4-7.4)	0.0048
	50-64 years old	2.3 (1.0-6.9)	0.047
	65 years and older	4.0 (1.4-10.8)	0.0095
Urbanicity Group	44-66% urban	0.36 (0.20-0.66)	0.0008
	22-44% urban	0.25 (0.12-0.51)	<0.0001
	0-22% urban	0.20 (0.059-0.69)	0.0053
Prevalence Group	0-6 cases/100,000	5.5 (1.2-24.6)	0.025
	6-12 cases/100,000	5.6 (1.7-18.6)	0.0013
	12-18 cases/100.000	1.9 (0.50-7.3)	0.54

*Reference Groups:
Age – under 5 age group, Urbanicity – 66-88% urban, Prevalence – 18-24 cases

significantly associated (p=0.0009) with the season of the year. Cases in the summer had 2.3 (95% CI: 1.1 - 4.6) times higher odds of reporting engaging in one of these activities than those in the fall and 4.7 (95% CI: 1.9-11.6) times higher odds than those in the winter. There was no significant difference (p=1.0) in the frequencies of reports of these activities between summer and spring.

While handling raw meat was only reported by 21.2% of the cases, it was significantly associated with sex (p=0.017) and age (p=0.0002). Women had 1.5 (95% CI: 1.0-2.7) times higher odds of reporting handling raw meat than men. Patients in the middle age groups (15-29, 30-49 and 50-64) had higher odds of reporting exposure to raw meat compared to the under 5 age group (Table 4.12). There was no significant difference in the odds of handling raw meat between the under 5 age group and the 5-14 and 65 and over age groups. Having a household member in daycare was also significantly associated (p=0.013) with the age group of the patient. Patients in the 15-29 and 50-64 age groups had significantly lower odds of reporting a family member in daycare than the under 5 age group. The other comparisons were not significant.

4.2.7 Prevalence Distribution

The mean crude prevalence of campylobacteriosis in the 16 county region during the study period was 10.4 per 100,000 (95% CI: 9.5 - 11.4). The crude prevalence estimate for all FoodNet sites in the US during the same period was 12.7 per 100,000 (95% CI: 12.4 - 13.0), while that of the entire state of Tennessee was 7.40 per 100,000 (95% CI: 6.7 - 8.1).

The county level age- and sex-standardized prevalences of campylobacteriosis are presented in Figure 4.1. Grainger and Jefferson counties had the highest prevalences at

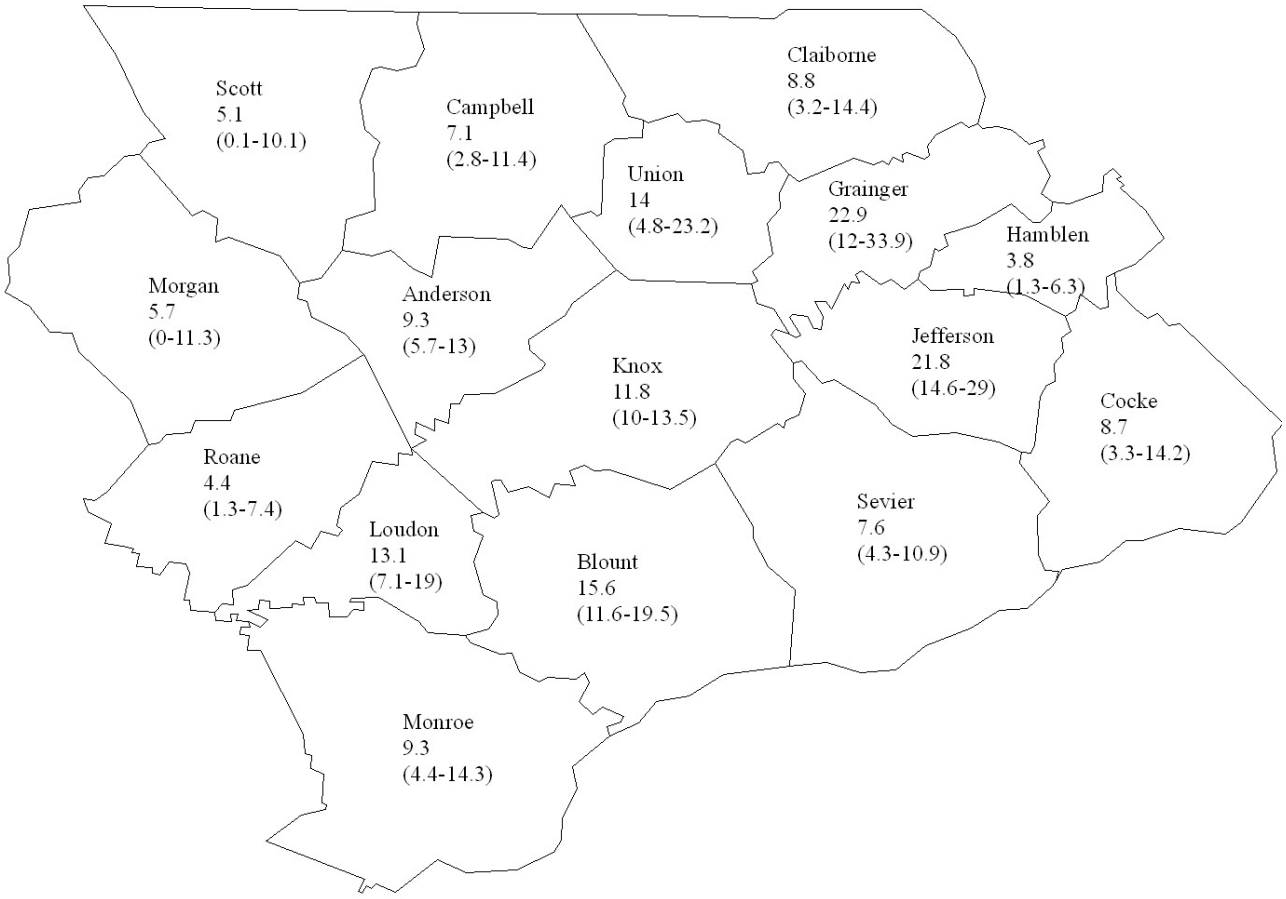


Figure 4.1: Age- and sex-standardized prevalence of campylobacteriosis and 95% confidence intervals for a 16 county region in East Tennessee, 2003-2006

22.9 and 21.8 cases per 100,000 population, respectively; while Hamblen and Roane counties had the lowest at 3.8 and 4.4 cases per 100,000 population, respectively. Some counties in the study area had prevalence values that were much higher than the national average. Age- and sex-standardized prevalence estimates for each CCD are show in Figure 4.2; no cases were reported in some CCDs (white) and the prevalence of campylobacteriosis was the highest in the Philadelphia CCD of Loudon County and the Washburn CCD of Grainger County at 67 and 57 cases per 100,000, respectively (dark blue). The CCDs were grouped based on the urbanicity of the residents, in 10% increments. The prevalence of campylobacteriosis in these groups ranges from 7.6 – 14.0 cases per 100,000, but none of the groups differed significantly and there was no trend in the prevalence of campylobacteriosis.

The age- and sex-specific prevalence estimates of campylobacteriosis for the entire study region are shown in Figure 4.3 for each age group. The highest reported prevalence estimate was observed among male children under the age of 5 at 47.7 cases per 100,000 (95% CI: 36.6 - 61.2), and the lowest was reported among females aged 65 and older at 5.95 cases per 100,000 (95% CI: 3.6 - 9.2). There was no statistically significant ($p>0.05$) difference in the prevalence of campylobacteriosis between males and females across all age groups.

4.2.8 Temporal Patterns

The mean annual prevalence of reported cases of campylobacteriosis in East Tennessee gradually declined over the 4 year study period, while the prevalence in the US and the state of Tennessee remained approximately the same (Figure 4.4). The overall crude prevalence of campylobacteriosis was 13.39 cases per 100,000 population

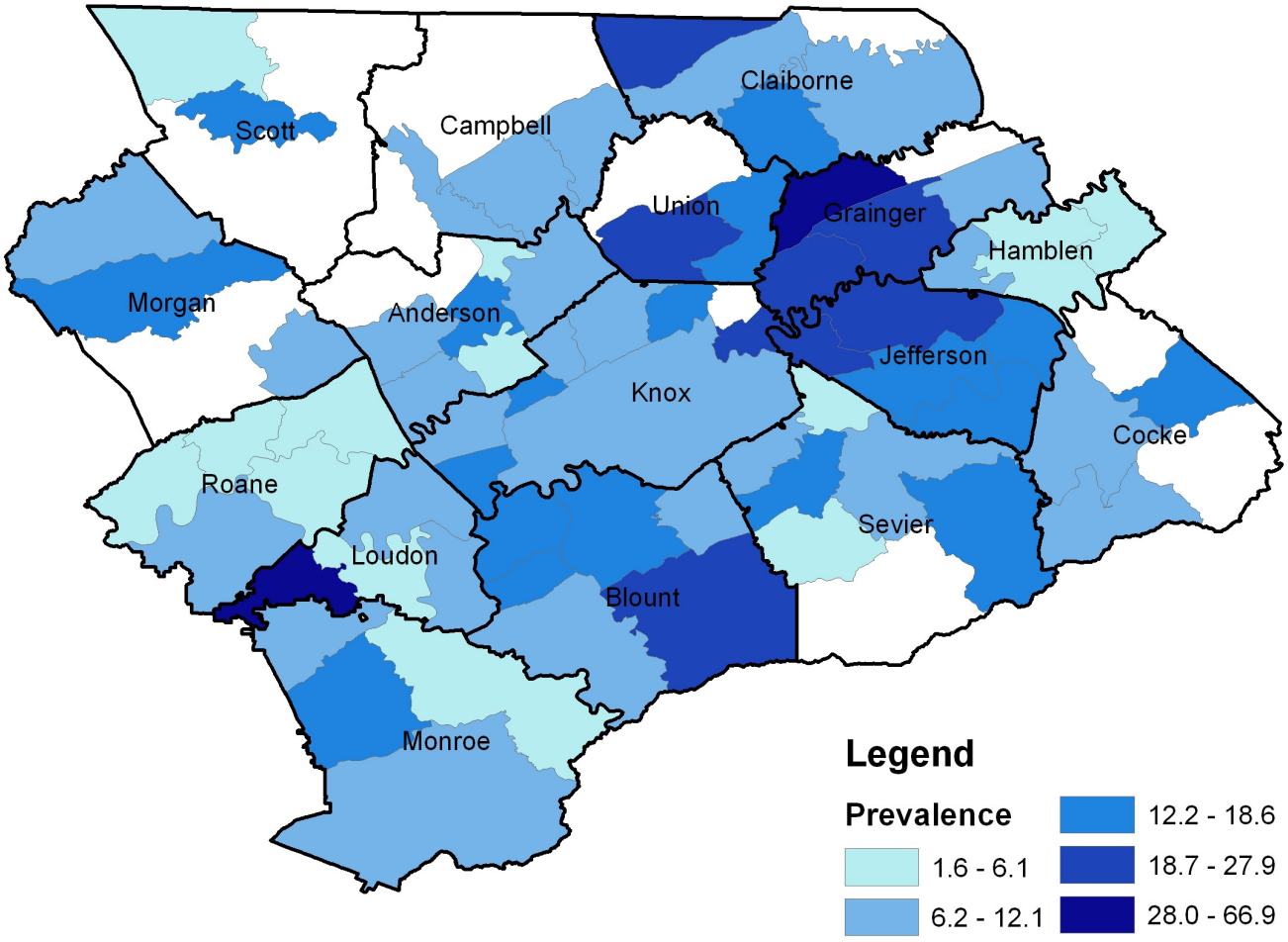


Figure 4.2: Age- and sex-standardized prevalence of campylobacteriosis for each census county division in a 16 county region in East Tennessee, 2003-2006

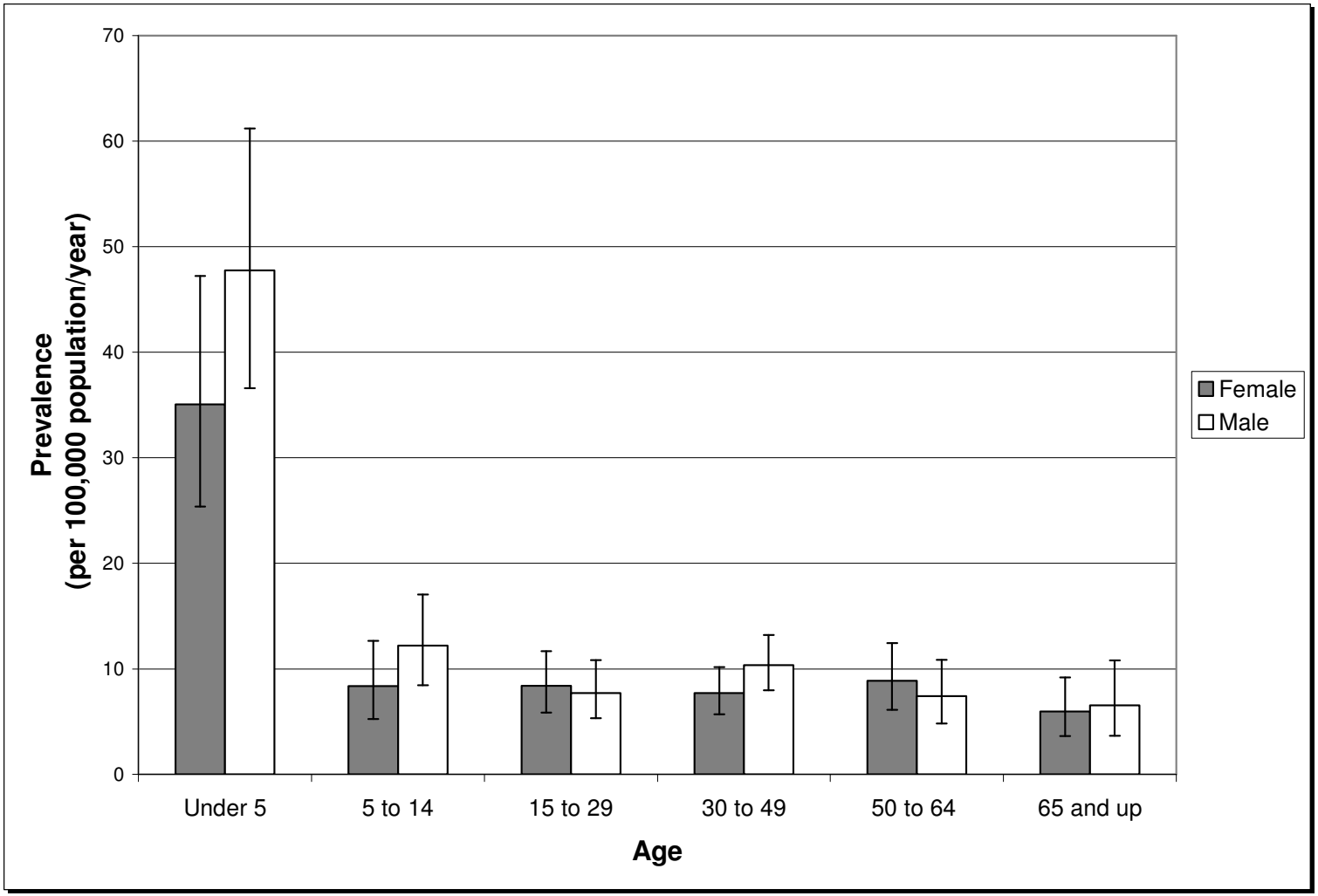


Figure 4.3: Age- and sex-specific prevalence of campylobacteriosis in a 16 county region in East Tennessee, 2003-2006. (Error bars represent the 95% confidence interval)

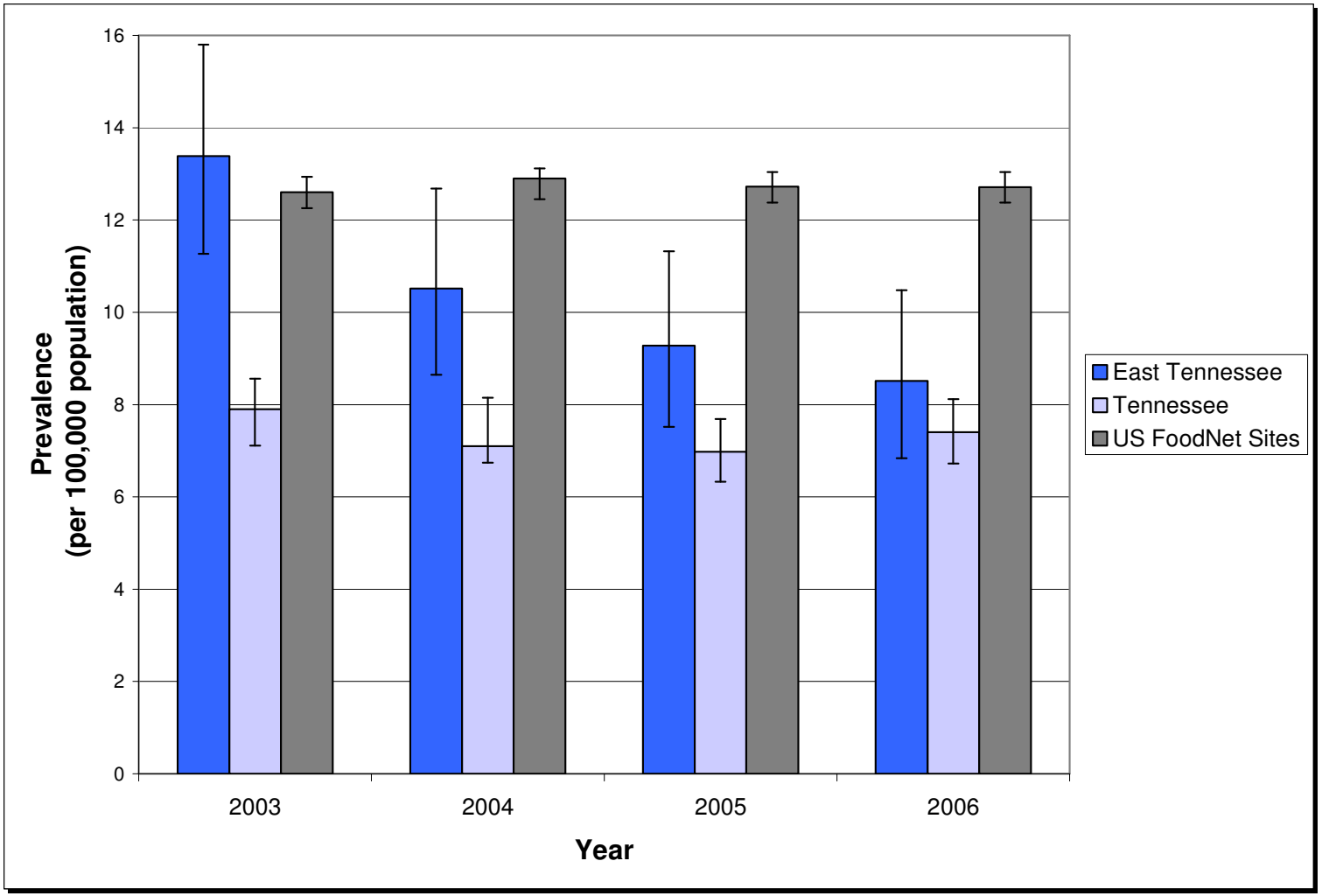


Figure 4.4: Annual prevalence of campylobacteriosis for a 16 county region in East Tennessee, the entire state of Tennessee and all US FoodNet sites, 2003-2006. (Error bars represent the 95% confidence interval)

in 2003 in ET. This prevalence was not statistically different from the prevalence of disease for the entire FoodNet area in the United States, of 12.60 cases per 100,000 ($p=0.4759$), but was significantly ($p<0.0001$) different from the prevalence of disease in the state of Tennessee (7.81 per 100,000). By 2006 the prevalence of disease in ET had decreased to 8.51 per 100,000. This was significantly lower ($p=0.0002$) than the prevalence in the US (12.71 per 100,000) but not significantly ($p=0.227$) different from that of the state of Tennessee (7.40 per 100,000). The annual prevalence of campylobacteriosis in ET in 2003 was significantly different from the prevalence in 2005 ($p=0.005$) and 2006 ($p=0.0008$). The critical p-value of Simes correction for multiple comparisons was 0.0167. There was no significant difference in the prevalence of disease among the other years of the study.

