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Geographic and Seasonal Variation in Campylobacteriosis

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Geographic and Seasonal Variation in Campylobacteriosis

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An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
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Abstract
Geographic and Seasonal Variation in Campylobacteriosis
By Elizabeth C. Ailes

Campylobacter infections are a major cause of bacterial gastroenteritis in the U.S., where approximately 2 million people become ill annually. As campylobacteriosis is not nationally notifiable and reporting requirements vary from state to state, laboratory-based surveillance data from the Foodborne Diseases Active Surveillance Network (FoodNet) is vital for assessing the magnitude of the disease burden and monitoring trends of this important pathogen. Since FoodNet was established in 1996, regional and seasonal variation in campylobacteriosis has been observed. In order to better understand these patterns, the first goal of this dissertation was to examine key factors that may explain the observed geographic variation in campylobacteriosis across the 10 FoodNet sites, such as geographic differences in surveillance artifacts (health care use, stool sample submission, and clinical laboratory practices) or risk factors for campylobacteriosis (high risk foods and other exposures). The second goal was to examine whether climatic factors were associated with campylobacteriosis and whether seasonal variation in risk factors existed. An analysis of multiple cross-sectional surveys conducted in the FoodNet sites did not find evidence to suggest that differences in healthcare utilization or stool sample submission practices explained the geographic variation in campylobacteriosis. Similarly, analysis of a survey of clinical laboratories found that variation in clinical laboratory methods is unlikely to account for the geographic variation in campylobacteriosis. Additionally, an examination of case-control study data showed no evidence of geographic effect modification of select risk factors for campylobacteriosis, although the frequency of some exposures did vary by FoodNet site. An analysis of surveillance and meteorological data showed a modest association between campylobacteriosis and both minimum temperature and extreme precipitation events. No evidence of seasonal effect modification of risk factors was found. The goal of this dissertation was to examine key factors that may explain the observed variation in campylobacteriosis, given the constraints of data collected for public health surveillance purposes. Reasons for the geographic and seasonal variation in campylobacteriosis in the FoodNet sites remain elusive. Potential areas of further exploration include differences in the circulating strains of *Campylobacter*, the quantity of *Campylobacter* on poultry, and immune response to *Campylobacter.*

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Campylobacter infections are the most common cause of bacterial gastrointestinal illness in the industrialized world and are a leading cause of bacterial foodborne illness in the United States (U.S.), where approximately 2 million people are infected annually.[1] Although outbreaks of *Campylobacter* infection can occur, infections in humans are usually sporadic[2] and person-to-person transmission is limited. Identified risk factors for *Campylobacter* infection include consumption of chicken, unpasteurized milk, and untreated water,[1, 3-6] as well as exposure to dogs, cats or poultry, working or living on a farm, and foreign travel.[1, 4, 6] In many industrialized countries the epidemiology of *Campylobacter* infections shows a bimodal age distribution, with increases in incidence in children less than one and in individuals between 20-35 years of age.[1, 4] Strong seasonal variation in incidence is notable as well, with peak incidence often observed during the summer months.[2, 3, 7 8-10] While most infections are self-limited, the symptoms of campylobacteriosis typically include diarrhea, fever, abdominal cramps, and muscle pain.[11] Furthermore, long-term sequelae, such as Guillan-Barré syndrome and reactive arthritis, can develop in ~ 1% of patients. In the U.S., the annual economic cost of campylobacteriosis related to days of work lost, medical visits, and death has been estimated at $0.6 - $1.0 billion.[12]

As campylobacteriosis is not a nationally notifiable disease in the U.S., and reporting requirements vary widely from state to state, information on the incidence of this disease is obtained from the Foodborne Diseases Active Surveillance Network (FoodNet). FoodNet began in 1996 in 5 sites and has grown to include 10 sites as of

2008. In addition to performing active surveillance for culture-confirmed *Campylobacter* infections, FoodNet has conducted a number of targeted studies to better understand the epidemiology of campylobacteriosis. Since the beginning of FoodNet, regional variation in *Campylobacter* incidence has been consistently observed. The average annual incidence of culture-confirmed *Campylobacter* infections from 1996-2006 ranged from 7/100,000 in both Maryland and Tennessee to 34/100,000 in California.[13] Reasons for this geographic variation are unclear. While differences in surveillance artifacts (health care access, stool culture submission, and clinical laboratory testing) may be a cause of geographic variation, it is also possible that there are true regional differences in the risk for infection.

The goals of my dissertation are to examine factors that may be related to regional and seasonal variation in *Campylobacter* incidence among FoodNet sites, given the constraints of data collected for public health surveillance and detection purposes.

CHAPTER 2. BACKGROUND

Campylobacter Microbiology

First identified as a *vibrio*-like cause of a illness in humans in the 1950s,[14] *Campylobacter* had been known to cause abortions and infertility in cattle and sheep and diarrhea in pigs and other animals for a number of years. With the advent of new laboratory techniques such as selective media and filtration, the importance of *Campylobacter* as a source of human illness came to light only in the late 1970s.[6, 15]

Members of *Campylobacteraceae* family include not only *Campylobacter,* but also the genus *Acrobacter* and the species *Bacteroides ureolyticus,* and are related to the *Helicobacter* genus.[16] These bacteria are similar morphologically to *Vibrio* and were first thought to be a novel *Vibrio* species. *Campylobacter* are spiral-shaped, flagellated, Gram-negative, bacterial rods that exhibit a darting motility. They are *microaerophilic* (requiring lower than atmospheric levels of oxygen) and many, particularly those responsible for human illness, are *thermophilic* (thriving at 37^{o} - 42^{o} Celsius), making them well-adapted to the intestinal tract of mammals and birds. Both the specific environmental conditions necessary for the growth of these bacteria and their fragility were partially responsible for the delay in the isolation of *Campylobacter* in the laboratory.

There have been over a dozen species of *Campylobacter* identified thus far, and with the development of more sensitive laboratory assays, new species are still being observed and categorized. While most species are causative agents of gastrointestinal disease, some species of *Campylobacter*, including *C. gracilis*, *C. concisus*, *C. curvus*,[17] *C. rectus* and *C. showae*,[18] have been associated with periodontal infections in humans.

Several species, such as *C. fetus*, are more often found in immunocompromised hosts.[19] Furthermore, a number of species have so far only been found in animal hosts and have yet to be associated with disease in humans. However, the clinical laboratory methods used in many areas currently favor particularly thermophilic *Campylobacter* species, such as *C. jejuni*, *C. coli* and *C. lari*.[20]

While it has been estimated that over ninety-percent of the gastrointestinal disease caused by *Campylobacter* is a result of infections of *C. jejuni* and *C. coli,* as laboratory methods for the isolation of *Campylobacter* are further refined, other *Campylobacter* species may become recognized as important causes of human illness. For example, co-infections of *C. coli* and *C. jejuni*, as well as infections with *C. upsaliensis, C. hyointestinalis*, and *C. lari*, have recently been identified in patients with diarrhea.[21] Newly identified *Campylobacter* species, such as *C. hominus*, found in the stools of healthy individuals,[22] and *C. lanienae*, isolated from healthy workers at a poultry abbattoir,[23] have also just been recognized. A better understanding of the impact of laboratory methods on the selection of certain *Campylobacter* species over others is needed. The majority of the following discussion of *Campylobacter*, unless specifically stated, deals primarily with *C. jejuni*, and to some extent *C. coli*.

Even within distinct species of *Campylobacter*, particularly within *C. jejuni* and *C. coli* (those most widely studied), there can be great strain-to-strain variation as is evidenced by genetic and phenotypic differences.[24] These bacteria have the ability to acquire and exchange genetic material through a number of mechanisms, resulting in an ever increasing array of different strains. Unlike other bacteria, such as *Salmonella*, for which the serotype often provides some indication of the potential source of infection,

Campylobacter serotypes are not a good marker for host-specificity or virulence. Therefore, a more refined genetic fingerprint of the bacteria has been needed.[25] Much of the work identifying various *Campylobacter* species, and specific genetic strains of *Campylobacter* within each species, has benefited from the development of new molecular subtyping techniques. Methods used to type *Campylobacter* include amplified fragment length polymorphism (AFLP), multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE), though new methods are also being developed.[26] Studies using these methods have allowed comparisons between strains found in various animal, environmental, and human sources and provide a better understanding of how various types of *Campylobacter* are dispersed through the environment, and ultimately the specific strains that are associated with human illness.[25,27-29] These "attribution" studies (which are able to associate human cases with specific environmental or animal sources due to the genetic similarity of the *Campylobacter* strains), have been conducted in a number of countries.

Survival Mechanisms

Given the stringent growth requirements necessary for *Campylobacter* to survive, these bacteria have developed a number of survival mechanisms that allow longer persistence in the environment. A number of bacteria, including *Escherichia coli, Salmonella*, and *Vibrio*, and it has been suggested for *Campylobacter* as well, exhibit the ability to enter a viable but non-culturable state (VNC).[30-33] Microorganisms in a VNC state are metabolically active but undetectable using laboratory culture methods. The role that the VNC form has in the development of clinical infections is still

undetermined. Baffone et al. (2006) were able to show that clinically relevant *Campylobacter* strains which were artificially induced to transform into a VNC state maintained the ability to return to a culturable state once ingested by mice.[34] However, other studies have shown no return to a virulent or infectious state.[35, 36] Verhoeff-Bakkenes et al. (2007) recently showed that *Campylobacter* organisms in a VNC state lacked the ability to adhere or invade cells in culture suspensions.[37] Thus, it remains questionable as to the importance of this form of the bacteria in the epidemiology of campylobacteriosis. Further evidence that *Campylobacter* in the VNC state can result in infections may suggest a new mechanism for the role of water in *Campylobacter* transmission. DNA-based assays have identified *Campylobacter* in samples of drinking and recreational waters even though cultures were negative.[38]

An additional survival mechanism of *Campylobacter* is their ability to develop biofilms, aggregates of bacteria enclosed in an extracellular matrix that acts to protect the interior cells.[39-41] Joshua and colleagues (2006) demonstrated that a number of *Campylobacter* strains, when placed in an aqueous solution, were able to form biofilms.[40] The development of these biofilms has been shown to enable bacterial populations to survive environmental stressors that individual bacteria are thought to be susceptible to.

Reservoirs

Campylobacter can replicate in a number of animal species and Campylobacteriosis is considered a zoonotic disease. While *Campylobacter* is not known to replicate in various non-animal environments, it can persist in them using the

survival mechanisms discussed earlier. This has important implications for human infections and therefore both animal and environmental reservoirs will be considered.

Animal Reservoirs

Campylobacter are well-suited to grow in the intestinal tract of many mammals and birds. Animal reservoirs of *Campylobacter* include, but are not limited to, poultry, wild birds, cattle, pigs, and domestic pets.[1, 42-44] Certain species of *Campylobacter* show greater affinity for specific animal species. An important implication for human *Campylobacter* infections is that domestic animals, such as cats and dogs, carry *Campylobacter*. Younger animals tend to shed higher amounts of *Campylobacter* than their elder counterparts and are more likely to develop symptomatic infections.[1, 45-47] *C. upsaliensis* is most often isolated from cats and dogs, while *C. jejuni* and *C. coli* also found less frequently.[48-50] Livestock animals such as cattle, sheep, and pigs are also important hosts for a number of *Campylobacter* species.[51, 52] Infections with *C. jejuni* and *C. coli* in many of these animals are asymptomatic, although younger animals, such as calves, may show symptoms such as diarrhea.[51] Manure from these animals has been found to contain varying amounts of *Campylobacter*.[50, 52] *C. fetus fetus*, *C. fetus veneralis,* and to some extent *C. jejuni,* infections in cattle and sheep can lead to abortive loss.[51]

Poultry are one of the most important reservoirs of *Campylobacter*, particularly of *C. jejuni*. These bacteria reside in the intestinal tract (jejunum, ileum, and cecum)[53] of poultry and are excreted in their feces. As with the other animals discussed above, chicks are more likely than hens to be symptomatic when infected with

Campylobacter.[51] Birds are particularly suited to carry *Campylobacter*, and these bacteria can also be found in a number of migratory birds species, as well as geese, ducks, and pigeons.[54] The importance of migratory bird carriage of *Campylobacter* for human infections remains unclear, although as molecular typing becomes more widespread, additional studies will likely consider their importance. Browman et al. (2004) compared genetic profiles of *Campylobacter* isolates from various migratory birds to those from human cases and found very few matches between the subtypes.[54] Additionally, while *Campylobacter* was linked in England to contamination of milk because of bird-pecked milk bottles, the reduction in this form of milk delivery has probably limited the contamination of milk by wild birds (though contamination of unpasteurized milk is still a source of infections).

Environmental Reservoirs

Given its restrictive growth requirements, *Campylobacter* is not thought to multiply in food or the environment. Persistence of *Campylobacter* in the environment is possible, however, and therefore human infection associated with exposure to certain environmental conditions is feasible. Contamination of the natural environment with *Campylobacter* is likely due to the shedding of the bacteria from infected animals. Lakes, ponds, streams, and estuaries have all been shown to harbor various concentrations of *Campylobacter*.[55-57] The likelihood of these aquatic environments to harbor *Campylobacter* is related to their proximity to livestock and poultry areas,[56] although *Campylobacter* can be deposited by migratory birds as well.

A number of mechanisms facilitate the survival of *Campylobacter* in water have been described, including the formation of VNC forms and biofilms, as discussed previously. Lehtola et al. (2006) showed that, when direct culture was used, *Campylobacter* could be detected in a water sample one day after it was spiked with *Campylobacter* but not thereafter. When fluorescent in-situ hybridization was used, the bacteria were found up to 3 weeks later (1 week later in biofilms).[58] Cools et al. (2005) showed that the survivability of *Campylobacter* in water at 4^{O} Celsius depended on the strain of *Campylobacter* studied.[59] They found that strains of poultry-origin survived longer (1-2 months) than those of water or human origin (~ 1 month). Another mechanism employed by *Campylobacter* to persist in water is internalization by invertebrates. Studies have shown that certain species of protozoa internalize *Campylobacter,* allowing the bacteria to be protected from environmental stressors,[60] and that *Campylobacter* can be engulfed by, and replicate within, amoebae.[61] Interestingly, novel enrichment techniques have been developed which capitalize on this feature of *Campylobacter.*[62]

Although *Campylobacter* are susceptible to desiccation, they have the ability to survive in animal manure. Studies of pathogen survival in manure have found that they can last for up to 63 days in dairy cattle slurry.[50] Once the manure is spread on soil, bacterial populations can persist for up to a month, although the type of soil may impact their ability to survive.[63] *Campylobacter* has also been identified in soil from sandy beaches and was found in samples of both wet and dry sand.[64]

Campylobacter on Food

Given the growth requirements and environmental sensitivity of *Campylobacter*, it is somewhat surprising that cases of illness are so often associated with consumption of contaminated food. The particular growth restrictions of *Campylobacter* with regard to atmospheric conditions and temperature prohibit these bacteria from readily growing outside their animal hosts; *Campylobacter* do not readily grow in food.[65] Cooking and pasteurization create temperatures too high for *Campylobacter* to survive, so these processes tend to eliminate *Campylobacter* in food products. Furthermore, these bacteria are also sensitive to desiccation[66] and ultraviolet light,[67] as well as to osmotic stress and low pH,[65] making survival in some foods less likely.

Poultry

Handling and consumption of (often undercooked) poultry is one of the main risk factors for *Campylobacter* infection.[1, 68] The mechanisms through which poultry are initially infected with *Campylobacter* remain unclear. Studies have shown that the likelihood of vertical transmission, from hens to chicks, of *Campylobacter* is low.[69] Therefore, the most likely route of contamination of poultry flocks is environmental. There is substantial variation both in the flock prevalence of *Campylobacter* (the percentage of flocks that have detected at least one infected bird), the within-flock prevalence (percentage of chickens within a flock that have *Campylobacter*) and the quantity of *Campylobacter* on poultry as it moves through the processing plant. In the U.S., flock prevalence among broiler chickens (chickens raised for meat as opposed for eggs) can range from 0-100%, depending on the flock being surveyed.[70] Despite high

prevalence of colonization in most U.S. flocks, studies have found several flocks that remain *Campylobacter*-free all the way to the poultry slaughtering houses (abbatoirs).[71] During processing, *Campylobacter* can be released from poultry intestines due to fecal spillage, leading to contamination of other parts of the carcass. The occurrence and concentration of *Campylobacter* on carcasses have been shown to vary throughout poultry processing. A 2007 study in the U.S. showed that the occurrence of *Campylobacter* ranged from 92% on "pre-scald" carcasses to 100% on "pre-chill" carcasses to 52% on "post-chill" carcasses.[72] While various processing steps have been shown to decrease the concentration of *Campylobacter* on carcasses, certain strains of *C. jejuni* are more resistant to these steps than others.[71] Furthermore, while carcass processing tends to reduce the overall load of *Campylobacter* on poultry, contamination of the processing equipment can occur; this may cause previously *Campylobacter*-free carcasses to actually become contaminated during processing.[71] The likelihood of cross-contamination from environmental surfaces to previously-clean carcasses seems to decrease with time, however.[73] Cools et al. (2005) showed that 120 minutes after a heavily contaminated flock of poultry was processed, the equipment surfaces were no longer contaminated.[73] Still, given the speed at which birds are processed, this may lead to a great number of newly-contaminated carcasses. Importantly, *Campylobacter* have been detected both on the outside and the inside of the poultry meat.[74, 75,76] Therefore, measures to reduce the burden of *Campylobacter* on poultry must not only consider environmental contamination during processing, but prevention of infection while poultry are still living.

Quantitative surveys of *Campylobacter* on chicken from grocery stores have shown the occurrence of the bacteria on retail chicken meat is high. A 2001 study in the Washington, D.C. metro area showed that 71% of chicken samples (and 90% of stores) tested had detectable levels of *Campylobacter*.[77] *Campylobacter* was also detected on turkey, beef and pork products although the occurrence was much lower. The National Antibiotics Resistance Monitoring System (NARMS), in collaboration with the Center for Veterinary Medicine at the U.S. Food and Drug Administration, has been conducting microbiological surveys of retail meat samples from a number of counties and states over the past few years. The data from 2004 (most recent available) shows that the occurrence of *Campylobacter* bacteria on chicken breasts was 60%, compared to <2% each for turkey, ground beef and pork, the other meat types surveyed.[78]

Milk

Unpasteurized milk has been shown to harbor a number of pathogenic bacteria, including *Campylobacter*. The importance of unpasteurized milk as a risk factor for *Campylobacter* infection was recognized in the late 1970s -- 1980s when a number of outbreaks of gastroenteritis were linked to unpasteurized milk contaminated with *Campylobacter*.[79-82] Cattle are reservoirs for a number of *Campylobacter* species. *Campylobacter* are commensal bacteria in cattle intestinal tracts but can also result in infection of the udder. Cattle excrete *Campylobacter* in their feces.[28] Contamination of milk can occur via contact with these feces,[82] and also through shedding of the organism directly into the milk if a cow has mastitis caused by *Campylobacter*.[83, 84] Some strains of *Campylobacter* have been shown to persist in raw milk for at least two weeks.[85]

Because pasteurization is effective at killing *Campylobacter* in milk,[79] the epidemiologic importance of milk as a vehicle has lessened over time with the decreases in the commercial sale of unpasteurized milk. However, there are individuals that prefer raw milk for various reasons, including taste and a belief that it is more beneficial nutritionally.[86] Additionally, dairy farm workers may also be more likely to drink unpasteurized milk.[87] These individuals continue to be at increased risk of infection, although the development of clinical illness will likely depend on their immunity to *Campylobacter*.

Drinking Water

Contaminated drinking water has been associated with a number of large outbreaks of *Campylobacter* infections in various communities. Depending on the source of the drinking water, contamination can occur through two main mechanisms. Surface water may become contaminated with feces from animals and birds that harbor *Campylobacter* through run-off and the use of water sources by waterfowl such as geese and ducks. Studies have detected *Campylobacter* in surface water sources such as streams, ponds and lakes.[48, 55, 57, 88] The mechanism by which treated drinking water becomes contaminated with *Campylobacter* is somewhat unclear, however disruptions in water treatment and leakage of sewage into the water supply have been documented to occur prior to *Campylobacter* outbreaks.[89-91] Additionally, the use of surface water for drinking water has been implicated as a risk factor for *Campylobacter* infections. *Campylobacter* in water is highly susceptible to photooxidative damage as a result of UV exposure via sunlight.[67, 92] As noted previously, the development of the VNC form

and biofilms, as well as localization within amoeba and other protozoa in water sources, greatly increases the viability of *Campylobacter*, though the impact of these characteristics on the risk of human illness remains unclear.[58] *Campylobacter* doenot readily multiply in water, although Tatchou-Nyamsi-Konig et al. (2007) showed that they could grow in natural mineral water when it contained organic material.[93]

Cross-contamination of Food

Cutting boards have been shown to harbor *Campylobacter* for more than two hours[73] and may serve to transfer *Campylobacter* to other food products that come into contact with the contaminated surface. DeBoer et al. (1990) showed that cutting boards previously used for raw chicken and then used to cut other products, such as vegetables and *Campylobacter*-free chicken, could transfer the bacteria to these other types of food.[94] Luber et al. (2006) found that knives, plates, and food preparer hands that touch naturally contaminated chicken pieces have also become contaminated after contact as well, with transfers of bacteria from raw chicken pieces to other items (plates, other foods) ranging from 0.3% to 28%, on average.[95]

Clinical Features

Transmission of thermophilic *Campylobacter* species (*C. jejuni* and *C. coli*), those which are most often linked to human illness, to humans is predominantly through the foodborne route.[96] The effects of infection with *Campylobacter* can range from simple asymptomatic carriage to paralysis. Infections in adults often appear similar to those of other foodborne infections such as salmonellosis, thus making empiric diagnosis of a *Campylobacter* infection difficult. Studies of the infectious dose of

Campylobacter are limited by ethical and immunological issues as well as by the lack of an appropriate animal model. The most widely cited figure is that from Black et al. (1988) who conducted a human-challenge trial and found that doses as low as 500 *Campylobacter* organisms were able to cause illness.[97] Based on this study, the median infectious dose (the number of organisms required to cause illness or infection in 50% of exposed hosts), was estimated to be approximately 800 bacteria.[98] A more recent study using mathematical models and outbreak data suggest that even fewer organisms could cause illness, although it is important to consider individual host immunity to *Campylobacter*.[99] Once enough bacteria have been consumed to cause illness, a fever prodrome develops approximately 3 days (range 18 hours to 8 days) after ingestion.[100, 101] The types of symptoms that develop depends on a number of factors, including the strain of *Campylobacter*, immune response to infection, and other patient characteristics. In most persons, the infection is acute and self-limited. Fever, cramps, malaise, and diarrhea, which in some cases (reported in 15%[100] to 45%[1]) can be bloody, are often present.[11, 101] Vomiting occurs in approximately 15% of cases.[100] In the majority of cases, the period of illness is about a week in length.[100] Relapses do occur in approximately 15-25% of cases.[11, 100]

Extraintestinal infections can arise. Although the abdominal pain that patients experience during *Campylobacter* infections has often been mistaken for appendicitis ("pseudo-appendicitis"), *Campylobacter* has also been isolated from removed appendixes in approximately 2% of cases.[100] Other infected individuals have developed bacteremia, hepatitis, pancreatitis, rashes, urinary tract infections and myocarditis as a result of infection, although these conditions occur rarely.[100] The majority of patients (~

90%) are not hospitalized,[102] and mortality associated with infection is low and tends to be limited to those who are elderly or have comorbidities. *Campylobacter* infection in children and neonates is somewhat different from those in adults. Diarrhea tends to be more bloody, and infections can be mistaken for intussusception.[100]

Long-term sequelae of *Campylobacter* infection, notably reactive arthritis, irritable bowel syndrome (IBS), as well as Guillain-Barré Syndrome (GBS) and other related neuropathies, also exist. Reactive arthritis (ReA), a swelling of the joints of the ankles, knees, and elbows,[100] has been estimated to occur in 1-5% of individuals stricken with *Campylobacter*,[103] although this proportion increases to over 10% in populations with a greater predominance of the Human Leukocyte Antigen (HLA) type B27.[100] ReA usually develops a month after the initial *Campylobacter* infection.[103] The incidence of *Campylobacter*-associated reactive arthritis has been estimated to be 4.3 per 100,000.[103] *C. jejuni*, *C. coli*, *C. lari* have all been associated with ReA.[103]

GBS is an acute malfunctioning of peripheral nerves due to an autoimmune reaction to a previous acute bacterial infection that causes demyelization of peripheral nerves. The disease is self-limiting, but an afflicted individual is ill for at least 3-4 weeks.[104] Miller-Fisher syndrome (MFS) is related to GBS and involves malfunctions of ocular nerves, uncoordinated movements, and deterioration of typical reflexive movements. Other neurological conditions, such as Bickerstaff brainstem encephalitis (BBE), and acute oropharyngeal palsy are related to GBS and MFS and also thought to be linked to previous infection with *Campylobacter jejuni*.[105] GBS is the most common form of acute flaccid paralysis in the world, with *Campylobacter* considered the leading bacterial cause.[104, 106] Campylobacteriosis precedes GBS by approximately 1-3 weeks.[101]

In the U.S., there are approximately 1-2 cases of GBS per 100,000 persons annually.[107] GBS is thought to occur as a result of previous *C. jejuni* infection, and is less strongly linked to earlier infection with other species of *Campylobacter*.[108] Antibodies to the lipo-oligosaccharide protein in the *C. jejuni* cell wall cross-react with human nerve ganglioside proteins because of the similarity in their molecular structures.[109, 110] Depending on the population studied and antibody detection method used, an estimated 25--80% of cases of GBS are attributable to previous infection with *C. jejuni.*[111, 112]

Antibiotic Resistance

Though most infections with *Campylobacter* are self-limited, antibiotic treatment may be helpful in some circumstances. Antibiotic treatment in the U.S. includes fluoroquinolones and macrolides (used mainly in children).[68, 113, 114] Antibiotic resistance among *Campylobacter* isolates has been observed in a number of countries, including the U.S.[68, 115] Given that campylobacteriosis is a zoonotic disease, the use of antimicrobial agents in food animals (particularly poultry) has important consequences for human infections.[113, 116] Not only do antimicrobial-resistant infections pose a risk because of treatment failures, but duration and severity of illness have been shown to increase in those with resistant infections.[117, 118]

In the U.S., the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1997 to monitor antimicrobial resistance, including fluoroquinolone resistance, among *Campylobacter*. Fluoroquinolones were approved for use in human medicine in the mid-1980s. A study of human *Campylobacter* infections in Minnesota in 1992 showed little fluoroquinolone-resistance (1.2% of isolates tested) among humans

C. jejuni isolates.[114, 119] Approval for fluoroquinolone use in animal medicine was granted in the mid-1990s, after which a rapid increase in fluoroquinolone-resistant human *Campylobacter* infections was observed.[114] As a result of this increase in resistant infections in humans and studies that show the harmful health effects of these infections, the Food and Drug Administration proposed a rule banning the use of fluoroquinolones in poultry in 2000. This rule was finalized and approved in 2005.[78] NARMS data are not yet available to determine whether there has been a corresponding decrease in fluoroquinolone-resistant infections in humans after the ban was instituted.

Immunity

Whether or not an individual develops symptomatic illness as a result of infection with *Campylobacter* is partially mediated by his/her immune response, although this process has not yet been fully elucidated. It is currently understood that both the humoral and the cellular compoentns of the immune system are important, although the humoral immune response has been studied more fully than the cellular. Initially, within approximately ten days of infection, serum immunoglobulin (Ig) A and IgM antibodies rise rapidly, and then subsequently decrease over the following weeks.[120, 121] IgA antibodies remain detectable for approximately one to three weeks.[121, 122] The IgM response may be influenced by the age of the individual, as younger individuals have been to show to have a significantly greater IgM response than older individuals, possibly due to an increased immunologic memory response in older individuals.[120] IgG antibodies also rise early in infection (generally within 20 days of infection[123]) and remain at high levels for weeks to months, and even years.[120, 121, 123]

This wide range in IgG persistence is likely a combination of the true variation in IgG responses between individuals and an artifact of varying study lengths.[120,122-124] In some persons, IgG antibodies have been found to increase, rather than decrease, over time, perhaps due to subsequent exposure to *Campylobacter*.[124] In others, antibody titers did not increase appreciably after infection, though this could be due to the use of inappropriate methods to detect the antibody response for a particular individual.[120, 123]

The specificity of immunity to a given strain or serotype of *Campylobacter* may also be important. Miller et al. (2005) examined infection rates of the specific serotypes of *Campylobacter* strains by age group, and compared the ratio of "uncommon" to "common" serotypes.[125] The common serotypes were the three serotypes which accounted for over 50% of the cases in the study. Older age groups, they found, were more likely to be infected with "uncommon" serotypes as opposed to younger age groups. The authors suggested that immunity may be the reason for this change in serotype distribution, as well as for the lower incidence in older ages.