The monthly prevalence of campylobacteriosis and 3-month moving average are shown in Figure 4.5. The linear regression line shows an overall decreasing temporal trend in the prevalence of disease in the region. Over the course of the study, the mean monthly prevalence had a decreasing trend at a rate of 0.0085 cases (per 100,000) per month. A consistent peak in the reported prevalence of campylobacteriosis occurred in the summer months of each year. In November of 2004 there appeared to be an off-seasonal peak in the prevalence of campylobacteriosis, but this peak was not significantly higher than the prevalence during the other winters of the study period (Figure 4.6). The average summer prevalence (3.9/100,000) was significantly higher ($p=0.0003$) than that in the fall (2.5/100,000), winter (2.2/100,000, $p<0.0001$) and spring (1.9/100,000, $p<0.0001$). The summer of 2003 had highest seasonal prevalence at 5.6 cases per 100,000 (CI: 4.2-7.2), while the spring of 2004 had the lowest at 1.3 cases per 100,000

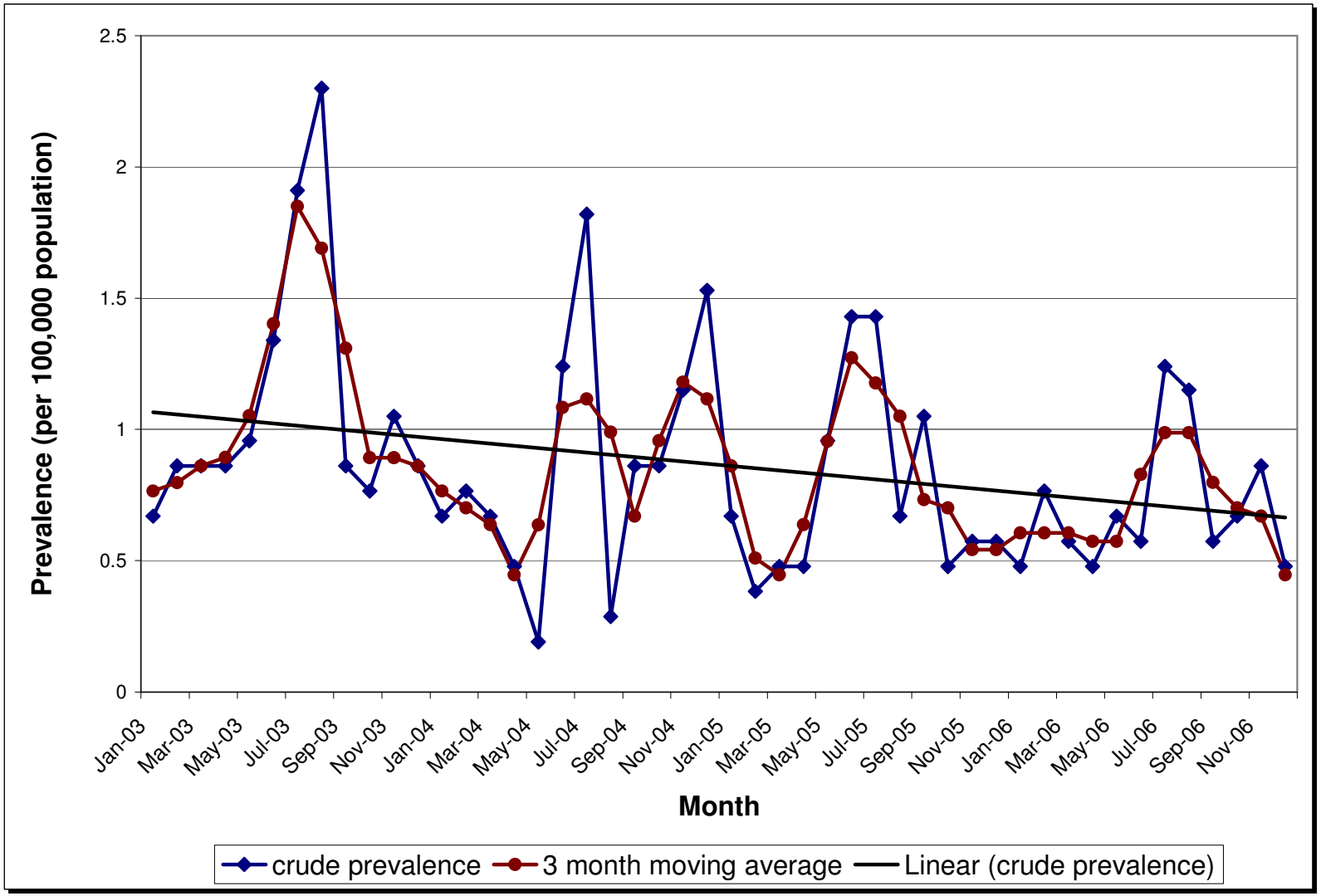


Figure 4.5: Monthly prevalence estimates of campylobacteriosis for a 16 county region in East Tennessee, 2003-2006.

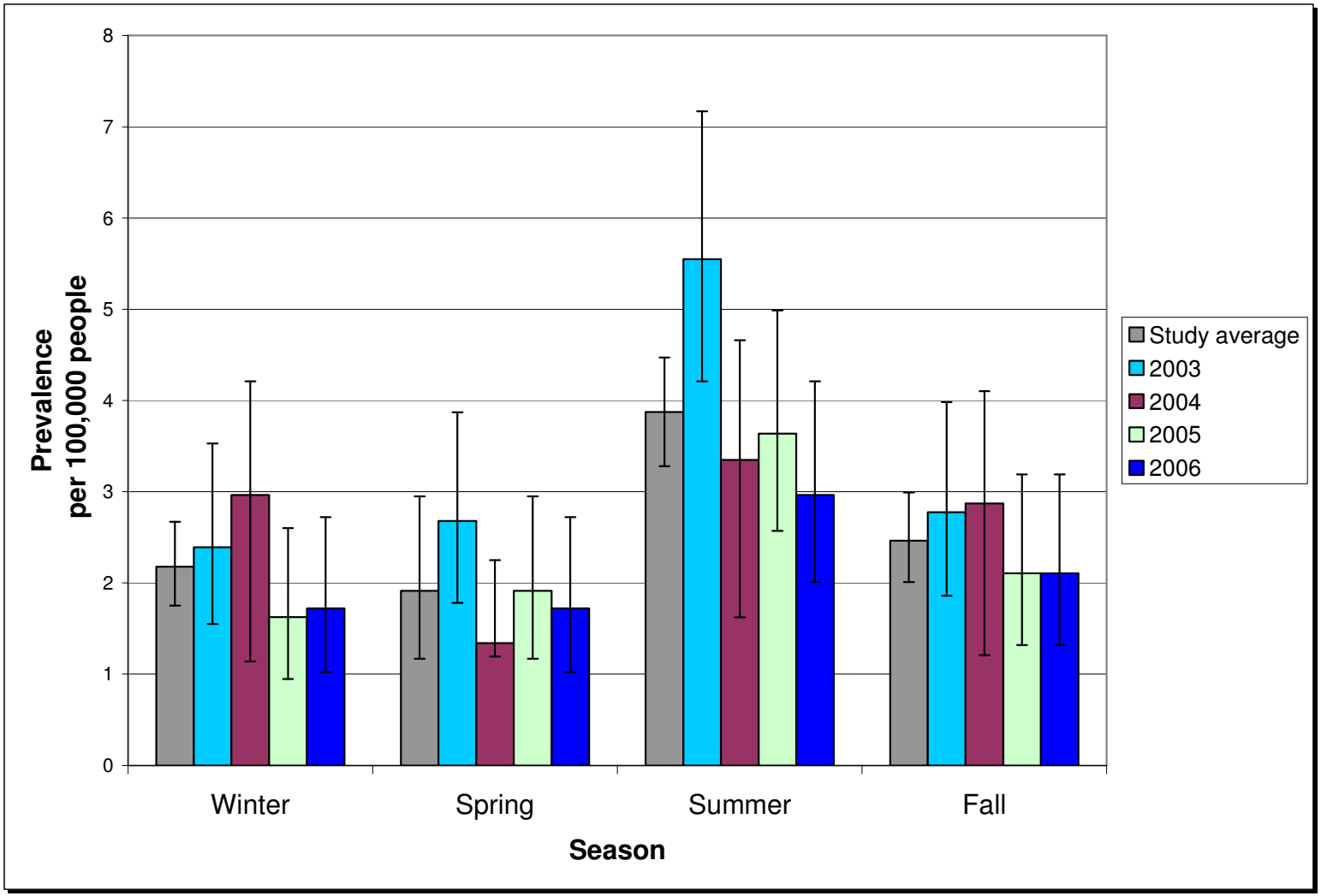


Figure 4.6: Seasonal prevalence of campylobacteriosis for a 16 county region in East Tennessee, 2003-2006. (Error bars represent the 95% confidence interval)

(95% CI: 0.73- 2.2) (Figure 4.6). The only significant difference in the monthly prevalences was identified between the summer months (June, and July) and the month of April (Figure 4.7). July had the highest average reported prevalence of 1.6 cases per 100,000 (95% CI, 1.3-2.1) followed by June (1.1 cases per 100,000; 95% CI, 0.86-1.5) and August (1.1 cases per 100,000; 95% CI, 0.80-1.5). April had the lowest average reported prevalence at 0.57 cases per 100,000 (95% CI, 0.37-0.85).

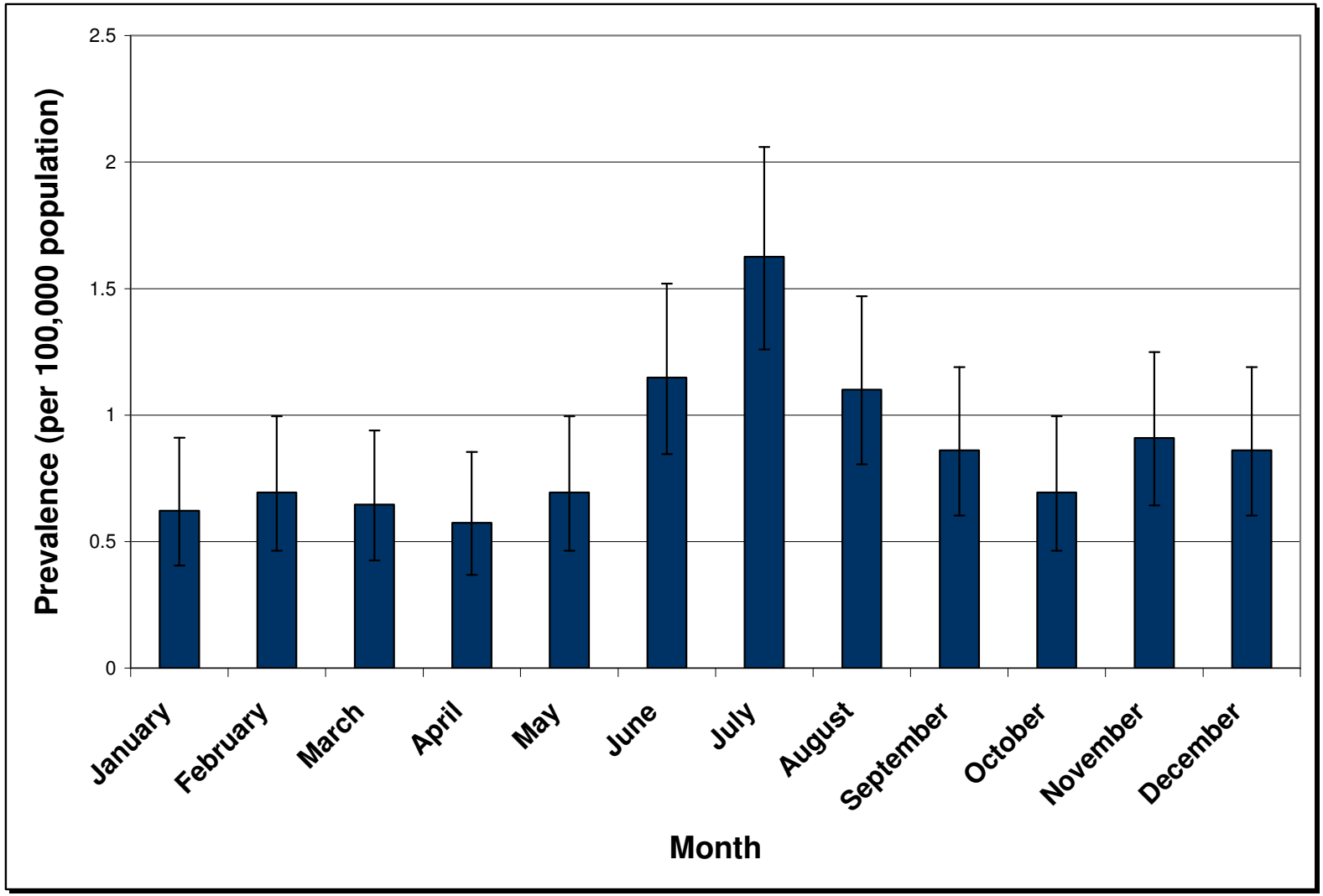


Figure 4.7: Average monthly prevalence of campylobacteriosis in a 16 county region in East Tennessee, 2003-2006. (Error bars represent the 95% confidence interval)

5.0 Discussion

The goal of this study was to perform a descriptive epidemiologic analysis of the cases of human campylobacteriosis reported in East Tennessee from January 2003 to December 2006. Information resulting from the study may be used by health departments in East Tennessee to help enhance their strategies for disease control and prevention. Generally, most of the epidemiologic characteristics of the campylobacteriosis cases observed in this study were similar to those in other studies conducted in the United States and around the world. For instance, the highest prevalence occurred in young children under the age of 5 and during the summer months. These are patterns typical of campylobacteriosis in the developed world.

The error rate observed in the data used in this study varied greatly depending on the year, the type of error (inaccuracy or missing information) and the health department (KCHD or ETRHD). Missing information or omissions in the computerized dataset, when information was present on the paper case report form, were classified as missing information errors since it can not be determined if the data was missing due to an oversight, a computer malfunction or purposeful omission. The highest proportions of errors, from both health departments, were observed in 2004. One reason for these high error rates could be a change in the database structure that occurred when a new data storage software application was adopted in 2004. When this change occurred, differences in the field names in between the two different databases may have caused data to be deleted from some entries. It is also possible that data could have shifted between entries, for example, the address for one patient was assigned to another patient

numerically ahead of that entry. Since this change in database structure occurred during 2004, only cases reported prior to the change were affected. These issues suggest that there needs to be safeguards put in place to take care of potential data integrity issues during system upgrades and improvements in data structure.

Another problem observed in the data entry was that many patients were entered into the database as white or Caucasian when the race or ethnicity box on the case report form was left unchecked. These patients were entered into the systems as "race = white" or "ethnicity = non-Hispanic", when they should have been entered in as "race = unknown" or "ethnicity = unknown". This resulted in the high inaccuracy error rate observed in the race (10/79, 13%) and ethnicity (30/79, 38%) variables. The "travel" variable had a high percentage of missing information errors (8/79, 10%). The paper case report form indicated the patient had reported travel and this was not in the computer database. Some of these errors were caused by the fact that the variable "travel" was added to the electronic database partway through the study. It was introduced into the computer database during 2005, but due to the way the data is stored, all cases prior to the introduction date still had the travel variable present in their database entry, but it was blank.

The inaccuracy error rates observed in this study were similar to that reported for data quality in electronic medical databases[266, 267]. A study of a voluntary participation database of low birthweight infants in Vermont, found inaccuracy error rates between 1.3 - 8.8%[266]. That study analyzed ten variables for disagreement between the medical record and the computerized database. Another study conducted in Pennsylvania analyzed the results of multiple published studies that reported the accuracy

of computer-based patient records (CPRs). The inaccuracy error rate of the CPRs ranged from 0 to 64%[267], but most of the studies analyzed reported an inaccuracy error rate between 3-7%. In this study the highest inaccuracy rate observed was 6.2% in the 2004 Knox County dataset, and the overall inaccuracy rate for all datasets was 3%. As with the missing data errors, the maximum and average percentages of inaccurate entries in the campylobacteriosis database in this study were within the range of inaccuracy errors of CPRs in the literature[267]. It was important for all the variables in the datasets used in this study to have complete and accurate information since they were used to create sub populations, to identify groups with a higher prevalence of campylobacteriosis.

Missing data error rates observed in this study were low (3.4%). When missing data is expanded to included unknowns the percentage ranges from 0-52% (Table 4.2), these are similar to other descriptive studies of gastrointestinal illness using surveillance data[268, 269]. In published reports the percentage values that are unspecified or missing has a wide range. A Canadian study of *Salmonella* serotype typhimurium reported 0-99.8%[268] missing or unspecified values depending on the variable. In that study, variables that had all data present were "Episode date" and "Disease", while the variables with the highest percentage of missing data were "Hospitalization" (71.5%) and "Risk factor" (99.8%). Another Canadian study of cryptosporidiosis reported a similar range of 0-89.7%[269] of cases with missing or unspecified values. In both Canadian studies the percentage of missing values was lower among the variables that were mandatory for reporting, especially demographic variables. Of the mandatory variables in the Canadian studies, "Risk Setting" had the highest percentage of missing values (49.6%). In the present study, the highest percentages of unknowns were observed for the variables

"Where was treatment sought" (52%) and Serotype (49%). These variables were not required for reporting, while demographic variables such as age, sex, and race were, and as such had low (0-5%) percentages of unknowns.

The median age of patients in this study (26 years) was within the range of median ages reported by two Norwegian studies (22 and 29 years)[270, 271], but lower than median age of a 2000 case-control study in Quebec (median: 31 years, range: 11days – 91 years)[197] and a 1999 United States FoodNet case-control study (median: 34, range: <1-96)[198]. In the current study, the median age was significantly lower in the more rural counties. The study area (ET), on average, has more of the population classified as rural compared to the other FoodNet sites and this difference and the lower median age observed in the rural areas may have influenced the lower median age observed in this study compared to other US studies.

The percentage of cases under the age of 1 (9%) was much higher in ET than other published studies that reported 1.9% in New Zealand[19], and 3.5-4.0% in US[239] of cases under 1 year old. In this study, the percentage of patients under 5 years old (24 %) was also higher compared to those of other studies: 16.9% in Norway[270], 13.4-13.8% in the US[239], 12.0% in New Zealand[19], and 11.5% in the United Kingdom[203]. A Polish study that analyzed the test results of all stool samples that were submitted for enteric disease isolation, found that 59% of the campylobacteriosis isolates were obtained from children under 2 years old[272]. That is much higher than the percentage observed in this study and may be due to differences in exposures or health care practices. In countries where there is universal heath care, more adults may seek medical care for their symptoms than in counties without universal health care

which can affect the percentage of children in the study. It is possible that the age differential observed in the US (more children seek medical care compared to adults) is more pronounced in Poland, or that children are exposed at a much higher rate in Poland so adult have some immunity and do not seek medical care.

The median age of patients from the most rural counties in ET was lower than that of the rest of the counties in this study (Table 4.3). This is similar to the findings of a previous study that reported that more children were reported in the rural areas compared to the urban ones and the odds of infection increased in the more rural areas, especially for young children[245]. The lower median age in the rural counties could be due to the fact that young children in the more rural counties may be exposed to *Campylobacter* at a higher frequency than children in the more urban counties due to higher risk of animal exposure. Moreover, the predominance of children in the rural areas of ET could be partially explained by the large percentage children in this study (>50% of children under 1 year old) who drink privately owned well water that may not be appropriately disinfected.

On average patients in ET waited 3 to 4 days to seek medical care after the onset of their symptoms, 52% waited 3 days or less. This delay may reduce the effectiveness of antibiotic treatment. A meta-analysis of studies of the treatment of campylobacteriosis found that patients who waited 3 days or more to seek care reported the duration of diarrhea to be twice as long as patients who sought care less than 3 days after the onset of symptoms[86]. That study also reported that in 50% of the studies, the patients waited less than 3 days, while in the other 50% of the studies, the patients waited an average of 6.5 days to seek care. The reason for the longer delay in seeking care observed in

patients in ET may be due to the fact that patients in the US normally have to wait several days before they can be seen in a private physician's office[273]. One survey of health care practices found that 50% of adults in the US had to wait for 2 or more days to see a physician[273]. Generally, most patients will wait for symptoms to warrant medical care (either in severity or duration), and then many will have to wait several additional days for an appointment to be seen by a physician. These delays may prolong the duration of symptoms.

More patients in the present study (50%) visited an emergency room for their illness compared with patients in the FoodNet case-control study (37%)[198]. It is possible that the lower percentage of patients seen in the emergency room in the 7 states involved in the FoodNet study could be due differences in the physician to patient ratio or differences in the proportion of patients with health insurance between ET and the FoodNet sites. The decision to seek medical care for their symptoms may be based on the patient's socioeconomic status as well. A US study of adult healthcare practices found that 57% of adults with "below average income" would choose not to visit a physician when ill due to the cost compared to only 12% of adults with "above average income"[274]. In Tennessee, there are programs for children to obtain health insurance through TennCare (the state healthcare provider) so more children may be seen by private practice physicians, since they have insurance, while those over 18 who do not have insurance may go to the emergency room to seek medical care. The percentage of patients seen in a physician's office, in this study, was higher for patients under 18 years of age (55%) than those over 18 (45%) but this difference was not statistically significant.

Most (99.5%) of the cases of campylobacteriosis in ET were diagnosed from a stool sample and the rest (0.5%) were identified from a blood sample; this percentage is identical to that observed in the FoodNet case-control study in 1998-1999[198]. The descriptive FoodNet study from 1996-1999 found similar results, 99% of samples were from stool and 1% were from blood[239]. Approximately half of the *Campylobacter* isolates in the present study were sent to the state laboratory for speciation. The percentages of each *Campylobacter* species observed in this study (*C. jejuni*: 98.6%, *C. coli*: 0.9%, dual infection: 0.4%) are similar to those observed in 7 states of the United States in 1998-1999. That FoodNet case-control study of 1316 patients, reported 95% *C. jejuni*, 4% *C. coli*, 1% *C. lari*, and 1 case of *C. mucosalis*[198]. In a Norwegian study, 92% of the 212 cases were infected with *C. jejuni* while the remaining 8% were infected with *C. coli*[271]. A study of 285 cases in France found 81.7% infected with *C. jejuni,* 15.3% with *C. coli*, 1.3% with *C. fetus*, and 1.7% with *C. lari*[275]. The lower isolation rate of *C. coli* (commonly identified in pigs) observed in the current study may be due to either fewer isolates being sent to the laboratory for species determination, or a lower prevalence of the bacteria in the region. The lack of *C. lari* and *C. upsaliensis* (reservoirs unknown) isolation in ET may be due to its low prevalence in the region or the growth inhibition of *C. upsaliensis* by the antibiotics used in the standard stool culture media[41].

Diarrhea is considered a hallmark symptom of campylobacteriosis, 97.5% of patients in ET who provided clinical information reported experiencing diarrhea. This is similar to other descriptive studies. A smaller study conducted in Denver, CO in 1979 reported 100% [117], a report from England and Wales observed 96% [276], and a

French study reported 96.5%[275] of patients experienced diarrhea. On the contrary, volunteers who were experimentally infected with *C. jejuni* all had positive stool cultures, indicating infection, but only 46% experienced diarrhea[76].

Just over a third of the patients in this study reported bloody diarrhea. This may be under-reported since some patients may not have examined their stool closely and may have missed seeing blood. This percentage is lower than other reports from the United States but similar to other worldwide reports and the results of experimental infections. In the FoodNet case-control study, 45% of patients reported bloody diarrhea[198], while a 1978 US study reported 42%[117]. Human experimental infections, conducted in Maryland, found that of volunteers with positive stool cultures, 36% experienced bloody diarrhea[76]. Worldwide, bloody diarrhea was reported by 35.2% of patients in New Zealand [199], 28% in England and Wales[276], 45% in Iran[277], 22% in Yemen[278], 10% in Bangladesh[279] and 15% in Thailand[280]. The percentage of patients who experience bloody diarrhea may depend on the virulence of the strain or species of *Campylobacter* and the immune status of the patient[281]. If the bacteria are able to damage the intestinal epithelial cells sufficiently by invasion or toxins, bleeding may occur.

The majority of patients in ET also reported fever (62.5%), and cramps (56.2%); only 37.5% of patients reported experiencing both. These symptoms were observed at a lower rate than the FoodNet case-control study in which over 80% of patients reported fever and cramps[198] and a smaller 1978 study of 35 patients where 96.6% experienced "abdominal pain" and 91% experienced fever[117]. Among studies conducted in other industrialized countries, these symptoms were also reported more frequently than the

findings of the current study[19, 276]. In a study conducted in Yemen, a lower percentage (21%) of patients reported fever but "abdominal cramps" were reported by a higher percentage (86.6%) when compared to patients in ET. Since symptoms in this study were self reported, differences in interviewer phrasing, interpretation of the responses or degree of emphasis of the questions could have influenced the response of the patient. One reason for the lower percentage of patients reporting cramps, in this study, may be the large proportion of children under 5 in the study dataset. These children may be unable to understand what the symptom is or be able to communicate the source of their discomfort to their parents who were interviewed for this information.

Associations between clinical symptoms in the present study were examined to determine if symptoms were commonly reported in combination, which may indicate similar strains of *Campylobacter* spp. A quarter of the patients reported nausea and vomiting together; this is logical since nausea is the conscious recognition of the subconscious excitation of the medulla associated with the vomiting center[179]. Also associated were the reporting of nausea with cramps, headache with fatigue and headache with muscle aches. None of these associations of symptoms formed temporal or spatial clusters that would indicate that the cases may have been associated or from the same strain.

Interviewer phrasing may have also influenced the percentage of patients reporting chills. In 2006, the symptom "chills" was added to the new campylobacteriosis case report form as a possible symptom in the form of a check box. After the new case report form was implemented, the interviewer specifically mentions chills, while prior to this time chills was only reported if the patient initiated the response when questioned for

"other". After this change in the case report form, the percentage of patients who reported chills dramatically increased. During the first 3 years of the study (2003-2005), 1.0% to 6.4% of patients reported experiencing chills, however in 2006 this percentage jumped to 22.4%. This increase was not likely due a true increase in percentage of patients experiencing chills, but more likely due to its inclusion on the symptom list on the case report form, about which patients are questioned.

There was an association between some symptoms of campylobacteriosis and the age of the patient, in this study. These associations were investigated to see if there was a pattern of symptoms being reported by patients in different age groups, since previous studies reported an association between age and bloody stool. Patients in the youngest age group (under 5) were more likely to report bloody diarrhea than those in the older age groups (Table 4.7). A similar observation was made in France where 54.8% of children 15 and under reported bloody diarrhea while only 35.1% of patients over the age of 15 reported it [275]. A study in Thailand also reported a similar result; 30% of children under 5 years old experienced bloody stool while only 15% of all ages experienced it[280, 282]. It has been hypothesized that this age related difference in the occurrence of bloody diarrhea, could be due to the infection being the first exposure to *Campylobacter* among young children which cause bloody diarrhea[283].

While a statistical association between age group and non-visible symptoms of campylobacteriosis exists, it may have no clinical relevance. These include the non-visible symptoms of nausea, cramps, muscle aches, headache and fatigue where is no physical evidence of the symptom. Since the parents may not recognize these symptoms

in their young child if the child could not communicate these symptoms to their parent, one or more of these symptoms may have been present but not reported.

The duration of symptoms varied widely among the patients in ET (range: 1 day-180 days). The patient that reported 180 days also reported experiencing gastrointestinal symptoms for several months before seeking medical care and may have attributed previous symptoms to their campylobacteriosis diagnosis. The median duration of disease (7 days) experienced by patients in this study is similar to reports from the US and other industrialized countries. In the FoodNet case-control study of 1999 the median duration was 6 days, (range: 1-31 days)[198]. The CDC reports that most cases recover completely in 2-5 days, but sometimes recovery can take up to 10 days[284]. A smaller study in Colorado reported that 80% of patients experienced a duration of illness of 1 week or less[117]. A 1982 study in West Germany found the average duration of diarrhea to be between 2 and 7 days[285]. The median duration of disease was also 7 days, but with a much shorter range(1-16 days) in New Zealand[199], while the range was much longer (0-701 days) in England and Wales where an average of 10.7 days of illness per patient was reported[276]. Reducing the duration of disease can have substantial effect on the cost of campylobacteriosis by reducing hospitalizations costs and lost wages.

When classified by the severity of disease index, almost half (48%) of the patients in this study experienced mild disease and less than 10% experienced severe disease. The scale was used to provide a method to classify patients according to their severity of disease and possibly identify population groups who experience more severe disease, however none were identified. One disadvantage of the severity of disease scale is that it

is heavily dependent on symptoms of disease, many of which are not visibly identifiable in young children such as: muscle aches, fatigue or cramps. The highest severity score for a child under 5 years old was 13. That child was 4 years old and may have been able to communicate more of the signs of illness to their parents; that child reported fatigue, cramps ad nausea. If the children in the youngest age group (under 5) are removed from the mean calculation, the mean severity score increases from 7.6 to 8.1±3. This increase may not necessarily indicate that adults experience more severe disease than children, but may be due to the fact that they are able to report symptoms themselves. A better severity of disease scale could have two possible values, one for adults and children over 5, and a different value for children under 5.

The following recommendation for the treatment of campylobacteriosis is found at the CDC website:

> "Almost all persons infected with Campylobacter recover without any specific treatment. Patients should drink extra fluids as long as the diarrhea lasts. In more severe cases, antibiotics such as erythromycin or a fluoroquinolone can be used, and can shorten the duration of symptoms if given early in the illness. Your doctor will decide whether antibiotics are necessary."[284]

The majority (84%) of patients in this study received at least one antibiotic. The most common antibiotics prescribed were fluoroquinolones and macrolides which are in line with the CDC's recommendations. No information on antibiotic sensitivity testing of the *Campylobacter* isolates was collected in this study, but sensitivity testing may become more common since antibiotic resistant *Campylobacter* are becoming more prevalent[286].

The percentage of isolates that are fluoroquinolone resistant is increasing worldwide, especially in developing countries[82]. In my travels in developing countries, I noticed it was common practice to take a single antibiotic pill, instead of a full course of several days of treatment, to treat minor infections and other illnesses such as a cold. Such practices may play a role in the higher *Campylobacter* resistance rates in these countries. This is also important since many adults in developing countries can be asymptomatically infected with *Campylobacter*. Due to the high level of *Campylobacter* resistance to fluoroquinolones outside the United States (84% in Thailand[287] and 72% in Spain[140]), doctors should consider travel history and exercise caution when prescribing fluoroquinolones to those who have traveled internationally[146]. It is important since some studies have shown that treating fluoroquinolone-resistant campylobacteriosis with a fluoroquinolone causes more severe disease[144].

The youngest age group (under 5) had the lowest percentage of patients who reported receiving an antibiotic (77%) while 100% of patients in the 65 and over age group received at least one antibiotic. The higher percentage of patients receiving an antibiotic in the oldest age group may be due to concerns of the physician that older patients may have other medical conditions that could increase the risk of complications if the infection is not treated aggressively[288]. The lower frequency of antibiotic treatment among young children may be due to fears of antibiotic complications[135]. In young children there is an increased risk of hemolytic uremic syndrome (HUS) if infected with *E. coli* O157:H7 which is the most common cause of bloody diarrhea in children[135]. Due to the risk of complications, antibiotics should not be prescribed to

children before the etiologic agent can be confirmed and a confirmatory diagnosis made[48].

Similar percentages of infants under age 1 were treated with antibiotics in ET (72%) and other FoodNet sites (72%) [14]; however the percentage for children under age 1 year old and 5 years (77%) were much higher than other countries. A Swedish case-control study of children under 6 reported that only 13.4% were prescribed an antibiotic[204]. This difference could be due to differences in the specific policy and treatment guidelines of the national public health agency of each country. In the US, the CDC recommends that an antibiotic could shorten duration and a doctor will decide if one is necessary. While the Swedish Society for Communicable Disease Prevention and Control and The Swedish Institute for Infectious Disease Control both state that the infection generally clears up on its own with oral rehydration and antibiotics are only given in rare cases[289, 290]. The emphasis of the Swedish recommendations is against the empiric treatment with antibiotics and this may be the reason why fewer children received them.

On average, 23% of the campylobacteriosis patients in this study were hospitalized each year during 2003-2006. This is higher than that reported in the descriptive FoodNet study of 12,707 patients from 1996 to 1999 (10%)[239], the FoodNet case-control study of 1316 patients in 1998-1999 (12%)[198] and a 2001 report of 286 patients in the Denver metropolitan area (9.8%)[291]. The percentage of hospitalizations in ET is also much higher than in other countries: reported percentages of hospitalizations are 3% in Ontario, Canada[292], 4.9 % in New Zealand[19], 10.2% in England and Wales[276], and 10.8% in Denmark[293]. In the present study, some (30%)

patients spent 1 day or less in the hospital, if these patients are removed, the proportion of patients hospitalized (LOS >1, 16.5%) was also higher than expected. Of the states participating in the descriptive FoodNet study, Georgia had the highest percentage of cases hospitalized at 15.2%[239]. Georgia borders part of Tennessee to the South and the percentage observed there is closer to that observed in ET. The higher than expected percentage of hospitalizations among the older patients (65 and up) may have led to a higher than expected overall percentage hospitalizations.

The observed high hospitalization percentage (60%) among the 65 and older age group of patients in this study was much higher than the overall percentage (23%) for all ages. This difference could be due to complications from other health conditions that are more common in older patients. The hospitalization proportion observed in the oldest patients (65 and up: 60%, 60 and up: 56.3%) was also much higher than that reported in the 1996-1999 FoodNet study (26.8% of patients 60 and older)[239] and a New Zealand study (9.6% of patients 60 and older)[19]. The lower percentage of hospitalization of older patients observed in New Zealand may be due to differences between the health care systems of the two countries. In New Zealand, on average, patients are seen by a doctor sooner, may be treated sooner, may not develop as severe of disease and therefore may not need to be hospitalized. In a study of worldwide healthcare practices, 58% of adults in New Zealand were seen by a doctor the same day and 81% were seen by the next day compared to the US where only 30% are seen on the same day and 47% by the next day[273].

The under 5 age group had the lowest percentage of patients hospitalized (11.5%). This may be due to the fact that parents take their younger children to the doctor at the

first sign of illness and the child is then treated early on in the course of disease thus reducing the need for hospitalization. One theory for the higher hospitalization percentage in the 5-14 age group (27.3%) was that older children may wait longer to seek medical care resulting in a higher hospitalization percentages, however this was not validated in this study as the median delay in seeking care was 3 days for both age groups.

Although, patients in the present study were hospitalized more often than the rest of the country and other parts of the world, the length of stay (LOS) was on average shorter for patients in this study (median: 2 days, range: <1 to 11 days). In the FoodNet study using data collected from 1996-1999 the mean LOS was 4.6 days and 8% of the cases that were hospitalized spent more than one week in the hospital[239]. Only 4 of the 101 (4%) hospitalized patients in ET remained in the hospital for 7 days or more. A Denver 2001 report also observed that 4% of patients had a LOS greater than 7 days[291]. The mean LOS was longer (3.9 days) in England and Wales[276]. It is possible that the patients who were hospitalized in this study were less severely affected as patients who were hospitalized in other states and countries and therefore did not require as long of stay in the hospital. Patients in this study who were hospitalized had a higher severity score than those who were not.

The highest percentage of hospitalizations (28.4%) occurred in the summer, and the fewest in the winter (19.8%). This difference may be due to a greater seasonal potential for exposure due to differences in behaviors, such as more outdoor activities and more travel. During the summer, due to the hot weather, more recreational water activities occur, in rivers and lakes, such as fishing, boating, and swimming. Also more

outdoor barbecues occur during the warmer months; during these events there is a higher risk of consumption of undercooked meat and cross contamination of food which could lead to a larger inoculum of the bacteria from the undercooked food. The seasonal differences in the hospitalization percentages observed in this study are unlike those described in New Zealand where the percentage of cases hospitalized remained almost constant between 4.7 and 5.0 percent in each season[19]. Although the seasons are reversed in New Zealand and the climate of the entire country is not exactly the same as ET, there are still large temperature differences between the seasons, similar to the temperature changes observed in ET.

During the 4 year study period, only 1 (0.3%) patient died. A 4 year FoodNet study of 5 sites reported that 11 patients died as a result of their illness[239]. The average annual campylobacteriosis specific mortality risk (number of deaths due to campylobacteriosis/total study population) was 0.096 deaths per 100,000 in ET and 0.074 per 100,000 in the FoodNet sites. While the campylobacteriosis specific mortality risk was higher in ET, this difference was not statistically significant, since both estimates have wide confidence intervals due to the low number of total deaths.

Contact with animals in the 10 days prior to the onset of symptoms was the most commonly reported risk factor in this study. This is an important health concern since *Campylobacter* is a zoonotic agent that can infect both animals and humans, and animals can be asymptomatically infected. One report warns that exposure to a dog with diarrhea triples a person's risk for campylobacteriosis[294]. Due to the increase in the number of stools for a dog with diarrhea, there may be a higher risk of fecal exposure, but studies

have shown that dogs with and without diarrhea are equally likely to carry *Campylobacter*[35, 295, 296].

Dogs (74%) and cats (33%) were the two most commonly reported animals that patients were exposed to in this study. Depending on the location and clinical conditions of the animals sampled and the *Campylobacter* species isolated, infection rates have been reported at 27.9%[297], 15-26% [298], and 51.1-87%[299] in dogs and 16.8% [300] and 24% [36] in cats. Cats have been found to be infected with *C. upsaliensis* more frequently than *C. jejuni* or *C. coli*; however this species is not frequently identified in humans. The lack of identification in humans could be due to growth inhibition of *C. upsaliensis* by the antibiotics used in the standard stool culture median used in the isolation of *Campylobacter* spp. In spite of this, cats still remain an important health concern of zoonotic campylobacteriosis. Almost 10% of patients reporting animal exposure came in contact with live chickens. Poultry are an important reservoir of *Campylobacter*. At a live poultry market in New York, 83% of the chickens were positive for *Campylobacter*[301] and 27% of broilers were positive at a farm in England[302]. Daily contact with poultry had borderline significance as a risk factor for campylobacteriosis in a case-control study in Denmark (OR:2.11, 95% CI: 0.99-4.49), but daily contact with a cat with diarrhea (OR:3.77, 95% CI: 1.03-13.83) or a cow (OR:3.09, 95% CI: 1.09-8.74) were found to be significantly associated with disease[13].