Epidemiology of Campylobacter Infections

In many industrialized countries, including the U.S., the epidemiology of *Campylobacter* infections shows a bimodal age distribution, with increased in incidence in children under one year of age and in individuals between 15-44 years of age.[4, 126-128] The incidence of *Campylobacter* infections tend to be slightly higher in males, although the reasons for this gender distribution are unclear.[47, 129] Additionally, a seasonal periodicity in infections is notable, with an increased incidence generally observed in summer.[2, 3, 7] Geographical patterns, such as a higher incidence in rural areas have also

been observed.[4] In less industrialized countries, *Campylobacter* infections are more endemic, leading to a different pattern of incidence. Individuals in these countries are generally infected in childhood and although re-infected later in life, these later infections tend to be asymptomatic.[130]

United States

Although *Campylobacter* receives perhaps less attention than other foodborne bacteria such as *Salmonella* and *E. coli* O157, in the U.S. the annual number of cases of *Campylobacter* has often been greater than those of *Salmonella or E. coli* O157.[131] The earliest reported incidence of *Campylobacter* infections in the U.S., obtained in 1983, was approximately 5 cases per 100,000 persons.[47] As campylobacteriosis is not a nationally notifiable disease in the U.S., and reporting requirements vary widely from state to state, information on the incidence of this disease is obtained from the Foodborne Diseases Active Surveillance Network (FoodNet). Since the beginning of FoodNet, regional variation in *Campylobacter* incidence has been consistently observed. The average annual incidence of culture-confirmed *Campylobacter* infections from 1996-2006 ranged from 7/100,000 in both Maryland and Tennessee to 34/100,000 in California.[13] The incidence of culture-confirmed *Campylobacter* infections in the FoodNet sites in 2006 was 12.3 per 100,000,[131] and has declined by approximately 30% since 1996-1998.[131] Furthermore, for every age group, the incidence of *Campylobacter* infections in males is higher than in females, and seasonal variation in incidence, with higher incidence during the summer, is often observed.[132]

Europe

The epidemiology of campylobacteriosis in European countries is similar to that of the U.S. with regard to the peak incidence rates in the very young and moderately aged, as well as the seasonal periodicity in incidence.[10, 133] The majority of the European Union countries who participated in a survey during 2001 reported having a surveillance system established for human *Campylobacter* infections.[134] A number of studies have been conducted in Europe on "attribution," linking specific reservoirs of *Campylobacter* infections to human infections by using genetic sequencing techniques.[27, 135, 136] Keller and colleagues (2007) compared genetic profiles creating using AFLP of *Campylobacter* isolates from a number of environmental, animal, and human sources in Switzerland.[27] They found that the human isolates clustered together and 43% matched other human isolates, 27% matched poultry isolates, 21% matched pet isolates (dog and cat), and 9% matched bovine isolates. A number of human isolates did not match any other patterns in the database, however, and the authors attributed these to travel-associated disease. In the Netherlands, *Campylobacter* risk assessment and attribution studies has occurred under the auspices of the on-going CARMA (*Campylobacter* Risk Management and Assessment) project. Numerous projects that use mathematical models to better examine the risks from various exposures and determine appropriate areas for intervention are being conducted (see CARMA website: http://www.rivm.nl/carma/).[137]

Developing Countries

The epidemiology of *Campylobacter* infections in developing countries is somewhat different from that in industrialized countries. While the age distribution in

industrialized countries is bimodal, in developing countries the second peak in adults is often not seen. Many studies in developing countries have shown that as age increases, there is a decrease in the illness-to-infection ratio associated with *Campylobacter*, suggesting that even though an individual becomes infected with the bacteria, s/he is less likely to become symptomatic.[126-128] Blaser et al. (1985) found that Bangladeshi children in every age group from less than one year of age to 15 years of age had higher levels of IgA, IgM, and IgG than a sample of children from the U.S. Additionally, these Bangladeshi children were less likely to have symptomatic illness.[138] Other evidence seems to indicate that the severity of illness may be inversely related to the level of humoral immunity.[139] Interestingly, *Campylobacter* serotype and subtype distributions have also been shown to differ between developing and industrialized countries.[139]

Risk and Protective Factors for Campylobacter Infection

Case-control studies have been conducted in various countries and populations to better understand the etiology of *Campylobacter* infections in humans. A number of risk factors have been consistently identified in these studies, as well as some protective factors.

Risk Factors

Risk factors for campylobacteriosis can be thought of as foodborne or environmental. Foodborne risk factors for campylobacteriosis include, but are not limited to, consumption of raw or undercooked chicken, unpasteurized milk, and untreated water.[1, 3-6] Environmental risk factors for campylobacteriosis include exposure

to dogs, cats or poultry, working or living on a farm or in a rural area, and foreign travel.[1, 4, 6]

Given that the reservoirs of *Campylobacter* include poultry and cattle, it is not surprising that consumption of the meat or products of these animals are risk factors for infection with *Campylobacter*. Consumption of unpasteurized milk has been identified as a risk factor for infection with *Campylobacter* in a number of studies [1, 43, 140-142] The proportion of cases in the population attributable to unpasteurized milk consumption is generally low, due to the low frequency of exposure, and was estimated to be 2% in the in the FoodNet population.[1] The effect of milk consumption might be modified by geographic residence and age, however. A study in Scotland in the mid-1980s showed a different age distribution of cases of *Campylobacter* among urban and rural inhabitants. While both showed a peak in incidence in children, a second peak in older ages was only seen in the urban inhabitants. The authors suggested that acquired immunity due to greater consumption of raw milk in rural areas was thought to be the cause of this difference.[143] Indeed, individuals who worked on dairy farms were shown to have an increased IgG and complement-fixing antibodies compared to those who did not, and the farm workers were also more likely to have asymptomatic infections.[140, 144] Furthermore, although the number of sporadic cases in the population attributed to raw milk consumption is somewhat low, large outbreaks have been associated with contamination of unpasteurized milk.[145]

Consumption of contaminated poultry products is also a large risk factor and accounts for anywhere from 5%[44]-25%[1] of cases, depending on the method of preparation and population studied. Eating undercooked chicken has been identified as a

risk factor for infection with *Campylobacter* in a number of studies.[1, 43, 141, 142, 146-150] A few studies have not found consumption per se to be a risk factor, but rather the handling of the raw poultry meat (that may lead to cross-contamination of other food products).[151, 152] Differences in risks associated with various preparations of chicken have also been found, notably an increased risk with chicken cooked over a grill or barbecued.[46, 153] In some studies, consumption of chicken at home is more likely to lead to infection and in others, consumption of chicken at a restaurant poses a greater risk.[154] A study published in 2009 from England suggests that the risks from chicken consumption are greater among individuals who do not "habitually" eat chicken as compared to those who are habitual chicken-eaters. Additionally, eating chicken outside of the home was found to be a risk factor for *Campylobacter* infection, but the risk was greater for non-habitual chicken eaters as compared to habitual chicken eaters.[155] Furthermore, some difference in risk appears to depend on whether the chicken was previously frozen or not, with fresh, unfrozen chicken associated with a higher risk of illness.[43, 147, 156] Freezing practices can contribute to differences in pathogen load on chicken because freezing results in a 2-log reduction in *Campylobacter*.[157] In one study in Denmark, eating fresh chicken was consistently found to increase the risk of *Campylobacter* infection whereas eating frozen chicken was only borderline significant.[156] Consumption of poultry was also identified as a risk factor for fluoroquinilone-resistant *Campylobacter* infections in the U.S.[158]

Drinking untreated water or well water has been associated with *Campylobacter* infections as well.[1, 46, 141, 142, 150, 159, 160] Environmental samples have identified *C. jejuni* in surface waters,[161] although *Campylobacter* tends to be confined to areas close to

farms with animals that had *Campylobacter*. Nevertheless, given the high prevalence of infection in many farm animals, the occurrence of *Campylobacter* in the environment could be substantial. The risk of *Campylobacter* infection due to the consumption of drinking water may be modified by other factors, however. One study found a significant interaction between private versus public drinking water and county, suggesting that perhaps different water treatment practices or distance from the water treatment plant could play a role.[4] Additionally, although a number of waterborne outbreaks of *Campylobacter* infection have occurred, drinking water is less often associated with sporadic cases of *Campylobacter* infection.

Contact with animals, including domestic cats and dogs and farm animals, have been identified as risk factors for *Campylobacter* infection.[1, 43, 46, 141, 142, 146, 149, 150, 159, 160, 162, 163] Exposure to cats and dogs (especially ill ones) has been frequently associated with *Campylobacter* infections, although the population attributable fraction for this exposure has been calculated to be somewhat low, with an estimate of about 5% in the FoodNet population.[1] Farm animal contact is also an important exposure. In one particular study, contact with poultry was seen to increase risk by over eleven-fold,[43] and a study in rural Michigan found that the risk among poultry workers was six-times that of individuals who worked in other occupations.[163] In addition, poultry, cows and sheep are also known to harbor *Campylobacter*.[48] Studies have shown that similar Penner serotypes of *Campylobacter* can be found in humans and bovine sources.[48] Foreign travel has been linked to infection with *Campylobacter*,[142, 159] and most case-control studies exclude travel-associated cases from further analysis.

Several other exposures were found to be significantly associated with *Campylobacter* infection or increased incidence in only a few studies. These included: consumption of salad vegetables;[149] consumption of bottled water;[149] ingestion of H2, H2 antagonists, and omeprazole;[164, 165] swimming in a natural water source;[166] increased amount of rainfall;[167] increased density of ruminants;[168] living in an areas with low population density;[4] and distance from the water treatment plant.[168] Although some risk factors are consistent across age groups, a recent FoodNet case-control study limited to infants (≤ 2 years of age) identified some novel risk factors for *Campylobacter* infection in this age group. These included drinking concentrated formula, eating fruits and vegetables prepared at home, and riding in a shopping cart next to meat or poultry.[159]

Protective Factors for *Campylobacter* Infection

Females and breastfed infants have repeatedly been shown to have a decreased risk of *Campylobacter* infection. Studies of surveillance data from a number of countries have shown the incidence of *Campylobacter* infections to be greater among males than females.[169, 170] Case-control studies have also shown females to be at lower risk of *Campylobacter* infection.[1] The reasons for this finding are still somewhat unclear. One study found young adult males are more likely to practice improper cooking practices, and thus they might be at a higher risk for infection with *Campylobacter* than females.[171] This difference in behaviors still would not explain the greater incidence in males at every age, however.[132] A recent study used a mouse model of *Campylobacter* infection to compare differences in illness between sexes, and found male mice to be more likely to be infected and shed greater amounts of *Campylobacter* than female mice.[172] Another

possibility is that there are immunological differences between sexes that modify the development of symptomatic versus asymptomatic infection after exposure to *Campylobacter*.[173] Lending credence to the importance of immunity in protection against infection against *Campylobacter* is the finding from a number of studies that breast-feeding is a protective factor for *Campylobacter* infection in infants.[159, 174, 175]

Some exposures found to be risk factors in various studies were found to have protective effects in others. For example, in one British study, farm animal contact was found to be a protective factor for *Campylobacter* infections.[160] The authors argued that this finding might suggest an increased immunity among those with greater frequency of farm animal contact. In the same study, handling of raw chicken was also found to be a protective factor for *Campylobacter* infections.[160] Again, the authors suggested that this finding might be due to an increased immunity among those who often handle raw chicken.

Outbreaks

Although many of the causes of *Campylobacter* outbreaks have also been identified as risk factors for sporadic infection in case-control studies (that generally exclude outbreak-associated cases), the population attributable fractions tend to be relatively low (<10%). When they have been identified, community outbreaks of campylobacteriosis have been linked to contaminated or untreated water,[90, 176-181] contaminated unpasteurized milk,[182, 183] and undercooked poultry.[184-186] Large waterborne outbreaks of *Campylobacter* associated with mass contamination of municipal water supplies have occurred,[91] and many of these outbreaks included

infections with other pathogens such as *E. coli* and norovirus.[89, 90] Environmental investigations suggested that in these instances there was contamination of municipal drinking water with sewage,[89] or with bird feces.[91]

Surveillance in the United States

Surveillance for *Campylobacter* infections began in the U.S. in 1982 with the implementation of a surveillance system through the Centers for Disease Control and Prevention (CDC). Electronic reporting of *Campylobacter* cases to CDC began in 1988 with the implementation of the Public Health Laboratory Information System, or PHLIS.[187] However, these reporting systems are passive, and reporting requirements vary widely from state to state and the disease is not nationally notifiable, so many states do not report cases. In 1973, CDC also developed a surveillance system to track foodborne disease outbreaks (two or more cases with similar symptoms due to consumption of a common food vehicle) whatever the cause.[188] While this system is nation-wide, the number of reports varies by state and a number of factors, including the etiologic agent, number of people affected, severity of illness, and how geographically dispersed the cases are. All of these factors affect whether an outbreak is identified or reported.

Since 1996, with the advent of the Foodborne Diseases Surveillance System (FoodNet), surveillance for culture-confirmed cases of *Campylobacter* improved through the use of active surveillance in select states and counties in the U.S. The surveillance area for FoodNet has increased steadily from its inception in 1996, which

included all or select counties in 5 states* (14,273,094 people, 5 % of the U.S. population), to 2006 when it included all or select counties in 10 states† (45.5 million people, 15 % of the U.S. population) (Figure 2.1). Through the use of active, laboratory-based, population surveillance for culture-confirmed infections, FoodNet is able to monitor the trends and burden of illness caused by *Campylobacter*.

The ascertainment of a case of laboratory-confirmed *Campylobacter* in FoodNet surveillance data depends on a number of factors. Initially an individual must become infected with *Campylobacter*, a process that depends on both the exposure pathway and dose of *Campylobacter* to which an individual exposed, in addition to whether the individual has some level of immunity to infection. Once a person has developed symptomatic illness, there are factors that modify whether this illness will eventually be captured as a culture-confirmed case in FoodNet surveillance data. These include whether the individual is sufficiently ill and has the means to seek medical care, whether the physician s/he sees requests a stool culture. whether the patient complies with the request and returns with a stool sample, and how well clinical laboratory tests the stool specimen for *Campylobacter*.

Clinical Laboratory Diagnostic Methods

The isolation and detection of *Campylobacter* in a clinical specimen is a complex process. A number of laboratory procedures need to be used to limit competing

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* select counties in California, Connecticut, and Georgia and the states of Minnesota and Oregon

† select counties in California, Colorado, Maryland, and New York and the states of Connecticut, Georgia, Minnesota, New Mexico, Oregon and Tennessee

microflora and to create an environment conducive to the growth of *Campylobacter*. Additionally, while most methods are better at isolating the thermophilic *Campylobacter* species that cause most diagnosed human disease (*C. jejuni* and *C. coli*), different methods may be required to detect the other more unusual species (i.e., *C. upsaliensis*). The general process involves transport of the specimen from the physician or hospital to the clinical laboratory, followed by identification using either culture- or other detection methods.