Children in this study under 5 years old were more than 3 times more likely than those over 50 to report animal exposure; this is most likely due to difference in behaviors of these age groups. One reason may be that children are more likely to have a family pet, visit friends with pets or go on a school trip that could put than in contact with

animals compared to older adults. Also children are less likely to wash their hands after handling animals, while older adults most likely will. One case-control of children in Sweden found the odds ratio for reporting dog exposure to be higher for children under 2 years (9.1, 95% CI:3.7-27.0) compared to the odds ratio for children aged 2-6 (2.4, 95% CI:1.1-5.1)[204]. While the present study did not include controls, the pattern observed in the Swedish study is similar to the pattern observed in this study, that children had the highest animal exposure.

Of all the animal exposures recorded in this study, it was not noted if the contact occurred in a work or recreational environment. The type of contact could change the amount of exposure that occurs. Another study defined contact with an animal as "handling or touching the animal or its excrements"[202]. No definition was used in this study, so it is possible that some patients, who reported contact, may not have been classified as exposed if the previous definition is used. A more thorough definition might include a quantification of the amount, duration, and type of exposure to the animal (in the same room as an animal, just hand contact, had animal on lap, facial contact with the animal, contact with animal excrement, if the animal sleeps on the bed). Since the animals in question were not tested for campylobacteriosis as well, it can not be assumed that the human infection was caused by to the animal exposure.

Travel outside the state or country was the next most commonly reported risk factor (21.6%). The percentage of patients in this study who reported international travel (11.3%) is similar to that of the FoodNet case-control study (13%). More patients in ET traveled to Central and South America (23%) compared to patients in the FoodNet study (10%) but fewer traveled to Europe. The difference in destination between the two

studies may be due to type of travel (business, pleasure or altruistic) or differences in the socioeconomic class of the populations. Several cases in ET noted that they contracted campylobacteriosis while on church mission trips, some of which were to Central and South American countries. Altruistic trips, such as church mission trips, are often made to the more rural parts of developing countries, where the risk of exposure is greater. These trips may be an important source of exposure and a factor in the prevalence of campylobacteriosis in the region and in the rest of the US.

A French case-control study found that 5.6% of the cases and only 2.4% of the controls reported international travel in the 8 days prior to the onset of symptoms, which was a borderline significant a risk factor (OR=2.5, 95% CI: 0.9-6.4)[275]. None of the patients in that study visited Central or South America. The percentage of international travel observed in that study is lower than that observed in the present study. This may be due to the destinations of the patients in the French study being less rural compared to those in this study. In Denmark, travel was identified as a risk factor in a 1996-1997 case-control study. In that study, travel was reported by 18.4% of the cases and only 9.4% of the controls[13]. The percentage of patients reporting international travel is much higher in Denmark where most cases had a history of travel to "Southern Europe, the Middle East or Asia." Since that study did not differentiate the percentages of patients traveling to each location, it can not be compared to the locations in the present study. The present study is also very different from a Norwegian study which reported that 53% of 12,327 cases that occurred between 1995 and 2001 were acquired abroad.

The differences in travel patterns observed among the various age groups (Table 4.13) may be related to behavioral patterns of the age groups. Many adolescents and

young adults in the 15-29 age group report international travel since many college students spend semesters abroad and members of this age group may be able to travel more due to more disposable income and many do not have children yet. Members of the 65 and over age group are past retirement age and therefore may have more time for travel, however the most of the patients in this study traveled locally, within the United States.

The odds of reporting any travel were 2.8 - 5.0 times higher for patients in the urban counties than those in the more rural counties. This may be due to differences in socioeconomic class; people in the more urban counties may be able to afford more travel. This is especially true for international travel, none of the patients in the least urban counties traveled internationally, while 59.6% of patients in the most urban did. It is possible that more patients in the urban counties had jobs that required international travel, or a higher income compared to patients in rural counties. There was also an association between the prevalence group of a county and travel; patients from counties with a lower prevalence were more likely to report travel. This result could be influenced by the urbanicity of the counties since the counties in the highest prevalence group were also some of the most rural counties.

Handling raw meat or poultry was reported by 21.6% of the cases in East Tennessee. This is lower than observed in the FoodNet case-control study where 48% of patients reported preparing raw chicken and 27% of patients reported touching raw chicken[198]. It is possible that some of the difference in the percentages between this study and the FoodNet study observed is due to differences in the phrases “prepare” and “handle”. There was a large difference in the FoodNet study, with more patients

reporting preparing than handling. It is possible that in this study someone may have reported preparing raw chicken, but not handling it which could explain the lower percentage observed for raw meat exposure. No information was provided in the FoodNet report on exposure to meat other than chicken. The percentage of patients who reported handling raw meat in East Tennessee is similar to that observed in France where 19.4% of patients prepared meat or poultry[275]. A case-control study in Norway found preparing raw poultry to be a significant risk factor for disease, 20% of the cases reported it, while only 2% of the controls did (OR: 9.55, 95% CI: 2.09-43.69)[202]. Due to the high level of *Campylobacter* contamination that has been reported in some retail poultry products, (81% of 525 chickens sampled in a Consumer Reports study[71]), care should be taken to avoid direct exposure from raw meat, through thorough hand washing. A Swedish case-control study observed that 16% of campylobacteriosis cases did not clean their hands during food preparation compared to only 10% of controls[208]. Another case-control study in Seattle, WA found that cases were less thorough than controls in cleaning meat cutting surfaces or using separate cutting surfaces[303]. Cutting surfaces should be thoroughly cleaned and separate surfaces should used for meat and produce. Meat should also stored on the lowest shelf in a sealed container to avoid cross contamination of other foods.

Reporting the risk factor "handling raw meat or poultry" was significantly associated with the sex of the patients with females having 1.5 times higher odds of reporting handling raw meat or poultry, when compared with males. This difference may be due to cultural and behavioral differences between the sexes; women are more likely to prepare meals at home. There was also an association between handling raw meat and

age; with members of the 30-49 age group reporting the highest percentage of raw meat handling (37.1%). Members of this age group had 12 times higher odds of reporting handling raw meat or poultry when compared to patients in the under 5 age group (4.4%). This difference could be due to behavioral factors since young children (under 5 years) rarely help in the preparation of the meal and when they do, it usually does not involve handling raw meat.

Swimming in water from natural sources has been shown to be associated with the acquisition of campylobacteriosis[207], and other recreational water use such as fishing or boating may pose a similar risk. Recreational water has also been associated with an outbreak of waterborne gastrointestinal illness caused by Cryptosporidium in ET. That outbreak occurred at a "splash pad" (an interactive fountain where children play in the water that comes up as jets from the ground) during the summer of 2006[304]. Recreational water exposure was included by the health department on the campylobacteriosis case report form under the risk factor variable "hike, camp, swim or fish". For this risk factor there was no distinction made between the type of water or which of 4 activities took place or if water was ingested. In ET 19% of patients reported at least one of the 4 activities in the 10 days prior to the onset of symptoms. This risk factor was found to be associated with the season in this study. During summer and spring 26.2% and 25.4% of patients, respectively, reported at least one of the 4 activities compared to only 13.5% in the fall and 7.1% in the winter. These differences are likely due to the climate of the area, very hot summers and cool winters, so participation in these activities is more likely to occur in the warmer months.

Due to the potential for fecal contamination, drinking untreated water is considered a high risk behavior for exposure to *Campylobacter*[65, 67]. In East Tennessee 10% of cases reported drinking untreated water from a stream, spring or other untreated source in the 10 days prior to the onset of symptoms. In the FoodNet case-control study, drinking untreated water from a lake, river or stream was identified as a risk factor in the univariate analysis; 4% of cases had engaged in this behavior compared to just 2% of controls (OR: 2.9, 95% CI: 1.6-5.3)[198]. Both of these percentages are much lower than the percentage of patients who drank untreated water in this study. The percentage of patients who drank untreated water may be higher in ET than other US sites because this type of water source and the practice of drinking untreated water may be more common in the rural areas of ET than the population of the FoodNet study that is more urban, on average. Patients who reported drinking untreated water were also likely to report hiking, camping, fishing or swimming, with 7% of all patients reporting both (OR: 13.5, 95% CI: 6.5-28.0). The wording on the case report form changed in 2006 from "Drink from a spring, stream or lake?" to "Did the patient drink untreated water?" This change could have resulted in a higher or lower percentage of patients reporting this risk factor so drinking untreated water cannot be analyzed across the years of the study.

Drinking well water has been identified as a risk factor for infection in case-control studies involving all ages in Finland[207] in children in Sweden[204], and in infants in the US[14]. In ET, 31.3% of patients reported that their household drinking water source was a private well and 3% reported it was a spring. Both of these sources can become contaminated if not properly protected. Information was not collected on the specific characteristics of each well or spring in this study; some patients noted that the

well had been tested and treated, while others stated they had never treated their well. No standardized data collection was performed regarding the timing or type of well testing or treatment. Drinking well water was especially common in young children, 40.2% of children under age 5 and 51.6% of children under age 1 had a well as their drinking water source. These percentages are much higher (p=0.0012) than those observed in the infant (<1 year of age) FoodNet case-control study where only 22% of cases and 8% of controls used well water[14]. The higher observed percentage of well water reported in this study may be due to an increased number of patients in the study from rural areas when compared to the average of the FoodNet sites. Adults in the rural areas with household well water may have developed immunity from past drinking water exposure and are therefore a lower proportion of the rural adults may develop campylobacteriosis symptoms. Immunity amongst the rural adults could also explain why there is a high percentage of young children in the rural areas who drank well water. The Swedish case-control study of children also reported a difference in the percentage of cases on well water based on age, 34% of children under 2 lived in a home with a well compared with 26% of children aged 2-6 years old[204]. While the percentages are still lower than those was observed in this study, the same age pattern was reported. Not every well can be considered the cause of the *Campylobacter* infection, however periodic monitoring and appropriate treatment of wells is important in reducing the prevalence of campylobacteriosis.

Contact with another person with similar symptoms was reported by 14.7% of the patients in this study, but most of the symptomatic contacts did not seek medical care so an exact diagnosis was not obtained. The type of contact that occurred was also not

identified so it is not known if the contact was a potential source of infection, if there was a common exposure or if the other person was even infected with *Campylobacter*, although it possible that contact with another infected individual may not have been the actual source of infection as well. In the FoodNet case-control study, 7% of cases reported a household member with diarrhea in the 4 weeks before illness compared to 11% of controls[198]. In that study, having a household member with similar symptoms was identified among the controls as a protective factor since the control patient may have been exposed previously and may have developed immunity but were not symptomatic during the earlier infection.

Due to the possibility of fecal exposure, contact with dirty diapers has been associated with transmission of campylobacteriosis, and therefore childcare or daycare centers could be important sources of its spread[305]. In ET, 8.4% of cases reported the risk factor "household member in daycare" (inclusive of the patient). The FoodNet case-control study of infants, reported that attending childcare was not significantly associated with campylobacteriosis in the multivariable analysis[14]. In this study, having a household member in daycare was associated with age; the 3 age groups with the highest percentages were: under 5 (13.5%), 30-49 (12.9%) and 5-14 (8.5%). The 15-29 (3.3%), 65 and over (2.9%), and 50-64 (0%) age groups had lowest percentages. This association may be due to differences in family structure, the 30-49 year olds may have children of the age that could be in daycare, while the children under age 14 could have siblings that are. Although it was not identified as a significant risk factor in previous case-control studies, several outbreaks of campylobacteriosis have occurred at daycare centers[225].

The average annual prevalence of campylobacteriosis over the 4 year study period in East Tennessee was 10.4 cases per 100,000 population. This is significantly lower than that of the entire FoodNet region (12.7 cases/100,000 population) and significantly higher than that of the state of Tennessee (7.4 cases/100,000 population). It is possible that these prevalence estimates reflect the true prevalence of disease in ET, Tennessee and around the US. There are also numerous factors that could influence disease reporting and the reported prevalence, which may be responsible for a portion of the observed differences in the prevalence of campylobacteriosis. Some parts of the state or country may have different or better reporting practices by the laboratories involved despite the active surveillance conducted by the health departments. This may result in a higher percentage of the cases identified in the laboratories and a higher percentage reported to the health department. Another possible factor is that doctors in some areas may be more likely to request a stool sample/culture from their patients which could lead to a higher percentage of cases identified in those regions. Worldwide a higher percentage of children seek medical care for their symptoms than adults, but it is possible that this difference may be more pronounced in some areas compared to others. Another factor that could influence the decision to seek care (and disease reporting) is access to medical care. In some states there is better state insurance and a difference in the percentage of the population that has private insurance exists. The areas with a higher percentage of the population insured may have a higher reported prevalence of campylobacteriosis since more patients are seeking medical care and therefore more are entered in the reporting database. In this study, the majority of patients were classified as

white (>95%), so comparisons across race and ethnicity could not be made due to the small sample size of the other races.

The patterns of age- and sex-specific prevalence estimates observed in this study are similar to those reported in previous studies. Children under 5 years of age had the highest prevalence of campylobacteriosis (41.6 cases per 100,000). This is significantly higher (p=0.0051) than reported in 1999 of 7 FoodNet sites which found a prevalence of 30.9 cases per 100,000[239]. While the prevalence of disease among children under 5 was higher in ET than the rest of the US, it was lower than that reported in Ontario, Canada (52.5 cases per 100,000)[292], the Eastern Townships of Quebec, Canada (169.2 per 100,000)[197] and Manitoba, Canada (males: 53.6 per 100,000, females:40.4 per 100,000)[244]. Although a bimodal age distribution has been reported in other studies in the US[239] and worldwide[4, 19, 292], a second peak (prevalence above the average) was not observed in the young adult population (ages 15-29) in the present study. Some authors have hypothesized that the second peak may be due to a higher proportion of young adults traveling to other countries where they may be exposed to *Campylobacter*. The lack of the second peak in ET may be due to a relative lower proportion of travel reported by young adults compared to other areas where the peak was observed.

The higher prevalence in young children in ET compared with other FoodNet sites may be due to differences in the age at first exposure. On average, ET is more rural than the other FoodNet sites and therefore children may be exposed to and infected with *Campylobacter* at a younger age in ET since there may be greater chance of exposure in the rural areas where animals, especially farm animals are more common. This is compared to more urban areas where a larger percentage of the population may not be

exposed until later in life. This hypothesis is supported by the findings of a study in Manitoba, Canada which reported that the incidence of campylobacteriosis was much higher among young children in the rural areas(males: 97.5 per 100,000, females: 72.8 per 100,000), compared to the urban ones(males: 13.2 per 100,000, females:10.5 per 100,000)[244]. In the urban areas of that study, the highest incidence was observed in the 20-24 year olds. Worldwide the highest prevalence estimates have been observed in young children. Some of the high reported prevalence estimates may be due to the lower inocula needed to infect an infant. Young children also often place foreign objects in toys in their mouths; this behavior could also lead to more young children becoming infected. Another reason for the high prevalence in young children may be due to a greater proportion of young children seeking medical care. Parents are more concerned when their 1-year-old is sick than when they (the parents) themselves are sick[233].

Grainger County had the highest age- and sex-standardized average annual prevalence of campylobacteriosis at 22.9 cases per 100,000 population. This prevalence is twice the average for the study region, and three times the average prevalence of the state of Tennessee. Several other counties had standardized prevalence proportions that were higher than the average prevalence of the study region. Jefferson County (21.8 cases/100,000 population) borders Grainger County to the South and Union County (14 cases/100,000 population) borders Grainger County to the West, these three counties could be grouped to form a group of high risk counties. These three high risk/prevalence counties are all rural areas (<25% urbanicity) which may explain the observed, above average prevalence proportions reported in these 3 counties. A similar pattern was observed in Manitoba, Canada where researchers reported that the prevalence of

campylobacteriosis was higher in almost every age group in the rural areas compared to the urban ones in the city of Winnipeg[244].

The standardized prevalence proportions were also above the average in Blount (15.6 cases/100,000 population) and Loudon (13.1 cases/100,000 population) Counties. Unlike Grainger, Jefferson, and Union counties, Blount and Loudon counties both fall into the 2nd urbanicity group (44-66% urban) since 63.2 and 50% of the populations live in an urban area, respectively. The reason for this finding is unknown. Geographically, in between these two areas (Grainger, Jefferson and Union, and Blount and Loudon counties) of above average prevalence is the most populous county, Knox County. Although Knox County is 87% urban and some studies have identified an association between urban dwellings and high prevalence of disease[239], the standardized prevalence in Knox County was only slightly above average at 11.8 cases per 100,000 population. The age- and sex-standardized prevalence estimates shown in Figure 4.2 for each CCD show how the areas of high prevalence extend across the county lines. The map shows that the area of high prevalence in Grainger and Jefferson counties is clustered toward the Western ends of the counties and this area of high prevalence crosses into Eastern Knox County. While some of the areas of the map are white, this does not indicate that campylobacteriosis is not occurring in these areas; just no cases were reported to the health departments from those areas. The prevalence estimates also show the areas of higher prevalence within each county that can be useful in health planning.

It appears that the reported prevalence of campylobacteriosis is decreasing in East Tennessee while it remains steady in the state of Tennessee and the rest of the US

FoodNet. Future years will show whether the prevalence of campylobacteriosis in ET will reach the average of the state or drop below it.

A marked seasonal pattern in the prevalence of campylobacteriosis was observed. A peak occurred each summer, which is consistent with the findings of several other published studies[4, 204, 231, 242, 243]. A study in Alberta and Newfoundland-Labrador, Canada found that there was a non-linear association between the ambient temperature and the case counts of reported campylobacteriosis[306]. For every degree increase in the mean weekly temperature, the number of cases of campylobacteriosis reported increased by 6% in Alberta and 4.5% in Newfoundland-Labrador. Another study that analyzed surveillance data from 15 countries in Europe, New Zealand, Australia and Canada, found that all countries showed a distinct seasonality and that countries with a milder winter were more likely to have a spring peak in the prevalence of campylobacteriosis[307].

The increase in the reported prevalence of campylobacteriosis during the warmer summer months may be due to an increase in risky behavior (hiking, camping, fishing or swimming) during the summer months. Outdoor activities, especially recreational water exposure, put the population at a greater risk for *Campylobacter* infections; since these activities bring the population into closer proximity with possible sources of infection, such as untreated water. Another reason for the summer increase in the prevalence may be an increase in the number of outdoor barbecues which could lead to an increase in the consumption of under cooked meat or greater opportunity for cross contamination of foods.

The highest peak in the prevalence of campylobacteriosis in East Tennessee occurred in the summer of 2003, the same year as the highest annual prevalence for the study. Identification of seasonal patterns among residents of ET can assist health department personnel in directing their scarce education resources and timing prevention efforts to have the greatest impact.

5.1 Strengths and Limitations

Under reporting of campylobacteriosis is a limitation of this study. There are numerous steps (Figure 2.1) that must be taken in order for a case to be reported and be entered into the surveillance database. As such, not every case of campylobacteriosis that occurred in ET is included in the database of this study. In addition, the proportion of cases that go unreported may not be the same for all age, sex and socioeconomic groups and geographic locations. So it is possible that certain population groups are over or under represented in the prevalence estimates. The differences in population groups that are reported may be due to differences in access to medical care and laboratory facilities, physician practice and patient compliance of each group. It is possible that doctors in rural areas may be less likely to request a stool sample due to the distance to the nearest laboratory, adults may be less likely to comply with a doctor's request for a stool sample due to their schedule, or less likely to comply with the request if their symptoms improve before sample collection, and some patients may be less likely to visit a doctor if their symptoms are not as serious. A Canadian study of physician practices found that doctors were more likely to request a stool sample if the patient reported

bloody diarrhea, recent overseas travel, an immunocomprised status or a duration of illness >7 days[308]. In that study physicians indicated that laboratory availability, the time required to get a laboratory diagnosis and cost also influenced their decision to request a stool sample. Regional differences in patient behavior and physician practice that affect disease reporting can make comparing the prevalence of campylobacteriosis in ET to other states and other countries difficult. These differences could include the more rural nature of ET that might indicate fewer diagnostic laboratories that are located a greater distance from the patients compared to other parts of the US. A big difference between the US and other countries is the difference in health care systems; countries with universal government funded health care may have higher rates of reporting since more patients have health insurance and are therefore able to visit the doctor. However; if surveillance system stable over time, reported prevalence can identify trends in true prevalence.

Another limitation of this study is that the data collected on clinical symptoms, and risk factors were self reported and voluntary. For other variables the data were not collected in a standardized manner, such as information on the health status of the animal the patient was exposed to or the treatment protocols of the private household wells; the information was recorded for some patients, but not for all. In some cases the respondent could not supply the information requested, therefore, unknowns and missing information occurred frequently for some variables. The missing information could lead to biased results if patients that did not provide the requested information were systematically different from the rest of study patients.

A strength of this study is that the data used were collected through active surveillance; this means that every stool culture that was positive for campylobacteriosis should have been reported to the health department. While the number reported is lower than the actual total number of campylobacteriosis cases that are occurring in the region, all cases identified during the study period were included in the study database, so the prevalence calculated should reflect the trends that are occurring in the prevalence of campylobacteriosis in ET. The data storage system and case report form changed during the study period, but the law governing disease reporting did not. While the wording of some of the risk factor variables changed, the basic information collected did not change over the 4 years of the study. All errors that were created by the change in the database structure in 2004 were corrected before analysis was conducted so this should not influence the conclusions either. Since all cases were reported to the health department in a similar fashion throughout study period, the data within ET can be compared across years of the study.

Another strength of this study was a data quality evaluation that was performed prior to analysis. Often the data obtained through surveillance systems is assumed to be accurate. The data quality evaluation of the data used in this study showed that the average error rate (6.5%) was within the range of error rates from other evaluations of computerized records[267]. This evaluation shows that the data used in this study is primarily accurate, can be trusted and can be used to analyze the trends of campylobacteriosis in ET.

5.2 Conclusions

In East Tennessee the reported prevalence of campylobacteriosis is declining. On average, the prevalence is lower than that reported in all of the FoodNet sites within the United States. However, the annual prevalence is, on average, 1.6 times higher than the whole state of Tennessee. Several counties (Grainger, Jefferson, Union, Blount, and Loudon) in the region have an average annual prevalence that is much higher than the regional average and some are higher than the national average. Similar to other studies, a large spike in the prevalence of disease occurred during the summer months.

The highest age-specific prevalence (41.57/100,000) was observed in young children under 5. This is higher than the prevalence for the same age group in the rest of the United States (24.01/100,000). The median age was lower in the more rural counties; children comprise a larger percentage of the cases in these areas than the urban ones.

While the quality of the data used in this study was within the range of previously published reports on data entry rates, some patterns were identified that affected data quality in this study. Changes in the database storage system, in 2004, affected data integrity. With the current database storage system, further errors such as these are not likely to occur. It was also noted that several patients were assigned a race or ethnicity in the computer dataset, when none was indicated on the paper case report form. In order to ensure the accuracy and integrity of the dataset, more care needs to be taken when transcribing information from the data collection form to the electronic database.

A larger percentage of campylobacteriosis patients were hospitalized in ET, but the LOS was shorter for patients in this study than the rest of the US and other countries.

Unlike what has been reported in other countries, the percentage of cases hospitalized, in this study, varied with the season, the highest percentage occurred in the summer.

The clinical characteristics of patients in this study were largely similar to those of other published reports. Almost all patients experienced diarrhea (97.5%) while only 37.8% reported blood in their stool, which is comparable to worldwide reports. Bloody stool was found to be significantly associated with age; young children had much higher odds of reporting this symptom than the rest of the population. Fever and cramps, however, were reported less frequently in this study than other parts of the US.

The most commonly reported risk factor was recent animal exposure, with young children 3 times more likely to report animal exposure than adults over 50. Other hygiene related risk factors (handling raw meat, a household member in daycare) were associated with age as well. Almost a third of the cases reported a well as the drinking water source of the household; this was higher in the more rural counties and among young children.

No factor or factors were identified that would explain why the prevalence of campylobacteriosis is higher in East Tennessee than the rest of the state.

5.3 Recommendations and Future Research

5.3.1 Recommendations to the Health Departments

- Focus education programs on areas of high risk, especially Grainger and Jefferson Counties. These programs could include school presentations or pamphlets focusing on hygiene (especially around animals). Pamphlets could also be

developed for distribution to parents on how to make hand-washing fun for children. These "games" could be distributed as posters to childcare centers and nursery schools to encourage thorough hand-washing. This information could also be included on a regional health department website with links to some of the many internet resources already available.

- In store education could be coordinated with area grocery stores to provide displays emphasizing the need to keep raw meat (and its juice) separated from other foods in the grocery cart, in the refrigerator and during preparation
- Promote private well protection and treatment information.
- Set a definition for the animal exposure risk factor and collect more information on the type of exposure (occupational, live on a farm, school trip, household pet or pet of a friend, or animal sleeps in bed), or average length of exposure to better quantify the animal exposure and possible *Campylobacter* transmission.
- Target altruistic and church groups with educational information for travelers to rural areas or international destinations where acquiring campylobacteriosis may be more common to ensure these groups are aware of the possible exposures for *Campylobacter* spp. and other enteric pathogens. Information has been by the CDC and this information could be assembled in a pamphlet or power presentation and given to groups that routinely travel overseas such as churches.
- Improve quality of surveillance data through more thorough collection and accurate data entry.

5.3.2 Future Research

In order to better understand the prevalence of campylobacteriosis in household pets of East Tennessee, a study should be conducted to randomly sample animals in their normal habitat for the presence of all thermophillic C*ampylobacters*. The study would investigate dogs that have frequent contact with other dogs to determine the prevalence of *Campylobacter* spp. This could be studied by routinely sampling dogs at parks or other areas where dogs frequently come in contact with each other. High prevalence of campylobacteriosis amongst the dogs at each park may be used to justify rules governing the pick up and sanitary disposal of dog feces in parks, and could be used to educate the public about *Campylobacter* transmission.

Since there is a large percentage of the patients in this study that have a well as the source of water for the household, a survey of wells in the region should be conducted to test for contamination. This survey could also include repeat sampling after periods of heavy rain to investigate if *Campylobacter* is washed into well water with rain water runoff.

References

1. Don A. Franco, C.E.W., *Campylobacter jejuni*, in *Foodborne Disease Handbook*, M.D.P. Y. H. Hui, J. Richard Gorham, Editor. 2001, Marcel Dekker, Inc: New York, NY. p. 83-105.
2. Skirrow, M.B., *Campylobacter enteritis: a "new" disease.* Br Med J, 1977. **2**(6078): p. 9-11.
3. Mead, P.S., et al., *Food-related illness and death in the United States.* Emerg Infect Dis, 1999. **5**(5): p. 607-25.
4. Kapperud, G. and S. Aasen, *Descriptive epidemiology of infections due to thermotolerant Campylobacter spp. in Norway, 1979-1988.* Apmis, 1992. **100**(10): p. 883-90.
5. Buzby, J.C., B.M. Allos, and T. Roberts, *The economic burden of Campylobacter-associated Guillain-Barre syndrome.* J Infect Dis, 1997. **176 Suppl 2**: p. S192-7.
6. Engberg, J., et al., *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**(1): p. 286-91.
7. Friedman, C.R., Nerimann, J., Wegener, H. C., Tauxe, R. V., *Epidemiology of Campylobacter jejuni Infections in the United States and Other Industrialized Nations*, in *Campylobacter*, I. Nachamkin, Blaser, M. J., Editor. 2000, American Society for Microbiology Press: Washington DC. p. 121-138.
8. Skirrow, M.B., Blaser, Martin J. , *Clinical Aspects of Campylobacter Infection*, in *Campylobacter*, I. Nachamkin, Blaser, Martin J., Editor. 2000, American Society for Microbiology: Washington, DC. p. 69-88.
9. Allos, B.M., *Association between Campylobacter infection and Guillain-Barre syndrome.* J Infect Dis, 1997. **176 Suppl 2**: p. S125-8.
10. Perez, G.P. and M.J. Blaser, *Campylobacter and Helicobacter*, in *Baron' Medical Microbiology*, S. Baron, Editor. 1996: Galvaston, TX.
11. Engberg, J., *Contributions to the epidemiology of Campylobacter infections. A review of clinical and microbiological studies.* Dan Med Bull, 2006. **53**(4): p. 361-89.
12. Neal, K.R. and R.C. Slack, *Diabetes mellitus, anti-secretory drugs and other risk factors for campylobacter gastro-enteritis in adults: a case-control study.* Epidemiol Infect, 1997. **119**(3): p. 307-11.
13. Neimann, J., et al., *A case-control study of risk factors for sporadic campylobacter infections in Denmark.* Epidemiol Infect, 2003. **130**(3): p. 353-66.
14. Fullerton, K.E., et al., *Sporadic campylobacter infection in infants: a population-based surveillance case-control study.* Pediatr Infect Dis J, 2007. **26**(1): p. 19-24.
15. Jones, K., *Campylobacters in water, sewage and the environment.* Symp Ser Soc Appl Microbiol, 2001(30): p. 68S-79S.
16. Tam, C.C., *Campylobacter reporting at its peak year of 1998: don't count your chickens yet.* Commun Dis Public Health, 2001. **4**(3): p. 194-9.
17. Miller, M., et al., *Australia's notifiable diseases status, 2003 annual report of the National Notifiable Diseases Surveillance System.* Commun Dis Intell, 2005. **29**(1): p. 1-61.

18. Nelson, W. and B. Harris, *Flies, fingers, fomites, and food. Campylobacteriosis in New Zealand--food-associated rather than food-borne.* N Z Med J, 2006. **119**(1240): p. U2128.
19. Baker, M.G., E. Sneyd, and N.A. Wilson, *Is the major increase in notified campylobacteriosis in New Zealand real?* Epidemiol Infect, 2007. **135**(1): p. 163-70.
20. Centers for Disease Control and Prevention, *Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2006.* MMWR Morb Mortal Wkly Rep, 2007. **56**(14): p. 336-9.
21. Sheeler, L. 2006.
22. Vandamme, P., et al., *Campylobacteraceae*, in *Bergy's Manuel of Systematic Bacteriology*, G.M. Garity, Editor. 2001, Springer: New York City. p. 1145-1168.
23. Vandamme, P., *Taxonomy of the Family Campylobacteraceae*, in *Campylobacter*, I.a.B. Nachamkin, M, Editor. 2000, American Society of Microbiology Press: Washington D.C.
24. Nachamkin, I., *Campylobacter and Arcobacter*, in *Manual of Clinical Microbiology*, P.R. Murray, Baron, E. J., Pfaller, M. A., Tenover, F. C., Yolken, R. H., Editor. 1999, American Society for Microbiology Press: Washington DC.
25. Escherich, T., *Beitraege zur Kenntniss der Darmbacterien. III. Ueber das Vorkommen von Vibrionen im Daemcanal und de Stuhlgaengen der Sacuglinge. [Articles adding to the knowledge of intestinal bacteria. III. On the existence of vibrios in the intestines and feces of babies].* Munch Med Wochenschr, 1886. **33**: p. 815-817, 833-835.
26. Kist, M., *[Who discovered Campylobacter jejuni/coli? A review of hitherto disregarded literature].* Zentralbl Bakteriol Mikrobiol Hyg [A], 1986. **261**(2): p. 177-86.
27. McFadyean, J. and S. Stockman, *Report of the Deparmental Committee appointed by the Board of Agriculture and Fisheries to inquire into epizootic abortion. Part II. Abortion in sheep*. 1913, HMSO: London.
28. Smith, T., and Taylor, Marian. S, *Some morphological and biochemical characters of the spiriall (Vibrio fetus n. sp.) associated with diesase of the fetal membranes in cattle.* Journal of Experimental Medicine, 1919. **30**(4): p. 299-312.
29. Jones, F.S., Little, Ralph B., Orcutt, Marion, *A Continuation of the Study of the Etiology of Infectious Diarrhea (Winter Scours) in Cattle.* Journal of the American Veterinary Medical Association, 1932. **81**: p. 610-619.
30. Sebald, M. and M. Veron, *[Base DNA Content and Classification of Vibrios.].* Ann Inst Pasteur (Paris), 1963. **105**: p. 897-910.
31. Mohammed, K.A., R.J. Miles, and M.A. Halablab, *The pattern and kinetics of substrate metabolism of Campylobacter jejuni and Campylobacter coli.* Lett Appl Microbiol, 2004. **39**(3): p. 261-6.
32. Logan, J.M., et al., *Campylobacter lanienae sp. nov., a new species isolated from workers in an abattoir.* Int J Syst Evol Microbiol, 2000. **50 Pt 2**: p. 865-72.
33. Bryner, J.H., P.A. O'Berry, and A.H. Frank, *Vibrio Infection of the Digestive Organs of Cattle.* Am J Vet Res, 1964. **25**: p. 1048-50.

34. Engvall, E.O., et al., *Isolation and identification of thermophilic Campylobacter species in faecal samples from Swedish dogs.* Scand J Infect Dis, 2003. **35**(10): p. 713-8.
35. Sandberg, M., et al., *Risk factors for Campylobacter infection in Norwegian cats and dogs.* Prev Vet Med, 2002. **55**(4): p. 241-53.
36. Bender, J.B., et al., *Epidemiologic features of Campylobacter infection among cats in the upper midwestern United States.* J Am Vet Med Assoc, 2005. **226**(4): p. 544-7.
37. Hald, B. and M. Madsen, *Healthy puppies and kittens as carriers of Campylobacter spp., with special reference to Campylobacter upsaliensis.* J Clin Microbiol, 1997. **35**(12): p. 3351-2.
38. Goossens, H., et al., *Characterization and description of "Campylobacter upsaliensis" isolated from human feces.* J Clin Microbiol, 1990. **28**(5): p. 1039-46.
39. Goossens, H., et al., *Is "Campylobacter upsaliensis" an unrecognised cause of human diarrhoea?* Lancet, 1990. **335**(8689): p. 584-6.
40. Carter, J.E. and N. Cimolai, *Hemolytic-uremic syndrome associated with acute Campylobacter upsaliensis gastroenteritis.* Nephron, 1996. **74**(2): p. 489.
41. Bourke, B., V.L. Chan, and P. Sherman, *Campylobacter upsaliensis: waiting in the wings.* Clin Microbiol Rev, 1998. **11**(3): p. 440-9.
42. Waldenstrom, J., et al., *Species diversity of campylobacteria in a wild bird community in Sweden.* J Appl Microbiol, 2007. **102**(2): p. 424-32.
43. Eyles, R.F., et al., *Comparison of Campylobacter jejuni PFGE and Penner subtypes in human infections and in water samples from the Taieri River catchment of New Zealand.* J Appl Microbiol, 2006. **101**(1): p. 18-25.
44. Endtz, H.P., et al., *Genotypic diversity of Campylobacter lari isolated from mussels and oysters in The Netherlands.* Int J Food Microbiol, 1997. **34**(1): p. 79-88.
45. Harvey, R.B., et al., *Prevalence of Campylobacter spp isolated from the intestinal tract of pigs raised in an integrated swine production system.* J Am Vet Med Assoc, 1999. **215**(11): p. 1601-4.
46. Krause, R., et al., *Recurrent septicemia due to Campylobacter fetus and Campylobacter lari in an immunocompetent patient.* Infection, 2002. **30**(3): p. 171-4.
47. Martinot, M., et al., *Campylobacter lari bacteremia.* Clin Microbiol Infect, 2001. **7**(2): p. 96-7.
48. Allos, B.M., *Campylobacter jejuni Infections: update on emerging issues and trends.* Clin Infect Dis, 2001. **32**(8): p. 1201-6.
49. Allos, B.M. and M.J. Blaser, *Campylobacter jejuni and the expanding spectrum of related infections.* Clin Infect Dis, 1995. **20**(5): p. 1092-9; quiz 1100-1.
50. Rollins, D.M. and R.R. Colwell, *Viable but nonculturable stage of Campylobacter jejuni and its role in survival in the natural aquatic environment.* Appl Environ Microbiol, 1986. **52**(3): p. 531-8.
51. Oliver, J.D., *The viable but nonculturable state in bacteria.* J Microbiol, 2005. **43 Spec No**: p. 93-100.

52. Boucher, S.N., et al., *Production and viability of coccoid forms of Campylobacter jejuni.* J Appl Bacteriol, 1994. **77**(3): p. 303-7.
53. Hazeleger, W.C., et al., *Temperature-dependent membrane fatty acid and cell physiology changes in coccoid forms of Campylobacter jejuni.* Appl Environ Microbiol, 1995. **61**(7): p. 2713-9.
54. Thomas, C., D.J. Hill, and M. Mabey, *Morphological changes of synchronized Campylobacter jejuni populations during growth in single phase liquid culture.* Lett Appl Microbiol, 1999. **28**(3): p. 194-8.
55. Cappelier, J.M., A. Rossero, and M. Federighi, *Demonstration of a protein synthesis in starved Campylobacter jejuni cells.* Int J Food Microbiol, 2000. **55**(1-3): p. 63-7.
56. Jones, D.M., E.M. Sutcliffe, and A. Curry, *Recovery of viable but non-culturable Campylobacter jejuni.* J Gen Microbiol, 1991. **137**(10): p. 2477-82.
57. Pearson, A.D., et al., *Colonization of broiler chickens by waterborne Campylobacter jejuni.* Appl Environ Microbiol, 1993. **59**(4): p. 987-96.
58. Saha, S.K., S. Saha, and S.C. Sanyal, *Recovery of injured Campylobacter jejuni cells after animal passage.* Appl Environ Microbiol, 1991. **57**(11): p. 3388-9.
59. Tholozan, J.L., et al., *Physiological characterization of viable-but-nonculturable Campylobacter jejuni cells.* Appl Environ Microbiol, 1999. **65**(3): p. 1110-6.
60. Thomas, C., D. Hill, and M. Mabey, *Culturability, injury and morphological dynamics of thermophilic Campylobacter spp. within a laboratory-based aquatic model system.* J Appl Microbiol, 2002. **92**(3): p. 433-42.
61. Chaveerach, P., et al., *Survival and resuscitation of ten strains of Campylobacter jejuni and Campylobacter coli under acid conditions.* Appl Environ Microbiol, 2003. **69**(1): p. 711-4.
62. Costerton, J.W., et al., *Microbial biofilms.* Annu Rev Microbiol, 1995. **49**: p. 711-45.
63. Buswell, C.M., et al., *Extended survival and persistence of Campylobacter spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining.* Appl Environ Microbiol, 1998. **64**(2): p. 733-41.
64. Somers, E.B., J.L. Schoeni, and A.C. Wong, *Effect of trisodium phosphate on biofilm and planktonic cells of Campylobacter jejuni, Escherichia coli O157: H7, Listeria monocytogenes and Salmonella typhimurium.* Int J Food Microbiol, 1994. **22**(4): p. 269-76.
65. Carter, A.M., et al., *Seasonal occurrence of Campylobacter spp. in surface waters and their correlation with standard indicator bacteria.* Appl Environ Microbiol, 1987. **53**(3): p. 523-6.
66. Bolton, F.J., et al., *A study of thermophilic campylobacters in a river system.* J Appl Bacteriol, 1987. **62**(2): p. 167-76.
67. Obiri-Danso, K. and K. Jones, *Distribution and seasonality of microbial indicators and thermophilic campylobacters in two freshwater bathing sites on the River Lune in northwest England.* J Appl Microbiol, 1999. **87**(6): p. 822-32.
68. Eyles, R., et al., *Spatial and temporal patterns of Campylobacter contamination underlying public health risk in the Taieri River, New Zealand.* J Environ Qual, 2003. **32**(5): p. 1820-8.