Transport Media

Once a stool specimen or rectal swab has been obtained from a patient, it often requires transport to a clinical laboratory. If the transport time of the specimen from the patient to the clinical laboratory is greater than 2 hours, the use of transport media is recommended.[189] A number of types of transport media are available, including Cary-Blair, Amies, Stuarts, Culturette, buffered glycol, and Campy-thio. They are all not equally effective at preserving *Campylobacter* organisms during transportation.[190, 191] The length of time in which *C. jejuni* survives in transport media was evaluated and shown to be less than 7 days for Culturette or buffered glycerol, but greater than 7 days for Cary-Blair.[190] Even though transport media is meant to preserve bacteria, storage in transport media for too long can result in the bacteria entering a viable but non-culturable state (VNC), or simply dying. If this occurs, there is decreased sensitivity of the culture-based techniques used subsequently to detect *Campylobacter*.[69] Storage of *Campylobacter* in the transport media is preferable if the specimen is not processed immediately. A few limited studies showed that *Campylobacter* will not persist for a

long time in storage media held at room temperature, but holding the sample in transport media at 4^{o} Celsius will enable the bacteria to survive.[189, 192]

Non-culture Methods

Once received by the clinical diagnostic laboratory, stool specimens can be tested for the presence of *Campylobacter* through culture or non-culture methods. Non-culture methods detect *Campylobacter* DNA or surface proteins on *Campylobacter* and include techniques such as polymerase chain reaction (PCR) and enzyme immunoassay (EIAs). They can be more sensitive than culture-methods for detecting *Campylobacter* in specimens with fewer numbers of organisms or for identifying bacteria that has already entered the VNC state or died.[69, 193] However, some species of *Campylobacter* (such as *C. upsaliensis)* may not be detected using non-culture methods.[194] Still, a number of studies have reported non-culture methods to be at least as good as culture-based techniques.[194-196] However, these methods do not permit subtyping or antimicrobial resistance determination.

Culture Methods

Culture-based methods rely on detecting living bacteria in a stool or other clinical sample. Thus, proper transportation of the stool samples must occur in order for the bacteria to still be viable at the time the culture is conducted. Stool samples may undergo an enrichment procedure or be directly plated onto a culture plate. Enrichment is recommended if: 1) the *Campylobacter* in the specimen are thought to have been environmentally stressed, 2) the samples were delayed in transport to the clinical laboratory, or 3) when the number of organisms in the sample is likely to be low (such

as food or environmental samples, and samples from convalescent patients).[197, 198] Studies have shown the use of enrichment prior to culture may increase detection rates by approximately 25% -50%.[199-201]

Whether enrichment broth is used or not, the next step in bacterial culture is to plate the specimen on culture media. The media can be either "selective" or "non-selective" for *Campylobacter* organisms. *Campylobacter* selective media contain combinations of chemicals or antibiotics that prohibit the growth of other bacteria and fungi that could obfuscate the presence of *Campylobacter*.[84] If non-selective media are used, an additional method, such as filtration, is often used to remove the competing microorganisms from the stool specimen.

Types of selective media include charcoal-cefoperazone-deoxycholate agar (CCDA), charcoal-based selective medium (CSM), Skirrow medium, Campy CVA medium (containing cefoperazone, vancomycin, and amphotericin B), Campy BAP (blood agar plates that contain Blaser agar or Campy agar with 5 antimicrobial agents), or Blood-free Campy selective agar (CAT). In laboratory studies, charcoal-containing media (i.e., CCDA, CSM, CAT) were found to be of superior quality to blood-containing media (i.e., Skirrow media) or other types, mainly because they provided better inhibition of competing microflora.[189, 202-206] Among charcoal-based media, CAT was found to isolate more unusual species of *Campylobacter*, such as *C. upsaliensis*, than media such as CCDA.[207, 208] Certain antimicrobial agents included in formations of selective media have been found to be deleterious to specific *Campylobacter* species, a finding which some have suggested explains the observed predominance of *C. jejuni* and *C. coli* in humans specimens.[209]

If selective media are not used, a filtration technique is employed to remove contaminants before plating the specimen on non-selective agar. *Campylobacter* bacteria are smaller in cross section than many other bacteria, allow them to be able to pass through filters of various sizes (0.45 μm, 0.65 μm pore sizes) while larger organisms are retained.[197] Results of studies comparing the isolation efficacy of membrane filtration to selective media are mixed. While some found 10-60% increases in isolation of *Campylobacter* with the use of filtration compared to selective media alone,[210, 211] others showed no difference,[212] or a worse performance. In general, however, filtration is recommended in order to detect the non-*C. jejuni* and non-*C. coli* species of *Campylobacter* that are not favored when selective media are used.

After a procedure to remove competing microorganisms has been completed, the culture plate is incubated in an atmosphere and temperature appropriate to encourage the growth of *Campylobacter jejuni/coli*. It is necessary to create a microaerobic environment of 5% oxygen, 10% carbon dioxide, and 85% nitrogen.[213, 214] Various methods are available to create this atmospheric environment, including candle jars or commercial kits, that have varying degrees of effectiveness. As the atmosphere created by a candle jar (a jar in which a lit candle is used to modify the atmosphere), is 17% oxygen and 3% carbon dioxide,[190] these are sub-optimal for creating the appropriate microaerobic environment to grow *Campylobacter*. Some studies found that adding hydrogen gas to the usual microaerobic environment increases the likelihood of finding species such as *C. upsaliensis*, *C. lari*, and *C. concisus*.[191]

Most *Campylobacter* organisms grow at temperatures ranging from 37 to 42 ^{o} Celsius. Many laboratory studies have found that incubating culture plates at 42 ºC is

effective to isolate thermophilic *Campylobacter* species.[210, 215-217] Richardson et al. (1982), however, found that bacterial colonies were larger at 42 ºC, but not more numerous.[218] Scates et al. (2003) also showed that there was no difference between the number of *C. jejuni* isolates obtained when plates were incubated at 37 and 42 °C, but the strains of *C. jejuni* that grew differed by temperature. The authors suggest that plates should be incubated at both 37 and 42 degrees to isolate the maximum number of genotypes of *C. jejuni*.[219] There does appear to be a modification of the effect of temperature by the manner in which atmospheric conditions are created. Wang et al. (1982) showed that a temperature of 42 ºC was satisfactory to isolate *Campylobacter* from candle jars whereas 37 ºC was not effective;[190] this finding was replicated in another study as well.[220]

The length of time during which plates are held in appropriate temperature and atmospheric conditions is also important. Culture plates are generally left in these conditions for up to 48 hours and occasionally exceeding 72 hours. Allowing plates to incubate for longer periods of time may increase the likelihood of detecting *Campylobacter* bacteria when they are present. Endtz and colleagues showed that increasing the incubation time from 48 to 72 hours led to increased isolation of *Campylobacter* for all of the media examined.[205]

American Society for Microbiology Guidelines

While conflicting results have been found about the relative importance of some of these methods, guidelines for clinical laboratory practices for *Campylobacter* detection have been established. The American Society for Microbiology (ASM)

recommends the following procedures for the isolation and identification of typical thermotolerant *Campylobacter* species (e.g., *C. jejuni* and *C. coli*) from a stool specimen (depicted in Figure 2.2): use of transport media (such as Cary-Blair) if the specimen transport time $\geq$ 2 hours; storage of samples at 4 ^{o}C (in transport media) once the specimen has arrived at the clinical lab if it will not be processed immediately; incubation of culture plates at 5% O_2, 10% CO_2, 85% N_2 using a method other than a candle jar to create these conditions; growth on selective media such as Campy-CVA or CCDA, and incubation at 42^{o} Celsius for at least 48 hours.[189]

Prevention of Campylobacter Infections

A number of countries with particularly high incidence of *Campylobacter* infections have instituted strict control measures on the most likely and significant vehicles of *Campylobacter* infection -- poultry. In 2001, for example, the Danish poultry broiler industry began participation in a voluntary control program that tests every chicken flock for the presence of *Campylobacter*. Beginning in 2003, flocks that were found to be *Campylobacter*-free were slaughtered and could be sold as fresh chicken meat. Flocks that had detectable levels of *Campylobacter* wee slaughtered either in different plants from the *Campylobacter*-free flocks or at different times in the same slaughtering plants, and the meat from these flocks was sold as frozen meat to the Danish public.[221] Consumers often prefer to purchase “fresh” chicken as opposed to frozen, and often the price for fresh chicken is higher than for frozen. The association of these control measures with decreased incidence of *Campylobacter* in Denmark has been dramatic. Since the program began in 2001, a 20% decrease in *Campylobacter* incidence

was observed by 2003.[156] Whether or not this program will effectively control the *Campylobacter* problem in this country is debatable, however, given the recent increases in the importation of poultry meat.[222]

Other countries have implemented control measures at various stages from farm to dinner table for *Salmonella* and *E. coli* O157. In the U.S., efforts by the poultry industry and regulators to control bacterial contamination of meat products may have contributed to the overall decrease of 30% in the incidence of *Campylobacter* infections observed in the FoodNet sites from 1996-1998 to 2006.[131] In 1996, the U.S. Department of Agriculture's Food Safety Inspection Service (FSIS) began implementation of the *Pathogen Reduction: Hazard Analysis and Critical Control Point System* (HACCP). This program required that meat and poultry plants increase efforts to sanitize plants, conduct microbiological testing for *Salmonella* and *E. coli* O157, implement quality controls, and create standards to help meet the performance standards for the amount of *Salmonella* allowed on meat products.[223, 224] Although these measures are aimed at *Salmonella* and *E. coli* O157, they may also result in a decrease of *Campylobacter* contamination.

As case-control studies have identified risk factors for infection with *Campylobacter*, these can then be used to target prevention efforts. In order to prevent infection with *Campylobacter*, the CDC recommends ensuring that poultry is cooked properly and that proper kitchen hygiene practices are implemented in order to reduce the cross-contamination of other foods with raw chicken meat or juices. Additional recommendations include avoiding consumption of unpasteurized milk or untreated

surface water and being sure to wash hands after contact with a young cat or dog, particularly one with diarrhea.[225]

Public Health Implications

The public health impact of *Campylobacter* infections is great. While most infections are self-limited, the symptoms of campylobacteriosiscan include diarrhea, fever, and muscle pain,[11] and long term sequelae can develop. The economic cost of campylobacteriosis related to days of work lost, medical visits, and death has been estimated at an annual $0.6 - $1.0 billion in the U.S.[12] The annual cost of *Campylobacter*-associated Guillain–Barré Syndrome has been estimated at $0.2 - $1.8 billion.[106] In the U.S., data from FoodNet was used to establish national goals for a 50% reduction in the 1996 incidence of *Campylobacter* infections by 2010, a "Healthy People" 2010 objective of 12.3 cases per 100,000 persons.[226] In 2006, FoodNet reported an average annual incidence of culture-confirmed *Campylobacter* infections of 12.7 per 100,000 persons, a 30% decline from the 1996—1998 baseline comparison, but still slightly above the Healthy People 2010 objective.[131] Furthermore, there is a need to better explain and understand the geographic variation observed in incidence in the FoodNet sites as well as the seasonal periodicity in incidence. The international geographic variation observed may indicate surveillance artifacts or true differences in risk. If it is the former, the interpretation of surveillance data can be adjusted to account for these artifacts. If it is the latter, then these risk differences indicate that risk is not fixed, but may be modified by policy or procedural changes in higher risk areas.

Possible Explanations for Geographic Variation in Campylobacter Incidence

Despite a decline in the incidence of *Campylobacter* infections in the U.S.,[131] there continue to be unexplained and consistent regional differences in the incidence of *Campylobacter* infection in the FoodNet sites. For example, in 2006, the incidence of culture-confirmed *Campylobacter* infection in select counties in California was 27 per 100,000, a rate close to five times that of the incidence in Maryland (6.3 per 100,000).[131] A sustained pattern of increased incidence in California and much lower incidence rates in sites such as Maryland, Tennessee and Georgia has been observed since the beginning of FoodNet surveillance for *Campylobacter*.[227]

Reasons for the observed site-to-site differences are unclear. One possible explanation is "surveillance artifacts", that is site-to-site variation in the likelihood with which a case will be diagnosed and reported (medical care-seeking behavior, stool sample submission frequency, or clinical laboratory practices). The active surveillance process of FoodNet with periodic laboratory audits means that all microbiologically diagnosed cases are captured, whether or not they reporting is required. To assess the degree to which cases go unreported because of surveillance artifacts, FoodNet conducts cross-sectional telephone surveys of residents of the FoodNet catchment area to assess health-seeking behaviors (FoodNet Population Survey) and surveys clinical laboratories serving the FoodNet catchment area to document laboratory practices.

Alternately, these differences in incidence could be due to true geographical differences in risk factors for *Campylobacter* infection or in the likelihood of exposure to these factors. FoodNet conducts case-control studies of infections under surveillance,

and a case-control study of *Campylobacter* infection was conducted in FoodNet sites during 1998-1999. A useful conceptual framework for assessing the mechanism by which a case of campylobacteriosis in the community may be detected by public health surveillance is the burden of illness pyramid (Figure 2.3). Disparities at any level of the pyramid, whether they are from differential surveillance for infections (surveillance artifacts) or from true differences in risk, could be responsible for an observed variation in incidence of laboratory-confirmed infections.

Surveillance Artifacts

Surveillance artifacts are factors that impact whether a case of *Campylobacter* infection is captured by public health surveillance. Examples of surveillance artifacts include increased awareness of a specific disease on the part of physicians, changes in diagnostic procedures, and improvements in disease screening. For *Campylobacter* infections, relevant surveillance artifacts could be whether an individual seeks medical care for his/her illness, whether the physician decides to obtain a stool culture to test for the presence of *Campylobacter*, and whether the clinical laboratory conducts the appropriate diagnostic tests. Various studies conducted as part of the FoodNet surveillance program allow for some estimation of the magnitude of these effects and the number of cases of campylobacteriosis that are essentially lost at each step in the pyramid. Using studies such as the FoodNet Population Survey and previous surveys of clinical laboratories, Samuel et al. (2004) were able to estimate that there were 34 cases in the community for every one case of culture-confirmed *Campylobacter* that was captured in FoodNet surveillance.[1]

Variation in Health Care Utilization

A number of studies in a variety of disciplines have identified factors that influence health care access and utilization. Individual characteristics that have been shown to impact health care utilization include race, residence in a rural as opposed to an urban environment,[228] extremes of age,[229] and health insurance coverage.[230, 231] Previous studies within the FoodNet population have found that, in general, approximately 20% of individuals who experience an acute diarrheal illness (defined as >3 loose stools in a 24 hours period) seek healthcare.[232] These studies also identified certain characteristics among individuals with diarrhea that are associated with seeking medical care: the presence of bloody diarrhea, duration of diarrhea for more than three days, being less than 5 or over 65 years of age, having medical insurance, and having a household income of less than $25,000.[229]

Variation in Stool Sample Submissions

The importance of obtaining stool cultures to determine the etiology of diarrhea was stressed in the 1999 Infectious Diseases Society of America guidelines for infectious diarrhea.[233] The authors emphasized that having knowledge of the causative agent of diarrhea is beneficial for a number of reasons. While some individuals will benefit from the use of correct antibiotics, such as those with an infection resistant to some antimicrobials, other infections may benefit from a lack of antimicrobial use. Studies of E. *coli* O157 infected patients suggest that those who receive antibiotic treatment could be at an increased risk of developing severe complications such as Hemolytic Uremic Syndrome (HUS).[234] Additionally, evidence of an etiologic agent is also important for public health surveillance and outbreak detection; having an etiologic

agent identified increases the chance of linking cases to each other and to the potential source of infection or outbreak.[233]

Among individuals within the FoodNet population, surveyed from 2000-2003, who sought care for an acute diarrheal illness, the proportion that reported submitting a stool specimen was 21%.[232] A survey of physicians serving the FoodNet population was conducted in 1996. The proportion of physicians who reported asking for a stool specimen from patients with acute diarrhea was 44%.[235] The large difference between the estimates from the patients and the physicians was thought to be due to recall bias, wherein the physicians were more likely to remember the patients from whom they requested a stool specimen. In surveying physicians, the factors associated with the request of stool specimen from a patient were found to be presence of HIV infection, occurrence of blood in stool, duration of diarrhea for longer than 3 days, and international travel.[235]

Variation in Clinical Laboratory Practices

All clinical laboratories in the FoodNet surveillance area reported that they routinely culture stools samples for *Campylobacter*.[236] However, as discussed previously, multiple steps are involved in the detection and identification of *Campylobacter* organisms from a clinical specimen. Variation at any of these steps could change the likelihood of finding *Campylobacter* or favor the growth of one species of *Campylobacter* over another. Although all of the clinical diagnostic laboratories in the FoodNet catchment area have been shown to routinely culture stool specimens for *Campylobacter* bacteria,[236] the methods used by these laboratories may vary.