69. Hutchison, M.L., et al., *Fate of pathogens present in livestock wastes spread onto fescue plots.* Appl Environ Microbiol, 2005. **71**(2): p. 691-6.
70. Nicholson, F.A., S.J. Groves, and B.J. Chambers, *Pathogen survival during livestock manure storage and following land application.* Bioresour Technol, 2005. **96**(2): p. 135-43.
71. Consumers Union of US, *Dirty Birds.* Consumer Reports, 2007. **72**(1): p. 20-23.
72. Davis, M.A. and D.E. Conner, *Survival of Campylobacter jejuni on poultry skin and meat at varying temperatures.* Poult Sci, 2007. **86**(4): p. 765-7.
73. Cogan, T.A., S.F. Bloomfield, and T.J. Humphrey, *The effectiveness of hygiene procedures for prevention of cross-contamination from chicken carcases in the domestic kitchen.* Lett Appl Microbiol, 1999. **29**(5): p. 354-8.
74. Cools, I., et al., *Persistence of Campylobacter jejuni on surfaces in a processing environment and on cutting boards.* Lett Appl Microbiol, 2005. **40**(6): p. 418-23.
75. Karenlampi, R. and M.L. Hanninen, *Survival of Campylobacter jejuni on various fresh produce.* Int J Food Microbiol, 2004. **97**(2): p. 187-95.
76. Black, R.E., et al., *Experimental Campylobacter jejuni infection in humans.* J Infect Dis, 1988. **157**(3): p. 472-9.
77. Moore, J.E., et al., *Campylobacter.* Vet Res, 2005. **36**(3): p. 351-82.
78. Martin B. Skirrow, a.M.J.B., *Clinical Aspects of Campylobacter Infection*, in *Campylobacter*, I. Nachamkin, Blaser, Martin J., Editor. 2000, American Society for Microbiology: Washington, DC. p. 69-88.
79. Mazick, A., et al., *An outbreak of Campylobacter jejuni associated with consumption of chicken, Copenhagen, 2005.* Euro Surveill, 2006. **11**(5): p. 137-9.
80. Sanz, J.C., et al., *[Description of an outbreak of Campylobacter jejuni gastroenteritis and molecular characterization of the implicated strain].* Enferm Infecc Microbiol Clin, 2006. **24**(7): p. 437-9.
81. Jimenez, M., et al., *An outbreak of Campylobacter jejuni enteritis in a school of Madrid, Spain.* Euro Surveill, 2005. **10**(4): p. 118-21.
82. Butzler, J.P., *Campylobacter, from obscurity to celebrity.* Clin Microbiol Infect, 2004. **10**(10): p. 868-76.
83. Blaser, M.J., *Campylobacter jejuni and Related Species*, in *Principles and Practice of Infectious Diseases*, G.L. Mandell, Bennett, J. E., Dolin, R., Editor. 2000, Curchill Livingstone: New York. p. 2276-2285.
84. CDC. *Guide to Confirming a Diagnosis in Foodborne Disease*. 2006 [cited 2007 May 27]; Available from: http://www.cdc.gov/foodborneoutbreaks/guide_fd.htm.
85. Mandel, B.K., De Mol P., Butzler, J. P., *Clinical Aspects of Campylobacter infections in humans*, in *Campylobacter Infection in Man and Animals*, J.P. Butzler, Editor. 1984, CRC Press: Boca Raton, FL. p. 22-30.
86. Ternhag, A., et al., *A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with Campylobacter species.* Clin Infect Dis, 2007. **44**(5): p. 696-700.
87. Guerrant, R.L., et al., *Practice guidelines for the management of infectious diarrhea.* Clin Infect Dis, 2001. **32**(3): p. 331-51.
88. Skirrow, M.B., et al., *Campylobacter bacteraemia in England and Wales, 1981-91.* Epidemiol Infect, 1993. **110**(3): p. 567-73.

89. Khan, M.A., *HLA-B27 and its subtypes in world populations.* Curr Opin Rheumatol, 1995. **7**(4): p. 263-9.
90. Koobatian, T.J., et al., *The use of hospital discharge data for public health surveillance of Guillain-Barre syndrome.* Ann Neurol, 1991. **30**(4): p. 618-21.
91. Rantala, H., M. Uhari, and M. Niemela, *Occurrence, clinical manifestations, and prognosis of Guillain-Barre syndrome.* Arch Dis Child, 1991. **66**(6): p. 706-8; discussion 708-9.
92. Briscoe, D.M., J.B. McMenamin, and N.V. O'Donohoe, *Prognosis in Guillain-Barre syndrome.* Arch Dis Child, 1987. **62**(7): p. 733-5.
93. Cole, G.F. and D.J. Matthew, *Prognosis in severe Guillain-Barre syndrome.* Arch Dis Child, 1987. **62**(3): p. 288-91.
94. Ropper, A.H., *Severe acute Guillain-Barre syndrome.* Neurology, 1986. **36**(3): p. 429-32.
95. Mishu, B. and M.J. Blaser, *Role of infection due to Campylobacter jejuni in the initiation of Guillain-Barre syndrome.* Clin Infect Dis, 1993. **17**(1): p. 104-8.
96. Rees, J.H., et al., *Campylobacter jejuni infection and Guillain-Barre syndrome.* N Engl J Med, 1995. **333**(21): p. 1374-9.
97. Vriesendorp, F.J., et al., *Electrophysiological studies in Guillain-Barre syndrome: correlation with antibodies to GM1, GD1B and Campylobacter jejuni.* J Neurol, 1995. **242**(7): p. 460-5.
98. Snijders, F., et al., *Prevalence of Campylobacter-associated diarrhea among patients infected with human immunodeficiency virus.* Clin Infect Dis, 1997. **24**(6): p. 1107-13.
99. Chang, C. and J.F. Miller, *Campylobacter jejuni colonization of mice with limited enteric flora.* Infect Immun, 2006. **74**(9): p. 5261-71.
100. Watson, R.O. and J.E. Galan, *Signal transduction in Campylobacter jejuni-induced cytokine production.* Cell Microbiol, 2005. **7**(5): p. 655-65.
101. Hickey, T.E., et al., *Campylobacter jejuni cytolethal distending toxin mediates release of interleukin-8 from intestinal epithelial cells.* Infect Immun, 2000. **68**(12): p. 6535-41.
102. Mellits, K.H., et al., *Activation of the transcription factor NF-kappaB by Campylobacter jejuni.* Microbiology, 2002. **148**(Pt 9): p. 2753-63.
103. Silverman, N. and T. Maniatis, *NF-kappaB signaling pathways in mammalian and insect innate immunity.* Genes Dev, 2001. **15**(18): p. 2321-42.
104. Johanesen, P.A. and M.B. Dwinell, *Flagellin-independent regulation of chemokine host defense in Campylobacter jejuni-infected intestinal epithelium.* Infect Immun, 2006. **74**(6): p. 3437-47.
105. Banchereau, J., et al., *Immunobiology of dendritic cells.* Annu Rev Immunol, 2000. **18**: p. 767-811.
106. Banchereau, J. and R.M. Steinman, *Dendritic cells and the control of immunity.* Nature, 1998. **392**(6673): p. 245-52.
107. Newell, D.G., *Campylobacter, Infection and Immunity*, in *Encylcopedia of Immunology*, P.J.R. Delves, Ivan M, Editor. 1998, Academic Press: San Diego, CA. p. 407-408.

108. Cawthraw, S.A., et al., *Long-term antibody responses following human infection with Campylobacter jejuni.* Clin Exp Immunol, 2002. **130**(1): p. 101-6.
109. Cawthraw, S.A., et al., *Antibodies, directed towards Campylobacter jejuni antigens, in sera from poultry abattoir workers.* Clin Exp Immunol, 2000. **122**(1): p. 55-60.
110. Everest, P., Ketley, Julian M., *Campylobacter*, in *Molecular Medical Microbiology*, M. Sussman, Editor. 2001, Academic Press: San Diego. p. 1311-1329.
111. Scott, D.A. and D.R. Tribble, *Protection Against Campylobacter Infection and Vaccine Development*, in *Campylobacter*, I. Nachamkin and M. Blaser, Editors. 2000, American Society for Microbiology Press: Washington, DC.
112. Blaser, M.J., et al., *Campylobacter jejuni-specific serum antibodies are elevated in healthy Bangladeshi children.* J Clin Microbiol, 1985. **21**(2): p. 164-7.
113. Ruiz-Palacios, G.M., et al., *Protection of breast-fed infants against Campylobacter diarrhea by antibodies in human milk.* J Pediatr, 1990. **116**(5): p. 707-13.
114. Walz, S.E., 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**(5): p. 652-6.
115. Dekeyser, P., et al., *Acute enteritis due to related vibrio: first positive stool cultures.* J Infect Dis, 1972. **125**(4): p. 390-2.
116. Butzler, J.P. and M.B. Skirrow, *Campylobacter enteritis.* Clin Gastroenterol, 1979. **8**(3): p. 737-65.
117. Blaser, M.J., et al., *Campylobacter enteritis: clinical and epidemiologic features.* Ann Intern Med, 1979. **91**(2): p. 179-85.
118. Bolton, F.J. and L. Robertson, *A selective medium for isolating Campylobacter jejuni/coli.* J Clin Pathol, 1982. **35**(4): p. 462-7.
119. Goossens, H., M. De Boeck, and J.P. Butzler, *A new selective medium for the isolation of Campylobacter jejuni from human faeces.* Eur J Clin Microbiol, 1983. **2**(4): p. 389-93.
120. Lastovica, A.J. and E. Le Roux, *Efficient isolation of Campylobacter upsaliensis from stools.* J Clin Microbiol, 2001. **39**(11): p. 4222-3.
121. Paisley, J.W., et al., *Dark-field microscopy of human feces for presumptive diagnosis of Campylobacter fetus subsp. jejuni enteritis.* J Clin Microbiol, 1982. **15**(1): p. 61-3.
122. Nachamkin, I. and S. Barbagallo, *Culture confirmation of Campylobacter spp. by latex agglutination.* J Clin Microbiol, 1990. **28**(4): p. 817-8.
123. Tolcin, R., et al., *Evaluation of the Alexon-trend ProSpecT Campylobacter microplate assay.* J Clin Microbiol, 2000. **38**(10): p. 3853-5.
124. Dediste, A., et al., *Evaluation of the ProSpecT Microplate Assay for detection of Campylobacter: a routine laboratory perspective.* Clin Microbiol Infect, 2003. **9**(11): p. 1085-90.
125. Endtz, H.P., et al., *Evaluation of a new commercial immunoassay for rapid detection of Campylobacter jejuni in stool samples.* Eur J Clin Microbiol Infect Dis, 2000. **19**(10): p. 794-7.

126. Robins-Browne, R.M., et al., *Treatment of Campylobacter-associated enteritis with erythromycin.* Am J Dis Child, 1983. **137**(3): p. 282-5.
127. Tribble, D.R., 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**(3): p. 338-46.
128. Robins-Browne, R.M., et al., *Treatment of acute nonspecific gastroenteritis of infants and young children with erythromycin.* Am J Trop Med Hyg, 1983. **32**(4): p. 886-90.
129. Pai, C.H., et al., *Erythromycin in treatment of Campylobacter enteritis in children.* Am J Dis Child, 1983. **137**(3): p. 286-8.
130. Ruiz, J., et al., *Trends in antimicrobial resistance in Campylobacter spp. causing traveler's diarrhea.* Apmis, 2007. **115**(3): p. 218-24.
131. Shlim, D.R., *Update in traveler's diarrhea.* Infect Dis Clin North Am, 2005. **19**(1): p. 137-49.
132. Dryden, M.S., R.J. Gabb, and S.K. Wright, *Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin.* Clin Infect Dis, 1996. **22**(6): p. 1019-25.
133. Mattila, L., et al., *Short-term treatment of traveler's diarrhea with norfloxacin: a double-blind, placebo-controlled study during two seasons.* Clin Infect Dis, 1993. **17**(4): p. 779-82.
134. Slutsker, L., et al., *Escherichia coli O157:H7 diarrhea in the United States: clinical and epidemiologic features.* Ann Intern Med, 1997. **126**(7): p. 505-13.
135. Wong, C.S., et al., *The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections.* N Engl J Med, 2000. **342**(26): p. 1930-6.
136. OIE/WHO/FAO, *Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific assesment,*. 2004, World Health Organization: Geneva.
137. Engberg, J., et al., *Quinolone and macrolide resistance in Campylobacter jejuni and C. coli: resistance mechanisms and trends in human isolates.* Emerg Infect Dis, 2001. **7**(1): p. 24-34.
138. Lucey, B., et al., *Trends in antimicrobial susceptibility among isolates of Campylobacter species in Ireland and the emergence of resistance to ciprofloxacin.* Vet Rec, 2002. **151**(11): p. 317-20.
139. Luber, P., et al., *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**(12): p. 3825-30.
140. Saenz, Y., et al., *Antibiotic resistance in Campylobacter strains isolated from animals, foods, and humans in Spain in 1997-1998.* Antimicrob Agents Chemother, 2000. **44**(2): p. 267-71.
141. Wagner, J., et al., *Susceptibilities of Campylobacter jejuni isolates from Germany to ciprofloxacin, moxifloxacin, erythromycin, clindamycin, and tetracycline.* Antimicrob Agents Chemother, 2003. **47**(7): p. 2358-61.

142. Hart, C.A. and S. Kariuki, *Antimicrobial resistance in developing countries.* Bmj, 1998. **317**(7159): p. 647-50.
143. Smith, S.I., T.I. Sansa, and A.O. Coker, *Antibiotic susceptibility patterns and beta-lactamase production of animal and human isolates of Campylobacter in Lagos, Nigeria.* Z Naturforsch [C], 1999. **54**(7-8): p. 583-6.
144. Smith, K.E., et al., *Quinolone-resistant Campylobacter jejuni infections in Minnesota, 1992-1998. Investigation Team.* N Engl J Med, 1999. **340**(20): p. 1525-32.
145. Dang, H. *National Antimicrobial Resistance Monitoring System (NARMS).* 2007 [cited 2008 February 25, 2008]; Available from: http://www.fda.gov/cvm/narms_pg.html.
146. Gupta, A., et al., *Antimicrobial resistance among Campylobacter strains, United States, 1997-2001.* Emerg Infect Dis, 2004. **10**(6): p. 1102-9.
147. Engberg, J., et al., *Quinolone-resistant Campylobacter infections: risk factors and clinical consequences.* Emerg Infect Dis, 2004. **10**(6): p. 1056-63.
148. Nelson, J.M., et al., *Prolonged diarrhea due to ciprofloxacin-resistant campylobacter infection.* J Infect Dis, 2004. **190**(6): p. 1150-7.
149. Nelson, J.M., et al., *Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story.* Clin Infect Dis, 2007. **44**(7): p. 977-80.
150. Gaunt, P.N. and L.J. Piddock, *Ciprofloxacin resistant Campylobacter spp. in humans: an epidemiological and laboratory study.* J Antimicrob Chemother, 1996. **37**(4): p. 747-57.
151. Poly, F. and P. Guerry, *Pathogenesis of Campylobacter.* Curr Opin Gastroenterol, 2008. **24**(1): p. 27-31.
152. Crushell, E., et al., *Enteric campylobacter: purging its secrets?* Pediatr Res, 2004. **55**(1): p. 3-12.
153. Drews, G., *Contributions of Theodor Wilhelm Engelmann on phototaxis, chemotaxis, and photosynthesis.* Photosynth Res, 2005. **83**(1): p. 25-34.
154. Engelmann, T.W., *Backterium photometricum. Ein Beitrag zur vergleichenden. Physiolodie des Licht- und Farbensinnes.* Pflugers Arch Gesamte Physiol Menschen Tierre, 1883. **42**.
155. Kretschmer, R.R. and M.L. Collado, *Chemotaxis.* Infection, 1980. **8 Suppl 3**: p. S 299-302.
156. Pfeffer, W., *Lokomotorische Richtengsbewegungen durchemische Reize.* Untersuchengen aus dem Botanischen Institut Tubingen, 1884. **1**: p. 363-482.
157. Hugdahl, M.B., J.T. Beery, and M.P. Doyle, *Chemotactic behavior of Campylobacter jejuni.* Infect Immun, 1988. **56**(6): p. 1560-6.
158. Hazeleger, W.C., et al., *Physiological activity of Campylobacter jejuni far below the minimal growth temperature.* Appl Environ Microbiol, 1998. **64**(10): p. 3917-22.
159. Hess, J.F., R.B. Bourret, and M.I. Simon, *Histidine phosphorylation and phosphoryl group transfer in bacterial chemotaxis.* Nature, 1988. **336**(6195): p. 139-43.

160. Stock, A.M., V.L. Robinson, and P.N. Goudreau, *Two-component signal transduction.* Annu Rev Biochem, 2000. **69**: p. 183-215.
161. Ferrero, R.L. and A. Lee, *Motility of Campylobacter jejuni in a viscous environment: comparison with conventional rod-shaped bacteria.* J Gen Microbiol, 1988. **134**(1): p. 53-9.
162. Zilbauer, M., et al., *Campylobacter jejuni-mediated disease pathogenesis: an update.* Trans R Soc Trop Med Hyg, 2008. **102**(2): p. 123-9.
163. Lee, A., et al., *Mucus colonization as a determinant of pathogenicity in intestinal infection by Campylobacter jejuni: a mouse cecal model.* Infect Immun, 1986. **51**(2): p. 536-46.
164. Pei, Z. and M.J. Blaser, *PEB1, the major cell-binding factor of Campylobacter jejuni, is a homolog of the binding component in gram-negative nutrient transport systems.* J Biol Chem, 1993. **268**(25): p. 18717-25.
165. Konkel, M.E., et al., *Identification and molecular cloning of a gene encoding a fibronectin-binding protein (CadF) from Campylobacter jejuni.* Mol Microbiol, 1997. **24**(5): p. 953-63.
166. Moser, I., W. Schroeder, and J. Salnikow, *Campylobacter jejuni major outer membrane protein and a 59-kDa protein are involved in binding to fibronectin and INT 407 cell membranes.* FEMS Microbiol Lett, 1997. **157**(2): p. 233-8.
167. Fry, B.N., et al., *The galE gene of Campylobacter jejuni is involved in lipopolysaccharide synthesis and virulence.* Infect Immun, 2000. **68**(5): p. 2594-601.
168. Jin, S., et al., *JlpA, a novel surface-exposed lipoprotein specific to Campylobacter jejuni, mediates adherence to host epithelial cells.* Mol Microbiol, 2001. **39**(5): p. 1225-36.
169. Russell, R.G., et al., *Early colonic damage and invasion of Campylobacter jejuni in experimentally challenged infant Macaca mulatta.* J Infect Dis, 1993. **168**(1): p. 210-5.
170. Babakhani, F.K. and L.A. Joens, *Primary swine intestinal cells as a model for studying Campylobacter jejuni invasiveness.* Infect Immun, 1993. **61**(6): p. 2723-6.
171. Konkel, M.E., et al., *The pathogenesis of Campylobacter jejuni-mediated enteritis.* Curr Issues Intest Microbiol, 2001. **2**(2): p. 55-71.
172. Lara-Tejero, M. and J.E. Galan, *Cytolethal distending toxin: limited damage as a strategy to modulate cellular functions.* Trends Microbiol, 2002. **10**(3): p. 147-52.
173. Lara-Tejero, M. and J.E. Galan, *A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein.* Science, 2000. **290**(5490): p. 354-7.
174. Everest, P. and J. Ketley, *Campylobacter*, in *Molecular Medical Microbiology*, M. Sussman, Editor. 2001, Academic Press: San Diego. p. 1311-1329.
175. Hickey, T.E., et al., *Campylobacter jejuni-stimulated secretion of interleukin-8 by INT407 cells.* Infect Immun, 1999. **67**(1): p. 88-93.
176. Everest, P.H., et al., *Roles of leukotriene B4, prostaglandin E2, and cyclic AMP in Campylobacter jejuni-induced intestinal fluid secretion.* Infect Immun, 1993. **61**(11): p. 4885-7.