An ongoing study in the FoodNet sites is designed to better describe the types of methods routinely used in clinical laboratories to detect *Campylobacter* from a stool specimen. Few studies of this nature have been conducted; however, preliminary data suggest that differences do exist.[237] A study conducted in 2003 in Los Angeles County found the methods used by the laboratories in this county were varied.[238] Studies of clinical laboratory practices in other countries, however, have shown little variation. The European Centers for Disease Control (ECDC) conducted a survey of the clinical laboratories serving the ECDC countries. They found the methods used by the laboratories were similar among the countries, but the proportion of samples that tested positive varied. The main factor associated with an increased proportion of positive tests was that the laboratory routinely tested all stool specimens for *Campylobacter*.[134] A survey of clinical laboratories in New Zealand was conducted in 1992-1993 to determine if there were regional differences in laboratory techniques and whether changes in detection methods could account for the recent increase in *Campylobacter* incidence in New Zealand. No meaningful differences in laboratory isolation techniques were identified, and the few changes that had occurred within the last 5 years could not account for the recent increase in incidence.[239]

Confirmed Case Reported to Public Health Authorities

Once an individual has been identified to be infected with *Campylobacter*, this information should be relayed to public health authorities and appropriate action taken. However, since *Campylobacter* is not a nationally notifiable disease, differences in the relay of this information among states certainly could exist. Nevertheless, within the FoodNet catchment area, any resident who has a culture-confirmed *Campylobacter*

infection should be included in the active surveillance data.[240] Personnel in FoodNet sites perform annual audits of clinical laboratories that serve their surveillance area to ensure that all cases are captured in the surveillance. Reviews of these audits have shown that FoodNet surveillance captures at least 95% of all culture-confirmed cases in all sites.[240]

True Differences in Incidence Rates

While surveillance artifacts might be one reason for the differences in *Campylobacter* incidence observed in the FoodNet sites, another is that there may be true differences in the risk of infection between the sites. This variation in risk could be the result of a number of factors. Differing occurrence or concentration of *Campylobacter* in foods or water known, different behaviors by individuals in the populations of each site that put them at higher or lower risk of exposure or infection, or differences in immunity which modify the effect of the risk on the development of clinical disease may all be important.

Variation in Exposure to Campylobacter

The likelihood of an individual coming into contact with *Campylobacter* may not be the same across all FoodNet sites. The amount of *Campylobacter* on various food items has been shown to vary. For example, the amount of *Campylobacter* on poultry products may be higher in California than other FoodNet sites, and this variation could have a substantial impact on *Campylobacter* incidence given that poultry is considered a major source of *Campylobacter* infection in the U.S.[1, 2, 159] A survey of retail meat samples in the FoodNet sites in 2003 showed that all sites had *C. jejuni* detected on over

90% of chicken samples surveyed.[241] Ongoing studies in the FoodNet sites are aimed at identifying whether the concentration of *Campylobacter* on retail chicken meat varies significantly by FoodNet site.

Variation in freezing practices of poultry meat may also be important, as consumption of fresh (unfrozen) chicken has been shown to be a stronger risk factor for *Campylobacter* infection than consumption of frozen meat,[43] and levels of *Campylobacter* on poultry decrease when meat is frozen.[242] Notably, in 1993, California instituted a unique state–specific regulation that states "No person who processes, butchers, slaughters, packs, repacks, or sells poultry or poultry meat shall advertise, hold out, distribute, or sell as 'fresh' any poultry or poultry meat whose internal temperature has been below 26 degrees Fahrenheit."[243] As other states do not have similar regulations, it is possible that poultry sold as "fresh" in those states has been at least transiently frozen. Furthermore, countries that have seen an increase in the consumption of "fresh", previously unfrozen, chicken have noticed a rise in *Campylobacter* incidence as well.[156, 244] Using molecular subtyping methods, Karenlampi et al. (2007) were able to show an increase in the amount of MLST subtypes associated with poultry during the same time that there was an increase in the consumption of fresh chicken in Finland.[245]

Another exposure that may vary geographically is the consumption of unpasteurized, or "raw", milk, also an important risk factor for *Campylobacter* infections. Interstate commerce of unpasteurized milk is illegal under a U.S. FDA code (Section §1240.61) entitled "Mandatory pasteurization for all milk and milk products in final package form intended for direct human consumption."[246] However, individual states are allowed to regulate the sale of unpasteurized milk within their own states.

State regulations for the sale of unpasteurized milk are varied. Two states in FoodNet (Maryland and Tennessee) have banned the sale of unpasteurized milk, others allow it only under specific conditions (Colorado, Georgia, Minnesota, and Oregon) and a few states allow the sale of raw milk (California, Connecticut, New Mexico, and New York). There appears to be an ecological association between milk regulations and disease risk. A study by Headrick et al. (1998) found that milk-borne outbreaks of disease, including campylobacteriosis (that accounted for the majority of outbreak cases), were more likely to occur in states where the sale of raw milk was legal.[247] Thompson et al. (1986) also found a much higher incidence of *Campylobacter* infection among rural residents of certain Canadian provinces than among their urban counterparts. The authors contributed this higher incidence to the fact that the rural residents reported recent consumption of unpasteurized milk.[169]

Proximity to farm animals and livestock or living on a farm have been shown to be a strong risk factors for *Campylobacter* infections. In a number of countries, the incidence of *Campylobacter* infection has been shown to be higher among individuals who live in a rural area compared to those who live in an urban area.[169, 248] There are likely to be differences in the proportion of the population living in rural as opposed to urban areas within the FoodNet sites as well.[28]

Not all case-control studies examined whether identified risk factors for *Campylobacter* show effect modification by geographic area. One Danish study, by Neimenn et al. (2003), did look at geographic modification of risk factors and found that they were modified by county of residence. Specifically, the risks due to consumption of

unpasteurized milk, and routine contact with cows, were much higher for one particular rural county compared to others being studied.[44]

Other exposures that are known risk factors for *Campylobacter* infection, such as drinking untreated water from lakes, streams or ponds and having well water as a main source of drinking water, may vary geographically as well.

Variation in Immunity

Once an individual has been exposed to *Campylobacter*, whether s/he develops infection and then clinical illness depends upon his/her immune status. Previous exposure to *Campylobacter* could result in immunity such that clinical illness does not develop upon repeated exposure. It remains unclear whether immunity prevents colonization of the human gut by *Campylobacter* or whether it allows colonization but prevents symptoms from occurring (asymptomatic shedding). Furthermore, the specificity of the immunity is to a given strain or serotype may also be important.

In the U.S., the importance of immunological protection against clinical illness due to *Campylobacter* infection is somewhat unclear. A recent study by FoodNet showed that breastfeeding protected against illness from *Campylobacter* in infants less than six months of age, perhaps due to transfer of maternal antibodies.[159] The seroprevalence of antibodies to *Campylobacter* in the general U.S. population is mainly undetermined, although some data exist on small samples or specific subpopulations. A study by Belongia et al. (2003) showed that by 15-18 years of age, close to 90% of children residing on farms in Wisconsin tested positive for antibodies to *Campylobacter*, compared to 60% in children not living on farms.[249] In a convenience sample of healthy controls in a study of GBS, the authors reported seroprevalences of 36% for anti-

Campylobacter IgA, 53% for IgG, and 55% for IgM.[250] While no population-based figures exist for the seroprevalence in the U.S. population, a Danish study found that the seroprevalence of antibodies to *C. jejuni* ranged from 20%-32%, depending on age.[251] How seroprevalence might vary geographically in the U.S. is unknown.

Seasonal Variation in Campylobacter Incidence

The incidence of *Campylobacter* infections in the U.S. shows seasonal fluctuations, with higher incidence observed in summer months.[2, 252] This seasonal pattern has remained consistent, despite a decrease in the overall incidence of *Campylobacter* infections since FoodNet surveillance began in 1996.[227] Campylobacteriosis is not alone in its seasonal periodicity; many other food- and waterborne (i.e., salmonellosis and cholera), and respiratory infectious diseases (i.e., legionellosis, measles) exhibit seasonal patterns of incidence.[253] Mechanisms leading to the observed seasonal periodicity of infectious disease are often multifaceted and poorly understood. Changes in environmental conditions that favor the survival, growth and spread of microbial pathogens, such as changing temperature or precipitation, have been postulated as causes of seasonality, as have changes in human behavior (for example, congregation of school children during the beginning of the school year, or increased outdoor recreational activities during warmer summer temperatures).[253, 254]

Seasonal Variation in Exposure to *Campylobacter*

For *Campylobacter*, the reasons for seasonal periodicity in incidence are likely to be multifaceted. Some identified risk factors for campylobacteriosis, such as eating chicken from a backyard barbecue, and swimming in natural water, are likely to occur

much more frequently in the summer. Additionally, it may be that more individuals are prone to travel internationally during the summer, another known risk factor for infection. Also, the occurrence and concentration of *Campylobacter* in food and the environment can also change throughout the year. For example, the proportion of poultry flocks that test positive for *Campylobacter*, and the occurrence of *Campylobacter* in natural surface water varies seasonally.[3, 255]

A recent case-control study in Finland was the first to identify swimming in natural sources of water as a risk factor for *Campylobacter* infection.[166] This risk factor may have only been identified because the case-control study was limited to the summer season (July 1st -- September 30th) when Finland experiences their highest incidence of campylobacteriosis and swimming is most common. Another case-control study conducted in Sweden found the proportion of individuals that reported various exposures, particularly eating meat that had been grilled or consuming water from a stream or a lake, was greater during the summer months.[46] Although the risks due to these exposures were found to be the same for each season, the population attributable fractions due to these exposures may vary due to an increase exposure during the summer.

The occurrence and strains of *Campylobacter* in food and environmental sources also has been shown to change seasonally. For example, the prevalence of *Campylobacter* in estuaries in coastal Georgia was shown to be higher in the summer months.[56] This increase in summer was positively associated with increased rainfall as well.Seasonal fluctuations in the prevalence of *Campylobacter* in poultry and lambs destined for slaughter have been observed.[256-258] A few studies that examined the genetic

profiles of *Campylobacter* isolates found that some strains predominated during the summer peak in incidence as during the rest of the year. A 1999 study in New Zealand, where incidence also shows marked seasonality, found differences in the strains that were detected during winter months compared to summer months.[259] A study in Scandinavia found similar results, with certain strains 16 times as likely to occur during summer months as compared to other strains of *Campylobacter*.[136] Whether these findings are the result of changes in human behavior, or other events, is still undetermined.

Because of these seasonal changes in the prevalence and strains of *Campylobacter* in food and environmental sources for *Campylobacter* infection, it is possible that the actual risks for infection and illness from exposures to these sources change seasonally as well. Effect modification by season of specific *Campylobacter* risk factors has been observed in a handful of case-control studies. A study conducted in Denmark by Neimann et al. (2003) found that certain risk factors, principally consumption of barbecued chicken, unpasteurized milk, and apples or pears, showed effect modification by season.[44] While barbecuing "in season" (June-October) was a strong significant risk factor for *Campylobacter* infection, barbecuing "off-season" (November-May) was found to be protective (although not statistically significant). Drinking unpasteurized milk "in-season" was protective, although not statistically significant while drinking unpasteurized milk "off-season" was a significant risk factor for infection.

Temperature-Dependence of *Campylobacter* Incidence

The seasonal trend of campylobacteriosis in the U.S. appears to follow that of temperature in general, with higher incidence during June and July. A closer examination of the relationship between changes in temperature and *Campylobacter* incidence is needed. While studies have been conducted in a number of countries to better understand the temperature-dependence of *Campylobacter* incidence, no systematic studies have yet been conducted in the U.S.[10, 260] With the continuing debate about the impact and potential causes of global warming, the importance of understanding climate and temperature effects on infectious disease incidence has increased. The relationship between temperature and campylobacteriosis is of interest, as it might shed light on the underlying ecological pathway from *Campylobacter* in the environment to human exposure and illness.

Weather conditions, such as temperature and precipitation, obviously change throughout the year. In particular, the temperature pattern in the U.S. is generally similar to that of the seasonal pattern of campylobacteriosis, with increases in both during the summer. The relationship between temperature and campylobacteriosis is likely to be complex, as there is little indication that ambient temperature directly impacts the ability of *Campylobacter* to survive and multiply in the environment (since most evidence indicates *Campylobacter* requires a human or animal host). Some investigators have suggested that, because many *Campylobacter* infections come from contaminated chicken, and there is seasonal variation in the prevalence of *Campylobacter* in broiler chicken,[8, 255-257, 261] that the seasonality in human cases simply mirrors that observed in poultry. While a number of studies have observed seasonality in the prevalence of

Campylobacter in broiler chicken flocks, one has found the seasonal peak in poultry to precede that in human cases.[256] Two have found the peak in prevalence in animal sources to follow that of human cases.[8, 255] These findings suggest that some "ecological driver" common to both poultry (and other livestock) and human infection may be the cause of this trend, rather than an increase in poultry contamination leading to an increase in human cases.[255]

One "ecological driver" put forth in a recent hypothesis by Nichols (2005),[262] as well as others,[263-265] is that flies are a large contributor to *Campylobacter* seasonality. Housefly (*Musca domestica*) development from larvae to adult is largely dependent on temperature, as well as moisture content.[266] At warmer temperatures, housefly development can take days, while at lower temperatures can take weeks. Flies have been shown to become active above a threshold of 20 ^{o}C (68 ^{o} F),[267] and are most active in the northern hemisphere from May-October.[264] Nichols showed that there was an association between an excess number of *Campylobacter* cases (> 170 per week) and shortened development time (< 3 weeks) of the housefly. A study in Iceland that looked at risk factors for *Campylobacter* contamination of boiler chickens similarly found a link between fly activity and poultry infection mediated by temperature.[268] Furthermore, flies were shown to be an important contributor to *Campylobacter* colonization of broiler chickens in a study by Hald et al. (2007) that found that the addition of fly screens to open vent areas in broiler houses significantly reduced the colonization of broiler chickens by *Campylobacter*.[269]

A number of studies in other countries have specifically examined the relation between temperature, precipitation, or humidity on *Campylobacter* incidence. Some of

these have controlled for the general seasonal trend in incidence,[260] while others have tried to examine the more broad impact of temperature on the seasonal pattern.[10] A Canadian study found a 2.2% increase in the incidence of *Campylobacter* infection for every 1^{o} C increase in mean weekly temperature, with a lag of one week, once a threshold of -10^{o} C (14^{o} F) was reached.[270] In the U.K., Tam et al. (2005) found a 5% increase in the average number of weekly reports of *Campylobacter* cases with every 1^{o} C increase in mean temperature, given a lag of 6 weeks, up to a threshold of 14 ^{o}C (52^{o} F).[260] Given the length of the lag period and the importance of the threshold, the authors suggested that the effect of temperature on *Campylobacter* incidence is indirect and complex. Tam and coauthors choose to control for the effects of season by including Fourier terms (up to the 8th harmonic), as well as a variable indicating weeks that included public holidays, and splines to model both temperature and humidity. Kovats et al. (2005) compared the temperature trends of *Campylobacter* infection in a number of countries.[10] They found that *Campylobacter* incidence peaks in the spring in most countries and that there was a weak association between temperature and the peak week of *Campylobacter* incidence.

Only one study thus far has examined the relationship between temperature and *Campylobacter* in the U.S. Naumova and colleagues (2006) conducted an analysis of six reportable enteric infections in Massachusetts using surveillance and temperature data from 1992-2001. They found that the maximum temperature during a given year and the maximum number of *Campylobacter* cases peaked at the same time.[271]

Summary

In summary, reasons for the geographic and seasonal variation in campylobacteriosis in the United States remain unclear. The goal of this dissertation is to examine key factors that may explain the observed regional and seasonal variations in *Campylobacter* rates in the FoodNet sites, given the constraints of data collected for public health surveillance purposes.

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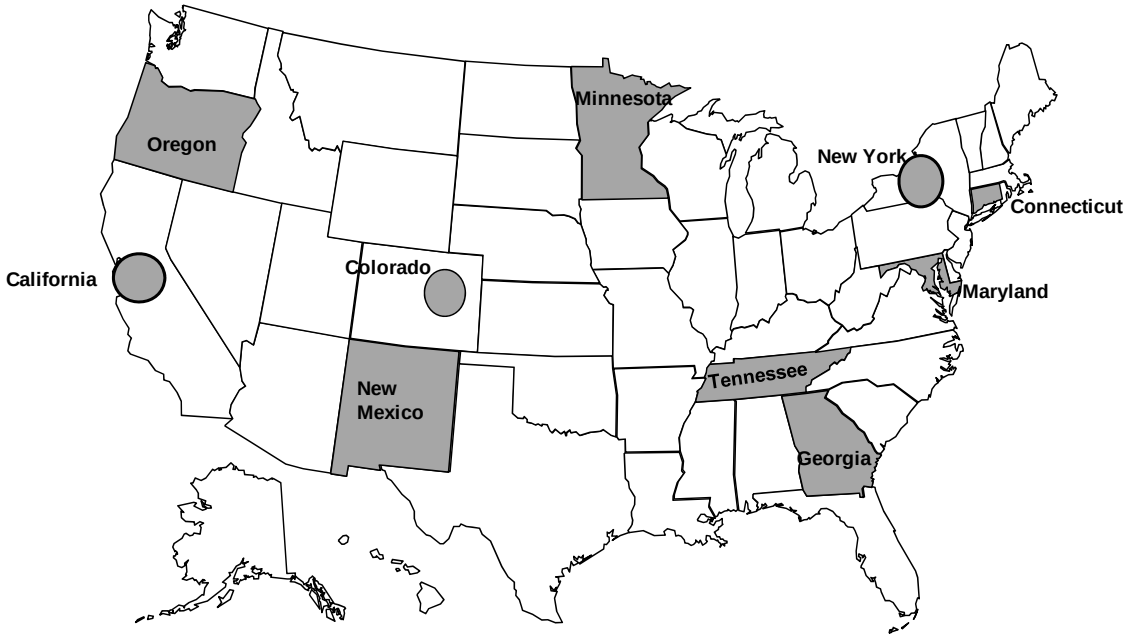
Tables and Figures

Figure 2.1 Map of FoodNet sites as of 2006*



* Connecticut, Georgia, Maryland, Minnesota, Oregon, Tennessee, and selected counties in California, Colorado, New York

Figure 2.2 The American Society for Microbiology (ASM) recommendations for the isolation and identification of typical thermotolerant *Campylobacter* species (e.g., *C. jejuni* and *C. coli*) from a stool specimen[189]

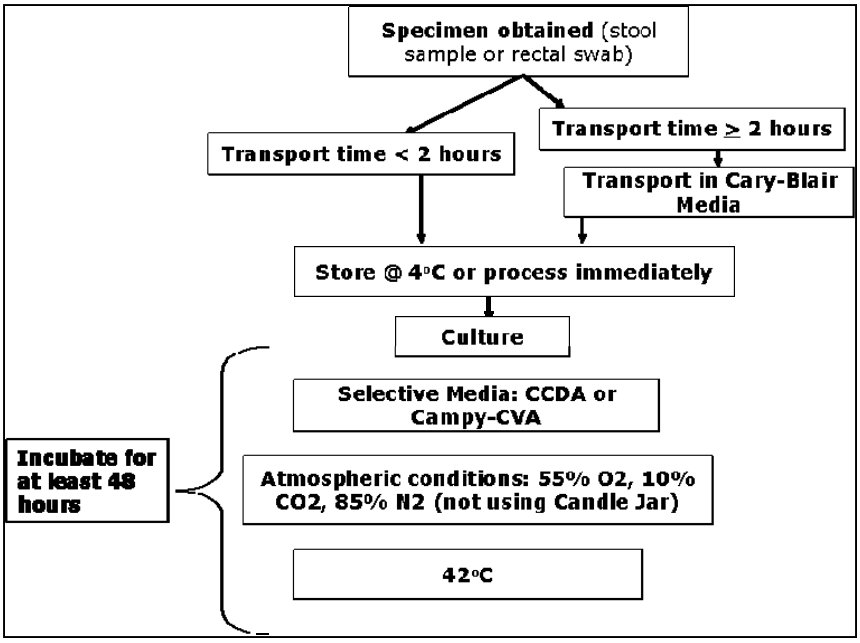
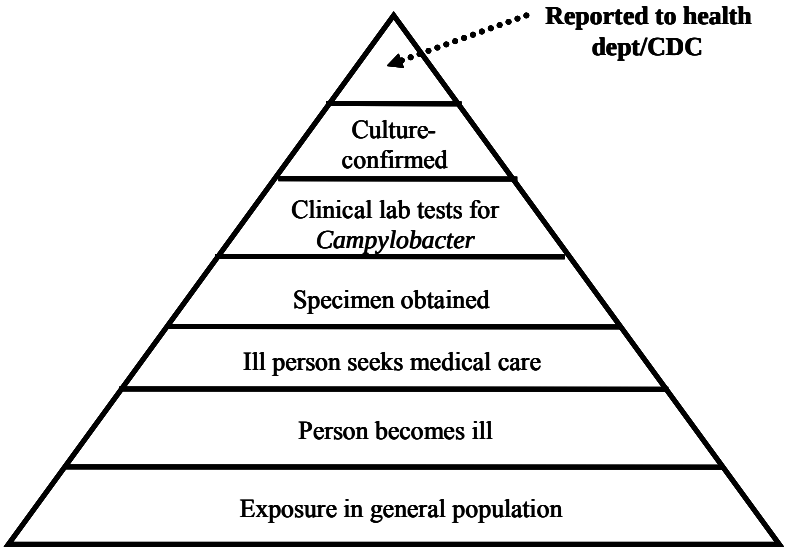


Figure 2.3 Burden of Illness Pyramid



CHAPTER 3. DISSERTATION OBJECTIVES

1. Do differences in risk factors or health care use explain the geographic variation in campylobacteriosis in the FoodNet sites?

2. Is there geographic variation in clinical laboratory practices for the detection of *Campylobacter* in the FoodNet sites?

3. What is the association between rainfall and temperature and *Campylobacter* infections in the FoodNet sites from 1996-2006?

4. Do differences in risk factors explain the seasonal variation in campylobacteriosis?

CHAPTER 4. DO DIFFERENCES IN RISK FACTORS OR HEALTH CARE USE EXPLAIN THE GEOGRAPHIC VARIATION IN CAMPYLOBACTERIOSIS IN THE FOODNET SITES ?

Abstract

In the United States, geographic variation in the rates of culture-confirmed *Campylobacter* infections has been consistently observed. Using population-based survey data from the Foodborne Diseases Active Surveillance Network (FoodNet), we examined whether there were geographic differences in health care use and stool culture submission practices that could explain the geographic variation in campylobacteriosis. We also used a case-control study conducted in the FoodNet surveillance area to examine whether the risk from various previously-identified risk factors for *Campylobacter* infection, and the prevalence of these exposures (proportion of individuals reporting exposure), varied geographically. No significant geographic differences were found in health care use or stool culture submission practices. There was no evidence of geographic effect modification of risk factors for campylobacteriosis (consumption of chicken at commercial eating establishments, raw milk, and untreated water, and exposures to farm animals and animal stool). The prevalence of some risk factors varied geographically, although they do not appear to fully explain the geographic variation in campylobacteriosis. The reasons for the geographic variation in campylobacteriosis in the United States remain unclear.

Introduction

Campylobacteriosis is one of the most common causes of bacterial gastroenteritis worldwide and in the United States (U.S.), where it accounts for approximately 2 million illnesses annually[1]. In the U.S., cases of campylobacteriosis are not nationally notifiable. Rather, information on this important disease is collected through the Foodborne Diseases Active Surveillance Network (FoodNet) that conducts active, population-based surveillance for culture-confirmed *Campylobacter* infections in select states and counties. Geographic variation has been consistently observed in the rates of culture-confirmed campylobacteriosis in the FoodNet sites. A five-fold difference in average rates of culture-confirmed campylobacteriosis exists between the California FoodNet site (reporting an average of 34 cases/100,000 persons) and the lower-incidence sites of

Maryland (6.8 cases/100,000 persons), Georgia (8.6 cases/100,000 persons) and Tennessee (7.3 cases/100,000 persons).[2] This variation has not been explained.

The numbers of culture-confirmed cases of campylobacteriosis captured in FoodNet surveillance only represents a fraction of those that occur. Samuel et al. (2004) estimated that there are 34 cases in the community for every one culture-confirmed case of campylobacteriosis captured in FoodNet surveillance. Differences in surveillance artifacts (health care utilization, stool sample submission, clinical laboratory testing procedures, or reporting of cases to public health authorities) or regional differences in exposure[1] to *Campylobacter* may result in overall differences in the reported rates of campylobacteriosis.[3] As FoodNet conducts active surveillance to capture every instance of a culture-confirmed case of campylobacteriosis in the surveillance area, reporting differences are not likely to exist. In a recent study by Ailes et al. (Chapter 5), differences in clinical laboratory practices did not account for the geographic differences in the clinical laboratory *Campylobacter* isolation rates. However, differences in health care utilization and stool sample submission practices or differences in risk factors for campylobacteriosis, as a result of variation in population exposure to *Campylobacter*, have yet to be fully examined.

Identified risk factors for *Campylobacter* infection in the FoodNet sites in the U.S. and other countries include consumption of raw or undercooked chicken, unpasteurized milk, and untreated water.[4-8] and exposure to dogs, farm animals, and foreign travel.[4, 6, 8] Geographic differences in risk factors for campylobacteriosis may exist because of differences in the occurrence or concentration of *Campylobacter*, variation in human behaviors that put individuals at higher or lower risk for infection, or

differences in immunity to campylobacteriosis. Geographic variation in factors related to health care use and stool sample submission practices could also potentially confer differences in rates of campylobacteriosis across the FoodNet sites. Previous studies have found that, in general, approximately 20% of individuals who experience an acute diarrheal illness seek healthcare[9] and, among these, 19% reported submitting a stool specimen for testing.[10] Factors associated with seeking medical care and submitting a stool sample included bloody diarrhea, duration of diarrhea, age, medical insurance, household income, Human Immunodeficiency Virus infection, and recent international travel.[10, 11]

The aims of this study were to determine whether there were differences across the FoodNet sites in the risks for campylobacteriosis from various exposures or in the prevalence of these exposure and, among individuals with acute diarrheal illness, whether there was geographic differences in health care use and stool specimen submission practices.

Methods

FoodNet

The Foodborne Diseases Active Surveillance Network (FoodNet) is a collaboration between the Centers for Disease Control and Prevention (CDC), the U.S. Department of Agriculture's Food Safety and Inspection Service, the U.S. Food and Drug Administration, and participating state health departments. It is conducted part of CDC's Emerging Infectious Diseases Program. FoodNet surveillance officers in the FoodNet sites routinely contact clinical laboratories serving the FoodNet surveillance

area to ensure that all incident cases of culture-confirmed campylobacteriosis among residents of the FoodNet catchment area are ascertained.

FoodNet began active laboratory-based surveillance for culture-confirmed *Campylobacter* infections in 1996. Initially the surveillance area was comprised of five sites: select counties in California, Connecticut, and Georgia and the entire states of Minnesota and Oregon (14.2 million persons, five percent of the U.S. population). Between 1996 and 2003, the FoodNet surveillance area expanded from a population of 14.2 million persons in five sites to 44.9 million persons in 10 sites.