177. Salyers, A.A. and D.D. Whitt, *Bacterial Pathogenesis, A Molecular Approach*. 2 ed. 2002, Washington, DC: American Society for Microbiology Press.
178. Porth, C.M., *Disorders of Gastrointestinal Function*, in *Pathophysiology, Concepts of Altered Health States*. 2005, Lippincott Wiliams and Wilkins: Philidelphia.
179. Guyton, A.C., *Textbook of Medical Physiolocy*. 8th ed. 1991, Philidelphia, PA: WB Saunders Company.
180. Blaser, M.J., P.F. Smith, and P.F. Kohler, *Susceptibility of Campylobacter isolates to the bactericidal activity of human serum.* J Infect Dis, 1985. **151**(2): p. 227-35.
181. Blaser, M.J., et al., *Extraintestinal Campylobacter jejuni and Campylobacter coli infections: host factors and strain characteristics.* J Infect Dis, 1986. **153**(3): p. 552-9.
182. Kiehlbauch, J.A., et al., *Phagocytosis of Campylobacter jejuni and its intracellular survival in mononuclear phagocytes.* Infect Immun, 1985. **48**(2): p. 446-51.
183. Perez, G.P. and M.J. Blaser, *Campylobacter and Helicobacter*, in *Baron's Medical Microbiology*, S. Barton, Editor. 1996: Galvaston, TX.
184. Aspinall, G.O., A.G. McDonald, and H. Pang, *Lipopolysaccharides of Campylobacter jejuni serotype O:19: structures of O antigen chains from the serostrain and two bacterial isolates from patients with the Guillain-Barre syndrome.* Biochemistry, 1994. **33**(1): p. 250-5.
185. Komagamine, T. and N. Yuki, *Ganglioside mimicry as a cause of Guillain-Barre syndrome.* CNS Neurol Disord Drug Targets, 2006. **5**(4): p. 391-400.
186. Kuroki, S., et al., *Campylobacter jejuni strains from patients with Guillain-Barre syndrome belong mostly to Penner serogroup 19 and contain beta-N-acetylglucosamine residues.* Ann Neurol, 1993. **33**(3): p. 243-7.
187. Wassenaar, T.M. and M.J. Blaser, *Pathophysiology of Campylobacter jejuni infections of humans.* Microbes Infect, 1999. **1**(12): p. 1023-33.
188. Wassenaar, T.M., et al., *Colonization of chicks by motility mutants of Campylobacter jejuni demonstrates the importance of flagellin A expression.* J Gen Microbiol, 1993. **139 Pt 6**: p. 1171-5.
189. Yao, R., D.H. Burr, and P. Guerry, *CheY-mediated modulation of Campylobacter jejuni virulence.* Mol Microbiol, 1997. **23**(5): p. 1021-31.
190. Ziprin, R.L., et al., *The absence of cecal colonization of chicks by a mutant of Campylobacter jejuni not expressing bacterial fibronectin-binding protein.* Avian Dis, 1999. **43**(3): p. 586-9.
191. Pei, Z., et al., *Mutation in the peb1A locus of Campylobacter jejuni reduces interactions with epithelial cells and intestinal colonization of mice.* Infect Immun, 1998. **66**(3): p. 938-43.
192. Johnson, W.M. and H. Lior, *A new heat-labile cytolethal distending toxin (CLDT) produced by Campylobacter spp.* Microb Pathog, 1988. **4**(2): p. 115-26.
193. Whitehouse, C.A., et al., *Campylobacter jejuni cytolethal distending toxin causes a G2-phase cell cycle block.* Infect Immun, 1998. **66**(5): p. 1934-40.

194. Bang, D.D., et al., *Prevalence of cytolethal distending toxin (cdt) genes and CDT production in Campylobacter spp. isolated from Danish broilers.* J Med Microbiol, 2001. **50**(12): p. 1087-94.
195. Hopkins, R.S., R. Olmsted, and G.R. Istre, *Endemic Campylobacter jejuni infection in Colorado: identified risk factors.* Am J Public Health, 1984. **74**(3): p. 249-50.
196. Stafford, R.J., et al., *A multi-centre prospective case-control study of campylobacter infection in persons aged 5 years and older in Australia.* Epidemiol Infect, 2006: p. 1-11.
197. Michaud, S., S. Menard, and R.D. Arbeit, *Campylobacteriosis, Eastern Townships, Quebec.* Emerg Infect Dis, 2004. **10**(10): p. 1844-7.
198. Friedman, C.R., et al., *Risk factors for sporadic Campylobacter infection in the United States: A case-control study in FoodNet sites.* Clin Infect Dis, 2004. **38 Suppl 3**: p. S285-96.
199. Eberhart-Phillips, J., et al., *Campylobacteriosis in New Zealand: results of a case-control study.* J Epidemiol Community Health, 1997. **51**(6): p. 686-91.
200. Effler, P., et al., *Sporadic Campylobacter jejuni infections in Hawaii: associations with prior antibiotic use and commercially prepared chicken.* J Infect Dis, 2001. **183**(7): p. 1152-5.
201. Vellinga, A. and F. Van Loock, *The dioxin crisis as experiment to determine poultry-related campylobacter enteritis.* Emerg Infect Dis, 2002. **8**(1): p. 19-22.
202. Kapperud, G., et al., *Risk factors for sporadic Campylobacter infections: results of a case-control study in southeastern Norway.* J Clin Microbiol, 1992. **30**(12): p. 3117-21.
203. Adak, G.K., et al., *The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of campylobacter infection.* Epidemiol Infect, 1995. **115**(1): p. 15-22.
204. Carrique-Mas, J., et al., *Risk factors for domestic sporadic campylobacteriosis among young children in Sweden.* Scand J Infect Dis, 2005. **37**(2): p. 101-10.
205. Ikram, R., et al., *A case control study to determine risk factors for campylobacter infection in Christchurch in the summer of 1992-3.* N Z Med J, 1994. **107**(988): p. 430-2.
206. Bruce-Grey-Owen Sound Health Unit, *Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May-June 2000.* Can Commun Dis Rep, 2000. **26**(20): p. 170-3.
207. Schonberg-Norio, D., et al., *Swimming and Campylobacter infections.* Emerg Infect Dis, 2004. **10**(8): p. 1474-7.
208. Studahl, A. and Y. Andersson, *Risk factors for indigenous campylobacter infection: a Swedish case-control study.* Epidemiol Infect, 2000. **125**(2): p. 269-75.
209. Tenkate, T.D. and R.J. Stafford, *Risk factors for campylobacter infection in infants and young children: a matched case-control study.* Epidemiol Infect, 2001. **127**(3): p. 399-404.
210. Altekruse, S.F., et al., *Food and animal sources of human Campylobacter jejuni infection.* J Am Vet Med Assoc, 1994. **204**(1): p. 57-61.

211. Workman, S.N., G.E. Mathison, and M.C. Lavoie, *Pet dogs and chicken meat as reservoirs of Campylobacter spp. in Barbados.* J Clin Microbiol, 2005. **43**(6): p. 2642-50.
212. Keller, J., et al., *Distribution and genetic variability among Campylobacter spp. isolates from different animal species and humans in Switzerland.* Zoonoses Public Health, 2007. **54**(1): p. 2-7.
213. Wieland, B., et al., *Campylobacter spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP.* J Vet Med B Infect Dis Vet Public Health, 2005. **52**(4): p. 183-9.
214. Sokolow, S.H., et al., *Epidemiologic evaluation of diarrhea in dogs in an animal shelter.* Am J Vet Res, 2005. **66**(6): p. 1018-24.
215. Burnens, A.P., B. Angeloz-Wick, and J. Nicolet, *Comparison of Campylobacter carriage rates in diarrheic and healthy pet animals.* Zentralbl Veterinarmed B, 1992. **39**(3): p. 175-80.
216. Wilson, I.G., *Airborne Campylobacter infection in a poultry worker: case report and review of the literature.* Commun Dis Public Health, 2004. **7**(4): p. 349-53.
217. Christenson, B., et al., *An outbreak of campylobacter enteritis among the staff of a poultry abattoir in Sweden.* Scand J Infect Dis, 1983. **15**(2): p. 167-72.
218. Potter, R.C., J.B. Kaneene, and W.N. Hall, *Risk factors for sporadic Campylobacter jejuni infections in rural michigan: a prospective case-control study.* Am J Public Health, 2003. **93**(12): p. 2118-23.
219. Schorr, D., et al., *Risk factors for Campylobacter enteritis in Switzerland.* Zentralbl Hyg Umweltmed, 1994. **196**(4): p. 327-37.
220. Gallardo, F., et al., *Campylobacter jejuni as a cause of traveler's diarrhea: clinical features and antimicrobial susceptibility.* J Travel Med, 1998. **5**(1): p. 23-6.
221. Riddle, M.S., et al., *Incidence, etiology, and impact of diarrhea among long-term travelers (US military and similar populations): a systematic review.* Am J Trop Med Hyg, 2006. **74**(5): p. 891-900.
222. Cohen, M.L. and R.V. Tauxe, *Drug-resistant Salmonella in the United States: an epidemiologic perspective.* Science, 1986. **234**(4779): p. 964-9.
223. Pavia, A.T., et al., *Epidemiologic evidence that prior antimicrobial exposure decreases resistance to infection by antimicrobial-sensitive Salmonella.* J Infect Dis, 1990. **161**(2): p. 255-60.
224. Blaser, M.J., et al., *Outbreaks of Campylobacter enteritis in two extended families: evidence for person-to-person transmission.* J Pediatr, 1981. **98**(2): p. 254-7.
225. Goossens, H., et al., *Investigation of an outbreak of Campylobacter upsaliensis in day care centers in Brussels: analysis of relationships among isolates by phenotypic and genotypic typing methods.* J Infect Dis, 1995. **172**(5): p. 1298-305.
226. Sobel, J., et al., *Pathogen-specific risk factors and protective factors for acute diarrheal illness in children aged 12-59 months in Sao Paulo, Brazil.* Clin Infect Dis, 2004. **38**(11): p. 1545-51.
227. Anonymous, *Annual Report on Zoonoses in Denmark, 2002*. 2003, Ministry of Food, Agriculture and Fisheries.

228. Swedish Institute for Infectious Disease Control. *Statistics on infectious diseases*. [cited July 6, 2007]; Available from: www.smittskyddsinstitutet.se.
229. DeNavas-Walt, C., B. Proctor, and J. Smith, *Income, Poverty, and Health Insurance Coverage in the United States: 2006*. 2007, US Census Bureau.
230. Centers for Disease Control and Prevention, *Preliminary FoodNet data on the incidence of foodborne illnesses--selected sites, United States, 2001.* MMWR Morb Mortal Wkly Rep, 2002. **51**(15): p. 325-9.
231. Louis, V.R., et al., *Temperature-driven Campylobacter seasonality in England and Wales.* Appl Environ Microbiol, 2005. **71**(1): p. 85-92.
232. Potter, R.C., J.B. Kaneene, and J. Gardiner, *A comparison of Campylobacter jejuni enteritis incidence rates in high- and low-poultry-density counties: Michigan 1992-1999.* Vector Borne Zoonotic Dis, 2002. **2**(3): p. 137-43.
233. Durant, D., *FoodNet Follow-Up.* The Food Safety Educator, 2000. **5**(3): p. 4.
234. Stafford, R.J., T.D. Tenkate, and B. McCall, *A Five Year Review of Campylobacter Infection in Queensland.* Communicable Diseases Intelligence, 1996. **20**(22): p. 478-482.
235. Skirrow, M.B., *A demographic survey of campylobacter, salmonella and shigella infections in England. A Public Health Laboratory Service Survey.* Epidemiol Infect, 1987. **99**(3): p. 647-57.
236. van Hees, B.C., et al., *Regional and seasonal differences in incidence and antibiotic resistance of Campylobacter from a nationwide surveillance study in The Netherlands: an overview of 2000-2004.* Clin Microbiol Infect, 2007. **13**(3): p. 305-10.
237. Altekruse, S.F., et al., *Campylobacter jejuni--an emerging foodborne pathogen.* Emerg Infect Dis, 1999. **5**(1): p. 28-35.
238. Albrecht, J.A., *Food safety knowledge and practices of consumers in the U.S.A*. 1995. p. 119-134.
239. Samuel, M.C., et al., *Epidemiology of sporadic Campylobacter infection in the United States and declining trend in incidence, FoodNet 1996-1999.* Clin Infect Dis, 2004. **38 Suppl 3**: p. S165-74.
240. Population and Environmental Health Group and Institute of Environmental Science and Research Limited, *Notifiable and Other Diseases in New Zeland: Annual Report 2006*. 2007, New Zeland, Ministry of Health.
241. Nylen, G., et al., *The seasonal distribution of campylobacter infection in nine European countries and New Zealand.* Epidemiol Infect, 2002. **128**(3): p. 383-90.
242. Meldrum, R.J., et al., *The seasonality of human campylobacter infection and Campylobacter isolates from fresh, retail chicken in Wales.* Epidemiol Infect, 2005. **133**(1): p. 49-52.
243. Naumova, E.N., et al., *Seasonality in six enterically transmitted diseases and ambient temperature.* Epidemiol Infect, 2007. **135**(2): p. 281-92.
244. Green, C.G., D. Krause, and J. Wylie, *Spatial analysis of Campylobacter infection in the Canadian province of Manitoba.* Int J Health Geogr, 2006. **5**(1): p. 2.
245. Ethelberg, S., et al., *Spatial distribution and registry-based case-control analysis of Campylobacter infections in Denmark, 1991-2001.* Am J Epidemiol, 2005. **162**(10): p. 1008-15.

246. Hearnden, M., et al., *The regionality of campylobacteriosis seasonality in New Zealand.* Int J Environ Health Res, 2003. **13**(4): p. 337-48.
247. WHO, E.P.H.R.i.D.R.T., *Methods for foodborne disease surveillance in selected sites : report of a WHO consultation 18-21 March 2002, Leipzig, Germany* 2002, World Health Organization: Geneva. p. 29.
248. Anonymous, *Enter-net annual report:2005- surveillance of enteric pathoggens in Europe and beyond.* 2007, Enter-net surveillance hub, HPA, Centre for Infections: London.
249. World Health Organization. *Global Salm Surv (GSS).* 2007 [cited 2007 June 27, 2007]; Available from: http://www.who.int/salmsurv/en/.
250. Centers for Disease Control and Prevention. *Nationally Notifiable Infectious Diseases* 2006 November 4, 2008 [cited 2008 November 21, 2008]; Available from: http://www.cdc.gov/ncphi/disss/nndss/phs/infdis2006.htm.
251. *Tennessee Department of Health Notifiable Disease Report*. [PDF] [cited 2008 5/21/08]; Available from: http://health.state.tn.us/Downloads/ph-1600.pdf.
252. Hardnett, F.P., et al., *Epidemiologic issues in study design and data analysis related to FoodNet activities.* Clin Infect Dis, 2004. **38 Suppl 3**: p. S121-6.
253. Jones, T.F., *The Tennessee Foodborne Illness Surveillance Network (FoodNet).* Tenn Med, 2000. **93**(9): p. 334-5.
254. Health, T.D.o., *Welcome New FoodNet Laboratories.* EIP Bulletin: Tennessee Emerging Infectious Disease Program, 2003.
255. *Tennessee Emerging Infections Program*. [cited 2008 September 14, 2008]; Available from: http://health.state.tn.us/ceds/EIP/programs.htm.
256. Voetsch, A.C., et al., *FoodNet estimate of the burden of illness caused by nontyphoidal Salmonella infections in the United States.* Clin Infect Dis, 2004. **38 Suppl 3**: p. S127-34.
257. *American Fact Finder Help, Census Data Information, Urban/Rural*. [cited August 13, 2008]; Available from: factfinder.census.gov.
258. US Census Bureau Geography Division. *Cartographic Boundary Files, DESCRIPTIONS AND METADATA*. 2001 [cited 2008 7/15/08]; Available from: http://www.census.gov/geo/www/cob/metadata.html.
259. CDC. *FoodNet Surveillance*. 2005 [cited 2008 April 6]; Available from: http://www.cdc.gov/foodnet/surveillance.htm.
260. *A matched case control study of Campylobacteriosis in southern Ontario*.
261. Centers for Disease Control and Prevention, *Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 States, United States, 2005.* MMWR Morb Mortal Wkly Rep, 2006. **55**(14): p. 392-5.
262. Centers for Disease Control and Prevention, *Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 sites, United States, 2004.* MMWR Morb Mortal Wkly Rep, 2005. **54**(14): p. 352-6.
263. Centers for Disease Control and Prevention, *Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--*

selected sites, United States, 2003. MMWR Morb Mortal Wkly Rep, 2004. **53**(16): p. 338-43.
264. CDC, *FoodNet Surveillance Report for 2004 (Final Report)*. 2006, CDC Division of Bacterial and Mycotic Disease: Atlanta, GA.
265. CDC, *FoodNet Surviellance Report for 2003*. 2005, CDC Division of Bacterial and Mycotic Diseases: Atlanta, GA.
266. Horbar, J.D. and K.A. Leahy, *An assessment of data quality in the Vermont-Oxford Trials Network database.* Control Clin Trials, 1995. **16**(1): p. 51-61.
267. Hogan, W.R. and M.M. Wagner, *Accuracy of data in computer-based patient records.* J Am Med Inform Assoc, 1997. **4**(5): p. 342-55.
268. Ford, M.W., et al., *A descriptive study of human Salmonella serotype typhimurium infections reported in Ontario from 1990 to 1998.* Can J Infect Dis, 2003. **14**(5): p. 267-73.
269. Majowicz, S.E., et al., *Descriptive analysis of endemic cryptosporidiosis cases reported in Ontario, 1996-1997.* Can J Public Health, 2001. **92**(1): p. 62-6.
270. Sandberg, M., et al., *Incidence trend and risk factors for campylobacter infections in humans in Norway.* BMC Public Health, 2006. **6**: p. 179.
271. Kapperud, G., et al., *Factors associated with increased and decreased risk of Campylobacter infection: a prospective case-control study in Norway.* Am J Epidemiol, 2003. **158**(3): p. 234-42.
272. Wardak, S., U. Duda, and J. Szych, *[Epidemiological analysis of campylobacteriosis reported by Sanitary Epidemiological Station in Bielsko-Biala, Silesia, in Poland].* Przegl Epidemiol, 2007. **61**(2): p. 417-24.
273. *2005 Commonwealth Fund International Health Policy Survey*. 2006, The Commonwealth Fund: New York.
274. Huynh, P.T., et al., *THE U.S. Health Care Divide:Diparities in Primary Care Experiances by Income. Findings from the Commonwealth Fund 2004 International Health Policy Survey*. 2006, The Commonwealth Fund: New York, NY.
275. Gallay, A., et al., *Risk factors for acquiring sporadic Campylobacter infection in France: results from a national case-control study.* J Infect Dis, 2008. **197**(10): p. 1477-84.
276. Gillespie, I.A., et al., *Point source outbreaks of Campylobacter jejuni infection--are they more common than we think and what might cause them?* Epidemiol Infect, 2003. **130**(3): p. 367-75.
277. Hassanzadeh, P. and M. Motamedifar, *Occurrence of Campylobacter jejuni in Shiraz, Southwest Iran.* Med Princ Pract, 2007. **16**(1): p. 59-62.
278. Al-Shamahy, H.A., A. Al-Robasi, and K.A. Al-Moyed, *Epidemiology,clinical features and antibiotic susceptibility of Campylobacter infections in Sana'a,Yemen.* Journal of Chinese Clinical Medicine, 2007. **2**(8).
279. Glass, R.I., et al., *Epidemiologic and clinical features of endemic Campylobacter jejuni infection in Bangladesh.* J Infect Dis, 1983. **148**(2): p. 292-6.
280. Taylor, D.N., et al., *Campylobacter immunity and quantitative excretion rates in Thai children.* J Infect Dis, 1993. **168**(3): p. 754-8.