Case-Control Study of Risk Factors for Campylobacteriosis

In 1998-1999, data were collected for a case-control study of sporadic *Campylobacter* infections in the FoodNet sites that has been described previously.[4] In 1998, the FoodNet surveillance area comprised seven percent of the U.S. population (21 million persons) and included the states of Connecticut, Minnesota, and Oregon and selected counties in California, Georgia, Maryland and New York. Each FoodNet site attempted to enroll $\geq$ 200 cases selected randomly from the culture-confirmed cases of campylobacteriosis that occurred during the study period. Cases were residents of the FoodNet catchment area with a sporadic culture-confirmed case of campylobacteriosis (as opposed to one linked to an outbreak). Controls were selected using progressive sequential digit dialing beginning with the telephone number of the case. Controls were matched to a case based upon county of residence and the following age strata: 2 to <6 years of age; 6 to <12 years of age; 12 to <18 years of age; 18 to <40 years of age; 40 to <60 years of age; 60 or older years of age.

Informed consent was obtained for all participants prior to interview. Information on cases less than 12 years of age was collected from a parent or guardian and all other cases were interviewed directly. Case-patient interviews were conducted within 21 days of the specimen collection date, and controls were interviewed within seven days of their matched case's interview. Cases and controls were asked about exposures during the seven days prior to the case's onset of illness, including symptoms experienced by the case, demographic characteristics, and recent food consumption patterns and animal exposures.

Differences in Risk Factors for Campylobacteriosis

Exposures of interest for this new analysis of the case-control study data were chosen because they were previously found to be associated with *Campylobacter* infection in the original analysis[4] and because the prevalence of exposure or risk from the exposure was posited to vary by FoodNet site. Conditional logistic regression models, accounting for the matching between cases and controls based on age group and county-of-residence, were used. Case-control pairs with the same age group and county of residence were pooled into the same strata.[12] In univariate analyses, each exposure was considered alone, in a model restricted to each FoodNet site (state), and again in a model including the entire dataset along with an interaction term between the exposure of interest and the FoodNet site. Children $\leq$2 years of age and persons reporting international travel within seven days of illness onset were excluded because risk factors for infants and travelers are different from other groups.[13]

To determine if there were differences in the risk due to a specific exposure, after accounting for other known risk factors for infection, multivariate models including other known risk and protective factors for infection (identified in the initial analysis of these data[4]) were also created. These factors included male sex, having a pet puppy, and consumption of the following: any chicken cooked at home, any pink chicken, fried chicken, any turkey meat at home, any turkey at commercial eating establishment, any meat at home, any meat at commercial eating establishment, any raw seafood, and any berries bought in a store. Using data from all FoodNet sites combined, multivariate models were developed that included an interaction term between the exposure of interest and the FoodNet site. The likelihood ratio test was used to determine if there was significant interaction between FoodNet site and the exposure of interest. The interaction analysis was complicated by the fact that cases were matched to controls on FoodNet site. While some might argue that including an interaction term between a matching factor and another factor of interest is not technically correct because the regression model is not strictly hierarchically well-formulated (i.e., it does not include the effects of FoodNet site as a separate variable in the model), the FoodNet site effects are incorporated into the model as components of the matching factors.[12]

The controls, if selected appropriately, should represent the exposure distribution among the general population. Therefore, the frequency of various exposures among the controls in each FoodNet site was calculated and a chi-square test was used to determine if exposures varied significantly by FoodNet site. When the number of exposed controls was < 5, a Monte Carlo simulation procedure (the MC option in the PROC FREQ procedure in SAS v. 9.2) of Fisher's exact test p-values was used to estimate exact p-

values for these comparisons because Fisher's exact test was too computationally intensive. Comparisons across the FoodNet sites were conducted with a SAS macro that used a Tukey-type multiple comparison procedure, once an overall chi-square indicated there was a significant difference in the prevalence of exposure among controls across the FoodNet sites.[14, 15]

Population Surveys of Diarrheal Disease and Healthcare Use

Four cross-sectional surveys of the general FoodNet population were administered between 1996 and 2003. The purpose of these surveys was to estimate the overall burden of diarrheal illness and to determine the frequency of health care use and stool sample submission. Individuals were eligible for selection into the survey if they resided in the FoodNet catchment area during the survey cycles (cycle 1: July 1996-June 1997; cycle 2: July 1998-June 1999; cycle 3: March 2000-February 2001; cycle 4: March 2002-February 2003). The methodology used to select individuals for the survey was similar to that employed by the Behavioral Risk Factor Surveillance System (BRFSS).[16, 17] Briefly, after removing business and non-working telephone numbers, households were contacted using a single-stage, random-digit dialing technique.[17] A computer algorithm was used to select one household member, based upon the total number of males and females in the household. All age groups were eligible for inclusion; if a child aged $\leq$ 12 years of age was selected, a parent was interviewed to ascertain information about the child's exposures. Approximately the same number of interviews (ten interviews per site per month) was conducted each month in all sites. The survey collected demographic information and asked survey respondents about episodes

of diarrhea or vomiting in the past month. Those reporting symptoms of diarrhea and vomiting were asked about other gastrointestinal symptoms, if they had sought medical care for their illness, and if they had submitted a stool sample for laboratory testing. For the 2002-2003 survey, interviews were conducted in both English and Spanish.

Differences in Health Care Utilization and Stool Sample Submission

The results of the four cycles of the population surveys conducted between 1996 and 2003 were combined, as has been done previously.[18] Individuals who reported having a chronic illness that has diarrhea or vomiting as a major symptom (e.g., Crohn's disease, Irritable Bowel Syndrome) were excluded from the analysis. To better inform the acute gastrointestinal illness case definition used in subsequent analyses (i.e., in order to select individuals that had an illness similar to campylobacteriosis), the proportion of cases from the case-control study that experienced various symptoms of infection (diarrhea, abdominal cramps, fever, bloody stool, and vomiting) and consequences of infection (any days missed from work or inability to perform usual activities) were calculated for each FoodNet site. A chi-square test was used to determine if the frequency of each of these symptoms and consequences of infection varied by FoodNet site. In the event that there were <5 observations at any site, a Fisher’s exact test was used. The median and interquartile range (IQR) for the duration of diarrhea, number of days missed from work, and number of days a case was unable to perform his or her usual activities were calculated; the Kruskall-Wallis test was used to determine if there were significant differences in the median across sites.

For the analysis of healthcare use, the dataset was restricted to individuals with an acute diarrheal illness (ADI) (defined as ≥3 loose stools in a 24-hour period with a duration of ≥ 3 days) within the month prior to interview. The duration of illness restriction was used in order to make the cases more similar to those with campylobacteriosis. For the analysis of stool specimen submission for testing, the dataset was restricted to those individuals with an ADI in the month prior to interview who sought health care for their illness.

The proportion of survey respondents who reported seeking health care use and submitting a stool sample for testing was calculated for each FoodNet site and survey cycle. Proportions were weighted to account for selection probabilities (related to the number of telephone lines and people within a household) and to generate population estimates (by adjusting for the age- and sex-distribution based on the Census data for the FoodNet surveillance area for each survey cycle).[19] In order to determine if there were differences in health care use by FoodNet site, a logistic regression model accounting for sample weights (PROC SURVEYLOGISTIC in SAS v9.2), was used. The association between FoodNet site and seeking care was determined alone, as well as after adjusting for survey cycle. In order to determine if there were differences in stool sample submissions by FoodNet site, a logistic regression model accounting for sample weights (PROC SURVEYLOGISTIC in SAS v9.2), was used. The association between FoodNet site and stool sample submission was determined alone and also after adjusting for survey cycle.

SAS v. 9.1 (Cary, NC) was used for statistical analysis; all hypothesis testing and p-values were two sided using a significance level of 0.05.

Results

Differences in Risk Factors for Campylobacteriosis

A total of 2,093 cases were contacted about enrollment in the case-control study. However, 780 either met one of the exclusion criteria or refused to participate, 178 reported traveling internationally in seven days prior to illness onset, 75 were $\leq$ 2 years of age, and 18 had missing county information. There was a total of 1,042 age group and county-of-residence-matched case-control pairs: California (140), Connecticut (237), Georgia (141), Maryland (98), Minnesota (180), New York (81), and Tennessee (165).

There was no evidence of effect modification of risk factors for campylobacteriosis by FoodNet site, either when exposures were considered alone (Table 4.1) or in multivariate models adjusted for other known risk factors for infection (data not shown). There were, however, differences in the frequency of exposure among controls by FoodNet site (Table 4.1 and Figure 4.1). The proportion of controls that reported eating chicken at a commercial eating establishment varied from 18.2% in the Oregon site to 46.1% in the Georgia site (p-value: <0.001). The proportion of controls that reported contact with any animal stool varied from 8.9% in Minnesota to 30.9% in New York (p-value: <0.001). The proportion of controls that drank water from a lake, river or stream varied from 0% in the California and Georgia sites to 5.1% in the Maryland site (p-value: 0.0011). The proportion of controls that reported having contact with a farm animal (chicken, turkey, cow, goat, horse or pig) varied from 2.1% in Georgia to 12.7% in Oregon (p-value: <0.001). There were no significant differences in the proportion of controls who reported drinking unpasteurized milk.

Differences in Health Care Utilization and Stool Sample Submission

The only difference in symptoms of culture-confirmed *Campylobacter* infection across FoodNet sites was in the proportion of cases in the case-control study that experienced three or more loose stools in a 24 hour period; no other marker was significant (Table 4.2). This varied from 84% of cases in the Oregon site to 99% of cases in the Maryland site (p-value: 0.004). No differences were observed in the median number of days missed from work or days during which cases were prevented from performing usual activities. Over 95% of cases reporting a duration of diarrhea of at least 3 days, and there were no differences in duration by FoodNet site.

The four population surveys included 50,757 respondents without a chronic illness that included vomiting or diarrhea. Of these, 1,023 reported having an acute diarrheal episode similar to campylobacteriosis (defined as $\geq$ 3 loose stools in a 24-hour period for $\geq$ 3 days, during the previous month or four weeks). Among all four population surveys combined, there were no differences in the likelihood of seeking health care for an acute diarrheal illness between FoodNet sites nor in submitting a stool sample for analysis (Table 4.3). Adjusting for survey cycle did not meaningfully changes these associations.

Discussion

Despite an overall decline in the rates of culture-confirmed infection, geographic variation in the rates of campylobacteriosis between the FoodNet sites has been consistently observed since FoodNet began surveillance in 1996.[1, 2] Reasons for this

geographic variation have remained unclear. This study found no evidence of effect modification of risk factors for campylobacteriosis or geographic differences in health care utilization or stool sample submission practices that explain the observed geographic variation in campylobacteriosis. In this study, no statistically significant geographic differences in the risks for culture-confirmed *Campylobacter* infection from eating chicken at a commercial eating establishment, contact with any animal stool, drinking water from a lake, river or stream, contact with a farm animal or drinking unpasteurized milk were found. Additionally, while the proportion of controls exposed to these risk factors did vary by FoodNet site, the pattern of variation did not clearly explain the overall geographic variation in culture-confirmed rates of campylobacteriosis.

Geographic differences in the virulence of *Campylobacter* strains is an unlikely explanation, as the clinical picture was largely consistent across sites. The only significant difference between sites in culture-confirmed case characteristics was in the proportion of cases reporting more than 3 loose stools per 24-hour period (and, even so, this was reported by over eighty percent of cases in all FoodNet sites). Other studies have not found any virulence differences in *Campylobacter* strains that could lead to differences in illness presentation.[20]

Geographic differences in health care utilization and stool submission practices were not found to statistically vary across the FoodNet sites. There was some suggestion that stool submission practices may be higher in some sites as compared to others. It is possible that a state's public health capacity has an impact on physicians' practices regarding ordering stool samples for testing, although we were unable to assess it in this

study. Anecdotal evidence from some high-profile foodborne disease outbreaks during the early 1990s suggested that states with strong public health departments were more likely to have physicians that were aware of and were testing for specific pathogens of interest.[21] Despite an increase in awareness regarding foodborne diseases, a 2005 survey of emergency department physicians found that approximately half were unaware of which bacterial illnesses were reportable in their respective states.[22] Additionally, only 40% and 56% were aware of the importance of stool testing for patients working in daycare or restaurant settings, respectively. A recent assessment of epidemiology capacity in the U.S. conducted by the Council of State and Territorial Epidemiologists suggested that the epidemiological capacity of state health departments has decreased; the impact of such changes in capacity on the reporting of diseases such as campylobacteriosis is not known.[23] It thus appears that neither health seeking behavior, nor risk factors exhibit geographic differences that explain the observed variation in incidence. Similarly, we found that geographic differences in laboratory practices do not explain the variation in incidence (Chapter 5).

Extremely high rates of campylobacteriosis have been observed in other countries, particularly Iceland and New Zealand. Studies conducted in these countries may help elucidate potential reasons for the geographic variation observed in the U.S. In Iceland, rates of campylobacteriosis reached 157/100,000 in 1999, and the majority of infections were thought to be a result of consumption of *Campylobacter*-contaminated poultry.[24] In order to curb such high rates, the government in Iceland instituted *Campylobacter* surveillance on poultry farms and required that any chicken from *Campylobacter*-positive poultry flocks had to be frozen before being sold to consumers.

(Studies have shown that freezing chicken meat can lead to a two-log reduction in the amount of *Campylobacter*.[25, 26]) Poultry from *Campylobacter*-free flocks could be labeled and sold as "fresh" chicken. After the implementation of these control measures, the incidence of campylobacteriosis in Iceland decreased dramaticically.[27]

During the 1990s, the incidence of campylobacteriosis in New Zealand increased steadily as well. According to Baker et al. (2007), this increase was not due to increased surveillance or testing, as a comparison between hospitalized and all cases showed a similar temporal pattern.[28] Reasons postulated as causes of the increase included changes in human behavior (increased consumption of poultry, increased consumption of meals outside of the home) and increased ambient temperature. As in Iceland, a large proportion of cases are attributed to consumption of contaminated poultry products,[29] and therefore recent efforts have focused on reducing the microbial load of *Campylobacter* on poultry in New Zealand.[30]

Given the focus on curbing *Campylobacter* contamination of poultry in other countries that have high rates of campylobacteriosis, perhaps differences in the occurrence or quantity of *Campylobacter* on poultry contribute to the substantially higher rates of campylobacteriosis in the California FoodNet site. Although no quantification of the amount of *Campylobacter* on poultry samples was conducted, at least one study has looked at the prevalence of *Campylobacter* in retail meat samples (including chicken) across the FoodNet sites and found little difference.[31]. Variation in freezing practices of poultry meat may also be important, as consumption of fresh (unfrozen) chicken has been shown to be a stronger risk factor for *Campylobacter* infection than consumption of frozen meat,[32] and levels of *Campylobacter* on poultry

decrease when meat is frozen.[26] Notably, in 1993, California instituted a unique state–specific regulation that banned the labeling of previously-frozen chicken as "fresh."[33] Future studies that better quantify the relative amount of *Campylobacter* on poultry across the FoodNet sites would be useful.