281. Tracz, D.M., et al., *pVir and bloody diarrhea in Campylobacter jejuni enteritis.* Emerg Infect Dis, 2005. **11**(6): p. 838-43.
282. Taylor, D.N., et al., *Influence of strain characteristics and immunity on the epidemiology of Campylobacter infections in Thailand.* J Clin Microbiol, 1988. **26**(5): p. 863-8.
283. Oberhelman, R.A. and D.N. Taylor, *Campylobacter Infections in Developing Countries*, in *Campylobacter*, I. Nachamkin and M. Blaser, Editors. 2000, American Society for Microbiology Press: Washington, DC. p. 139-153.
284. CDC. *Campylobacter General Information*. 2008 May 21, 2008 [cited 2008 July 8, 2008]; Available from: http://www.cdc.gov/nczved/dfbmd/disease_listing/campylobacter_gi.html.
285. Kist, M., *Campylobacter enteritis: epidemiological and clinical data from recent isolations in the region of Freiburg, West German*, in *Campylobacter, Epidemiology, Pathogenesis, and Biochemistry*, D.G. Newell, Editor. 1982, MTP Press: Southamptom, England.
286. Nachamkin, I., H. Ung, and M. Li, *Increasing fluoroquinolone resistance in Campylobacter jejuni, Pennsylvania, USA,1982-2001.* Emerg Infect Dis, 2002. **8**(12): p. 1501-3.
287. Hoge, C.W., et al., *Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years.* Clin Infect Dis, 1998. **26**(2): p. 341-5.
288. Blaser, M.J., *Epidemiologic and clinical features of Campylobacter jejuni infections.* J Infect Dis, 1997. **176 Suppl 2**: p. S103-5.
289. SMI. *Sjukdomsinformation om campylobacterinfektion*. [cited 2008 August 28, 2008]; Available from: http://www.smittskyddsinstitutet.se/sjukdomar/campylobacterinfektion/.
290. The Swedish Society for Communicable Disease Prevention and Control. *Campylobacter, patient information*. 2004 March 7, 2007 [cited 2008 July 28, 2008]; Available from: http://www.lakarforbundet.se/templates/AssociationPage.aspx?id=17115.
291. *Hospitalization Status of Campylobacter Cases Denver Metropolitain Area^: 2001*. 2001 [cited 2008 July 8, 2008]; Available from: http://www.cdphe.state.co.us/dc/eip/FoodNet/Campy/Campy_hosp_01.html.
292. Rajda, Z. and D. Middleton, *Descriptive epidemiology of enteric illness for selected reportable diseases in Ontario, 2003.* Can Commun Dis Rep, 2006. **32**(23): p. 275-85.
293. Helms, M., J. Simonsen, and K. Molbak, *Foodborne bacterial infection and hospitalization: a registry-based study.* Clin Infect Dis, 2006. **42**(4): p. 498-506.
294. Brooks, W.C. *Campylobacter, Salmonella, & E. Coli: Causes of Diarrhea in Puppies & Kittens*. Pet Health Library 2006 [cited 2008 September 15, 2008]; Available from: http://www.VeterinaryPartner.com/Content.plx?P=A&A=2232.
295. Gruffydd-Jones, T.J., M. Marston, and E. White, *Campylobacter jejuni enteritis from cats.* Lancet, 1980. **2**(8190): p. 366.
296. Prescott, J.F. and M.A. Karmali, *Attempts to transmit campylobacter enteritis to dogs and cats.* Can Med Assoc J, 1978. **119**(9): p. 1001-2.

297. Rossi, M., et al., *Occurrence and species level diagnostics of Campylobacter spp., enteric Helicobacter spp. and Anaerobiospirillum spp. in healthy and diarrheic dogs and cats.* Vet Microbiol, 2008. **129**(3-4): p. 304-14.
298. Guest, C.M., J.M. Stephen, and C.J. Price, *Prevalence of Campylobacter and four endoparasites in dog populations associated with Hearing Dogs.* J Small Anim Pract, 2007. **48**(11): p. 632-7.
299. Acke, E., et al., *Prevalence of thermophilic Campylobacter species in cats and dogs in two animal shelters in Ireland.* Vet Rec, 2006. **158**(2): p. 51-4.
300. Gargiulo, A., et al., *Survey of Campylobacter jejuni in stray cats in southern Italy.* Lett Appl Microbiol, 2008. **46**(2): p. 267-70.
301. Grant, I.H., N.J. Richardson, and V.D. Bokkenheuser, *Broiler chickens as potential source of Campylobacter infections in humans.* J Clin Microbiol, 1980. **11**(5): p. 508-10.
302. Pearson, A.D., et al., *Microbial ecology of Campylobacter jejuni in a United Kingdom chicken supply chain: intermittent common source, vertical transmission, and amplification by flock propagation.* Appl Environ Microbiol, 1996. **62**(12): p. 4614-20.
303. Seattle-King County Health Department, *Surveillance of the flow of Salmonella and Campylobacter in a community*. 1984, Communicable Disease Control Section, Seattle-King Country Department of Public Health: Seattle, WA.
304. Goddard, J. *Water-Borne Illness Becoming More Common*. 2007 [cited 2008 August 28,2008]; Available from: http://www.volunteertv.com/home/headlines/10009501.html.
305. *Diagnosis and management of foodborne illnesses: a primer for physicians.* MMWR Recomm Rep, 2001. **50**(RR-2): p. 1-69.
306. Fleury, M., et al., *A time series analysis of the relationship of ambient temperature and common bacterial enteric infections in two Canadian provinces.* Int J Biometeorol, 2006. **50**(6): p. 385-91.
307. Kovats, R.S., et al., *Climate variability and campylobacter infection: an international study.* Int J Biometeorol, 2005. **49**(4): p. 207-14.
308. Edge, V.L., et al., *Physician diagnostic and reporting practices for gastrointestinal illnesses in three health regions of British Columbia.* Can J Public Health, 2007. **98**(4): p. 306-10.

Appendices

Appendix A

Information Obtained by East Tennessee Regional and Knox County Health Departments on Campylobacteriosis Case Report Forms

A.1 Information collected on campylobacteriosis case report form used from 2003-2005

Demographics
Full name
Complete address
Phone number
Date of birth
Sex
Race (white, black, native-American, Asian/pacific islander, unknown)
Ethnicity (Hispanic, non-Hispanic, unknown)
Employer, School, Daycare or After-school center attended

Clinical Data
Date/Time of symptom onset
Symptoms (diarrhea, bloody stool, fever, headache, nausea, vomiting, cramps, muscle aches, fatigue, other)
Duration of symptoms
Treatment
Physician visit (doctor's office, urgent care, emergency room, none)
    Physician name
Hospitalized (yes, no, unknown)
    Hospital name, Admission date, Discharge date

Laboratory Data
Specimen source (stool, blood, urine, none, other)
Date of sample collection
Laboratory test conducted (culture, ova & parasite, serological, other)
Name of the reporting laboratory
Date of laboratory report

Exposures
Travel to another state or country in the two weeks prior to onset? (location, date)
Contact with any animals? (list)
Handle raw meat/poultry? (yes, no, unknown)
Household member in daycare?
Contact with person with similar symptoms?
Hike, camp, fish, or swim?
Drink from a spring, stream, or lake?

Household water supply (city, well, spring, other)

List of household members (age, relationship, symptomatic, date of onset, also tested, occupation)

Food History for 3 days prior to onset of symptoms
List of restaurants dined at 1 week prior to onset of symptoms

A.2 Information collected on campylobacteriosis case report form, 2006

Demographic
Full Name
Date of Birth
Reported Age
Sex
Full Address
Phone Number
Ethnicity (Hispanic, not Hispanic)
Race (American Indian/Alaskan, Asian, Black/African American, Hawaiian/Pacific islander, White, other)
Employer/School/Daycare
Occupation

Laboratory Report
Reporting facility
Ordering facility
Ordering provider
Laboratory report date
Date received by Health Department
Specimen source (blood, stool, cerebral-spinal fluid, urine, other, unknown)
Date sample collected

Clinical Information
Physician
Was the patient hospitalized for this illness? (yes, no, unknown)
    Hospital, Admission date, Discharge date
Diagnosis date
Is the patient pregnant?
Did the patient die from this illness?

Epidemiologic Information
Is this patient associated with a daycare facility?
Is this patient a food handler?
Is this case part of an outbreak?
Where was the disease acquired? (indigenous, out of jurisdiction, out of state, out of country, unknown)
Transmission mode (foodborne, waterborne, zoonotic, indeterminate, other)

Symptom History
Date/Time of illness onset
First symptoms
Symptoms (abdominal cramps, backache, bloody diarrhea, chills, constipation, diarrhea, fatigue, fever, headache, muscle aches, nausea, vomiting)

Date/Time of recovery
Duration

Travel History
Did patient travel prior to onset of illness?
Type (domestic, international)
Destination (date of arrival, date of departure)
Mode of travel (airplane, bus, car, cruise, ship, train)

Related Cases
Does the patient know of any similarly ill persons?
Are there any other cases related to this one?

Possible Source(s) of Infection During Exposure Period
Consumed any poultry? Undercooked?
Handled raw poultry
Consumed food at a group meal
Consumed food from restaurants
Contact with diapered children
Contact with any other persons having diarrhea
Occupational exposure to human or animal excreta

Drinking Water Exposure
Household water source (do not use tap water, municipal or city, private well, other, unknown)
If private well, how was the well treated?
What is the source of tap water at school/work?
Did the patient drink untreated water in the 7 days prior to onset of illness?

Recreational Water Exposure
Was there recreational water exposure in the 7 days prior to illness?
(hot spring, hot tub-whirlpool-jacuzzi-spa, interactive fountain, lake-pond-river-stream, ocean, recreational water park, swimming pool, other, unknown)

Animal Contact
Did the patient visit or live on a farm?
Did the patient visit a live animal exhibit?
Dif the patient come in contact with any animals?
Type of animal (cat, cattle, chicken, dog, goats, lizard, other, other amphibian, other mammal, other reptile, rodent, sheep, turkey, turtle, unknown)
Location of animal contact
Did the patient acquire a pet prior to onset of illness?

Patient Prophylaxis/Treatment
Was the patient treated with any antibiotics for this illness?

A.3 Variables included on the campylobacteriosis case report form that were added to the electronic dataset

Laboratory test ordered (stool culture, ova and parasite, serological)
Date of onset
Clinical symptoms (yes, no; fever-specific temperature)
Duration of symptoms
Treatment
Location of physician visit
Animal exposure
Other risk factor exposures (raw meat, other cases, daycare, outdoor activity, drinking untreated water)
Household water source (well, city, spring, other)

A.4 Variables present in the electronic campylobacteriosis datasets obtained from the East Tennessee Regional and Knox County Health Department

Variable*	Years in the dataset
City	All
Address	2003-2004
State	All
County	All
Zip code	All
Birth date	All
Age	All
Race	All
Ethnicity	All
Gender	All
Serotype	2004-2006†
Specimen source	All
Date of specimen collection/diagnosis date	All
Date reported	All
Outcome	All
Patient Status (hospital, outpatient, unknown)	All
Patient hospitalized?	2003, 2005-2006
Hospital name	2003-2004
Admission and discharge dates	All
Travel	2004-2006
Destination and travel dates	2005-2006
Reporting laboratory name	2004-2006

* Some variables, that had no pertinent information to this study, were excluded from the list

† Some information on serotype was collected in 2003 in the "other" variable field, but data were incomplete

A.5 New variables created from information provided on the campylobacteriosis case report form

Antibiotic prescribed (yes, no, unknown)
Class of antibiotic
Intravenous fluids given (yes, no, unknown)
Anti diarrheal or anti nausea medication given (yes, no, unknown)
Number of treatments
Age group
Severity (mild, moderate, severe)
Time waited before seeking medical care
Days spent in hospital (Length of stay)
Season

Appendix B

Counties Groupings Determined by Urbanicity and Prevalence of Campylobacteriosis Created for Analysis

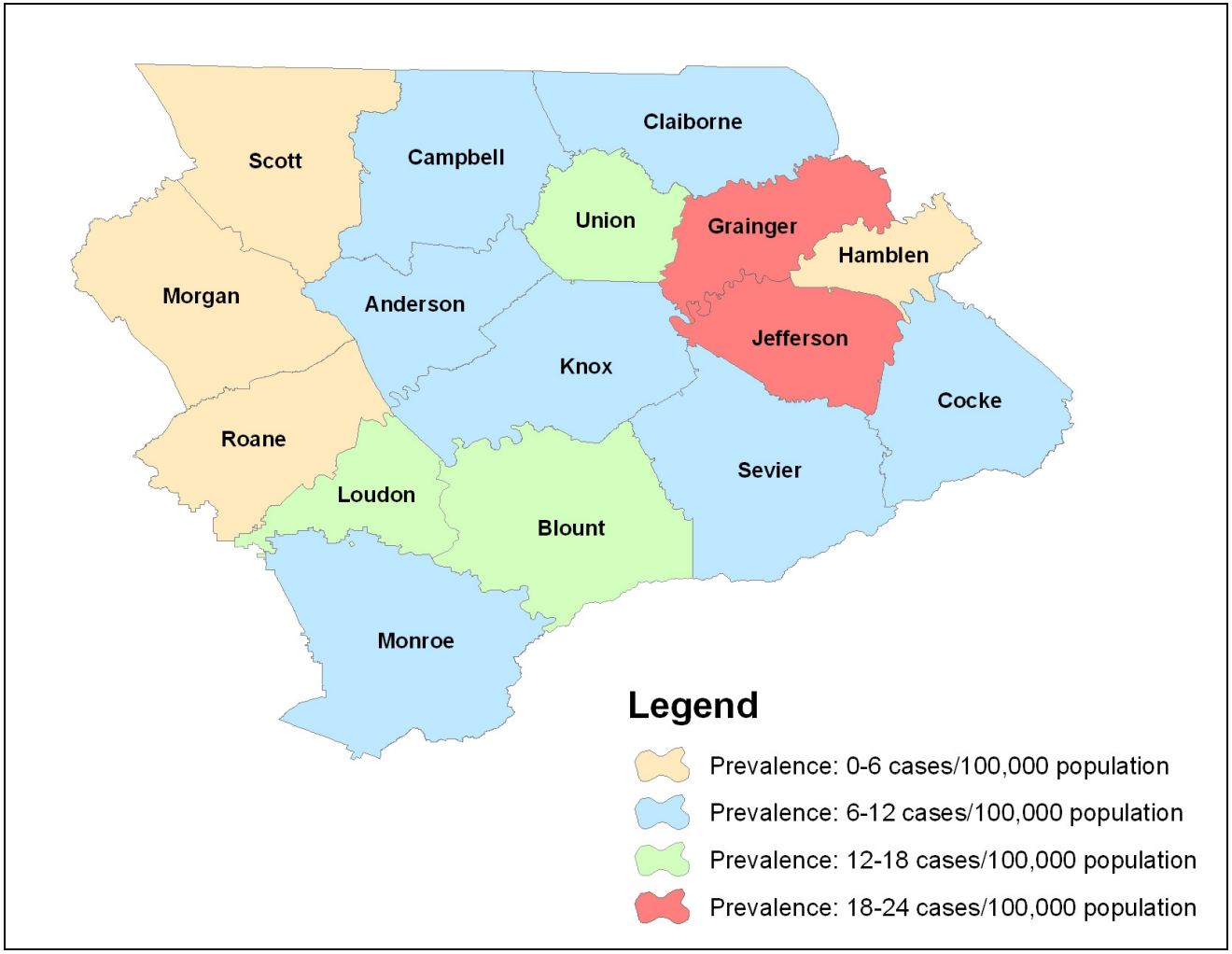
B.1 Groupings of the study region based on the urbanicity of the county

Grouping	Percentage of population living in an urban area
Group 1 (66% or more)	
Knox County	87%
Hamblen County	75%
Group 2 (44 – 66%)	
Blount County	63%
Anderson County	58%
Roane County	51%
Loudon County	50%
Group 3 (22 – 44%)	
Campbell County	43%
Sevier County	35%
Cocke County	33%
Claiborne County	30%
Jefferson County	25%
Monroe County	23%
Group 4 (0 – 22%)	
Morgan County	18%
Scott	15%
Grainger	0%
Union	0%

B.2 Groupings of the study region based on the prevalence of campylobacteriosis in the county

Grouping	Age- and Sex-Standardized Mean Annual Prevalence (cases/100,000 population)
Group 1 (0 – 6 cases/100,000 population)	
Hamblen County	3.8
Roane County	4.4
Scott County	5.1
Morgan County	5.7
Group 2 (6 – 12 cases/100,000 population)	
Campbell County	7.1
Sevier County	7.6
Cocke County	8.7
Claiborne County	8.8
Anderson County	9.3
Monroe County	9.3
Knox County	11.8
Group 3 (12 – 18 cases/100,000 population)	
Loudon County	13.1
Union County	14.0
Blount County	15.6
Group 4 (18 – 24 cases/100,000 population)	
Jefferson County	21.8
Grainger County	22.9

B.3 Map of the counties in the study region grouped based on the age- and sex-standardized prevalence of campylobacteriosis



Vita

Karissa Dawn Laughter was born in Portland, Oregon on April 19, 1981. She graduated from Half Moon Bay High School in 1999. Karissa attended the Massachusetts Institute of Technology in Cambridge, MA and received her BS in Biology with minors in Biomedical Engineering and Chemistry in 2003. She spent two years in rural Paraguay serving with the Peace Corps in the Beekeeping Extension program. She attended graduate school at the University of Tennessee in Knoxville, TN and received her Masters in Comparative and Experimental Medicine with a minor in Statistics in 2008.