Additional studies might also focus on geographic differences in immunity to *Campylobacter*, as a recent review by Havelaar et al. (2009) suggested that ignoring immunity to *Campylobacter* in epidemiological studies may lead to biased study results.[20] For instance, a recent case-control study of campylobacteriosis in the U.K. found that individuals who were habitual consumers of chicken had a lower risk of infection with *Campylobacter* than those who consumed chicken less often, suggesting that this risk for illness might be modified by protective immunity.[34] In the same study, recent dog owners had an increased risk of campylobacteriosis, whereas those who had owned dogs for a longer period of time did not have the same risk. Immunity to *Campylobacter* does not appear to prevent infection with the bacteria, but, rather, to reduce or eliminate illness symptoms. Previous studies have shown that habitual unpasteurized milk drinkers and long-term poultry abattoir workers are less likely to develop symptoms of *Campylobacter* infection and that they mount a strong antibody response to *Campylobacter* infection.[35, 36] The specificity of an individual's immunity to a specific *Campylobacter* strain may also be important. Miller et al. (2005) compared the age of infection with the infecting *Campylobacter* serotype from a collection of over two thousand stool samples.[37] Individuals 40 years of age and older were more likely than individuals less than 40 years of age to be infected with "uncommon" (found in at less than 10% of cases, compared to common serotypes found in at least 10% of cases)

serotypes. The authors suggested that immunity may be the reason for this change in the serotype distribution, as well as for the lower incidence of campylobacteriosis in individuals of older ages. The seroprevalence of antibodies to *Campylobacter* in the general U.S. population is mainly undetermined, although a study by Belongia et al. (2003) showed that by 15-18 years of age, close to 90% of children residing on farms in Wisconsin tested positive for antibodies to *Campylobacter*, compared to 60% in children not living on farms.[38] A recent study conducted in the Netherlands found that by 20 years of age, close to 100% of individuals surveyed had a measureable serological response to *Campylobacter*. The effect of this could be assessed with a targeted serosurvey. If geographic differences in population immunity play a role, we would predict that Maryland would have higher immunity levels, and California would have the lowest. If they did not play a role, we would expect the higher incidence in California to have produced the higher population immunity.

This study has several limitations. The analysis of geographic differences in risk factors for campylobacteriosis is limited to the identification of factors associated with culture-confirmed infection with *Campylobacter*. Cases may have been more likely to recall potentially risky food exposures that they attribute as the cause of their illness. Additionally, the cases likely represented the sickest individuals infected with *Campylobacter* because they visited a health care professional, had a stool sample taken, and the sample yielded *Campylobacter*. The requirements used for control selection, primarily that individuals who reported diarrheal illness within 28 days prior to interview were excluded, may have biased the control group in that they may have represented generally healthier individuals. Despite combining multiple cycles of the

population survey, the number of individuals who sought care for an acute diarrheal illness was small, particularly when stratified by FoodNet site. While there appeared to be some differences in the frequency of stool sample submission by site, these were not statistically significant. As the number of individuals included in the general population survey has increased over time[18] and surveys continue to be conducted, future analyses may be better able to examine whether differences in stool sample submission practices exist. However, if differences in healthcare seeking behavior or stool sample submission practices were a large contributor to the geographic variation in culture-confirmed rates of campylobacteriosis, one would expect to see a similar pattern with other enteric infections.

Future studies should examine differences in the quantification of *Campylobacter* on poultry between the FoodNet sites. Additionally, while this study did not find evidence that differences in risk factors or surveillance artifacts explain the geographic variation in culture-confirmed *Campylobacter* rates among the FoodNet sites, future studies that take into account the immunological response to *Campylobacter* should be considered.

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Tables and Figures

Table 4.1. Association of exposures with campylobacteriosis, stratified by FoodNet site. Conditional logistic regression models (adjusting for FoodNet site, county and age group) were used. Where indicated, exact models were used if case or control counts were less than 5. FoodNet sites have been listed in order of highest average incidence (1996-2006) to lowest according to Ailes et al. (2008).[2]

Exposure	FoodNet Site	Exposed Cases N (%)	Exposed Controls N (%)	Odds Ratio (95% Confidence Interval)	P
Any chicken at commercial eating establishment (excluding deli meat, pot pie, salad)	California	63 (45.0%)	50 (35.7%)	**1.44** (0.90, 2.32)	
	Minnesota	72 (40.0%)	43 (23.9%)	**2.36** (1.45, 3.82)*	
	Oregon	54 (32.7%)	30 (18.2%)	**2.12** (1.26, 3.55)*	
	Connecticut	103 (43.5%)	50 (21.1%)	**2.95** (1.96, 4.45)*	0.2273
	New York	29 (35.8%)	17 (21.0%)	**2.79** (1.27, 6.16)*	
	Georgia	101 (71.6%)	65 (46.1%)	**3.68** (2.13, 6.34)*	
	Maryland	39 (39.8%)	18 (18.4%)	**2.64** (1.36, 5.13)*	
Contact with any animal stool	California	22 (15.7%)	20 (14.3%)	**1.13** (0.58, 2.20)	
	Minnesota	39 (21.7%)	16 (8.9%)	**3.35** (1.69, 6.65)*	
	Oregon	63 (38.2%)	42 (25.5%)	**1.78** (1.12, 2.82)*	
	Connecticut	39 (16.5%)	34 (14.4%)	**1.17** (0.71, 1.93)	0.0979
	New York	25 (30.9%)	25 (30.9%)	**0.83** (0.41, 1.67)	
	Georgia	30 (21.3%)	19 (13.5%)	**1.68** (0.90, 3.15)	
	Maryland	17 (17.4%)	10 (10.2%)	**1.99** (0.85, 4.70)	

Drank any unpasteurized (raw) milk	California	1 (0.7%)	1 (0.7%)	**1.00** (0.01, 78.50)^	
	Minnesota	8 (4.5%)	1 (0.6%)	**9.09** (1.17, 414.00)*^	
	Oregon	3 (1.9%)	0 (0.0%)	**3.85** (0.41, ∞)^	
	Connecticut	3 (1.3%)	1 (0.4%)	**3.02** (0.24, 158.59)^	0.8239
	New York	3 (3.8%)	1 (1.3%)	**2.84** (0.23, 149.61)^	
	Georgia	1 (0.7%)	1 (0.7%)	**1.07** (0.01, 90.50)^	
	Maryland				
Drank untreated water from lake, river or stream	California	2 (1.5%)	0 (0.0%)	**2.85** (0.20, ∞)^	
	Minnesota	8 (4.6%)	2 (1.1%)	**3.39** (0.66, 33.26)^	
	Oregon	19 (11.9%)	7 (4.3%)	**3.29** (1.31, 8.24)*	
	Connecticut	3 (1.3%)	1 (0.4%)	**3.03** (0.24, 158.85)^	0.5605
	New York	2 (2.6%)	1 (1.2%)	**1.87** (0.09, 116.18)^	
	Georgia	4 (2.8%)	0 (0.0%)	**6.20** (0.75, ∞)^	
	Maryland	1 (1.0%)	5 (5.1%)	**0.24** (0.00, 2.49)^	
Had contact with a farm animal (chicken, turkey, cow, goat, horse or pig)	California	3 (2.2%)	5 (3.6%)	**0.56** (0.08, 3.09)^	
	Minnesota	32 (18.0%)	13 (7.3%)	**3.43** (1.50, 7.82)*	
	Oregon	33 (20.5%)	21 (12.7%)	**2.12** (1.10, 4.08)*	
	Connecticut	10 (4.2%)	7 (3.0%)	**1.39** (0.52, 3.77)	0.3990
	New York	11 (13.6%)	7 (8.6%)	**1.34** (0.33, 5.45)	
	Georgia	9 (6.4%)	3 (2.1%)	**3.36** (0.61, 34.26)^	
	Maryland	10 (10.4%)	4 (4.3%)	**3.31** (0.81, 19.35)^	

* P<0.05 ^ Exact odds ratio

Table 4.2. Frequency of case characteristics and consequences of infection, by FoodNet site, 1998 FoodNet case-control study of risk factors for campylobacteriosis. FoodNet sites have been listed in order of highest average incidence (1996-2006) to lowest according to Ailes et al. (2008).[2]

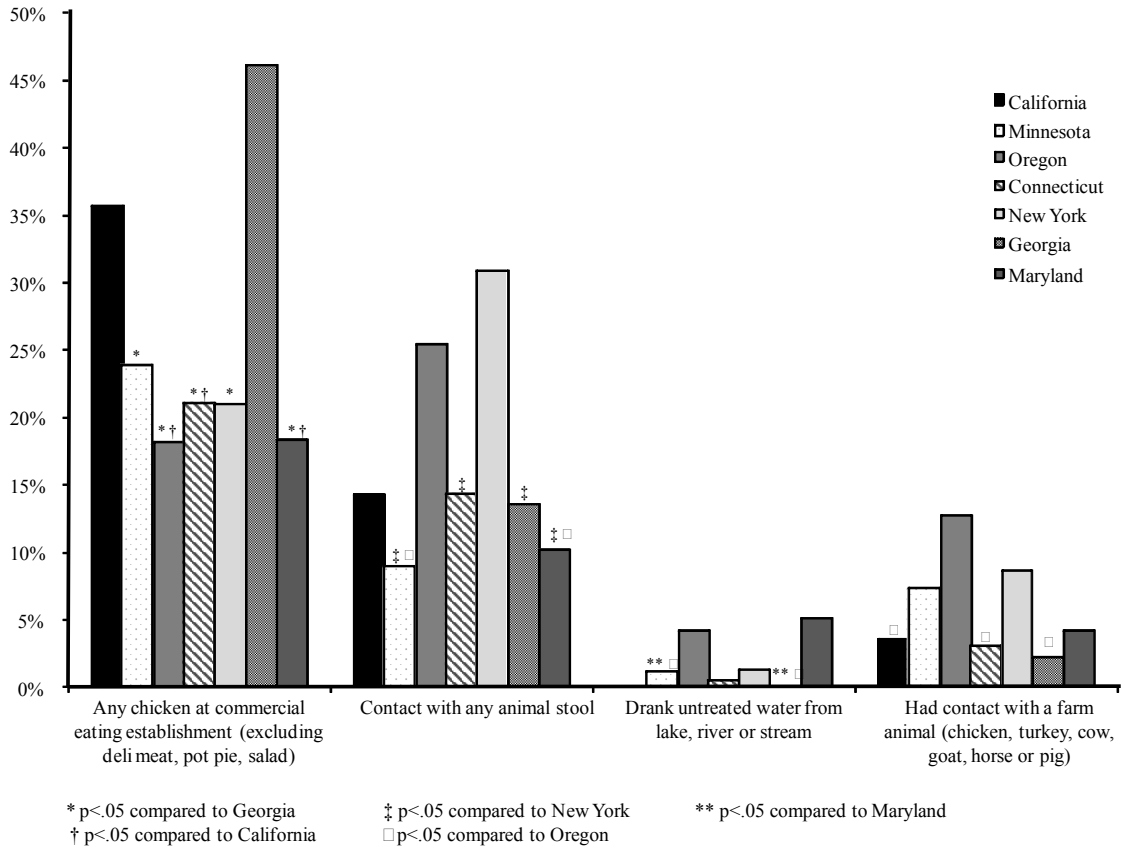
	CA (N=140) N (%)	MN (N=180) N (%)	OR (N=165) N (%)	CT (N=237) N (%)	NY (N=81) N (%)	GA (N=141) N (%)	MD (N=98) N (%)	P
Diarrhea	140 (100)	180 (100)	165 (100)	237 (100)	81 (100)	141 (100)	97 (99)	0.171
Abdominal cramps	120 (86)*	155 (87)*	144 (91)*	201 (85)*	72 (89)	132 (94)	79 (85)*	0.004
≥ 3 stools in 24 hour period	120 (86)	159 (89)*	139 (84)	204 (86)	70 (88)*	122 (87)	95 (99)*	0.214
Fever	109 (79)*	146 (85)*	130 (81)*	191 (85)*	66 (88)*	123 (90)*	86 (90)*	0.104
Blood in stool	58 (45)*	84 (50)*	73 (47)*	86 (39)*	37 (49)*	65 (50)*	45 (51)*	0.315
Vomiting	45 (32)	46 (26)*	54 (33)*	61 (26)*	23 (28)	49 (35)	34 (36)*	0.284
Duration of diarrhea ≥ 3 days	121 (94)*	163 (99)*	143 (97)*	205 (95)*	77 (97)*	125 (95)*	88 (97)*	0.119
No. days (Median (IQR))	5 (4-7)	6 (5-8)	6 (5-8)	6 (4-8)	7 (5-8)	6 (4-10)	6 (4-8)	0.308
Miss any work	74 (93)*	85 (88)*	81 (88)*	133 (90)*	45 (90)*	92 (93)*	46 (87)*	0.804
No. days (Median (IQR))	3 (2-4)	3 (2-5)	3 (2-4)	3 (2-5)	3.5 (2-5)	3 (2-5)	3 (2-5)	0.619
Prevented from usual activities	120 (86)	157 (88)*	148 (90)	211 (89)	67 (83)	128 (91)	87 (89)	0.589
No. days (Median (IQR))	5 (4-7)	5 (4-7)	5 (4-7)	5 (3-7)	5.5 (4-7)	5 (3-7)	5 (4-7)	0.888

Table 4.3. Association between FoodNet site and seeking care or being asked to submit a stool sample for testing, among those who reported an acute diarrheal episode with duration of three or more days during the previous one month or four weeks.

Outcome variable	FoodNet Site	Percent (weighted)	Std. error	OR (95% CI)		*P*
Seeking Care (Among those with an acute diarrheal illness last ≥ 3 days) (N=1,023 survey respondents)	California	27.09	4.74	**1.12**	(0.45, 2.79)	0.1768
	Colorado	24.89	7.42	**Referent**		
	Minnesota	27.81	4.55	**1.16**	(0.47, 2.86)	
	Oregon	26.13	4.66	**1.07**	(0.43, 2.66)	
	Connecticut	38.54	5.08	**1.89**	(0.78, 4.60)	
	New York	38.32	6.24	**1.88**	(0.73, 4.79)	
	Georgia	25.41	4.33	**1.03**	(0.42, 2.53)	
	Tennessee	30.95	6.49	**1.35**	(0.51, 3.62)	
	Maryland	43.50	7.08	**2.32**	(0.89, 6.10)	
Asked to submit a stool specimen (Among those who sought care) (N=326 survey respondents)	California	31.62	8.43	**2.02**	(0.29, 14.29)	0.7542
	Colorado	18.64	13.74	**Referent**		
	Minnesota	40.99	9.53	**3.03**	(0.43, 21.53)	
	Oregon	23.80	10.60	**1.36**	(0.16, 11.58)	
	Connecticut	23.83	7.16	**1.37**	(0.19, 9.70)	
	New York	37.65	11.01	**2.64**	(0.35, 19.96)	
	Georgia	23.00	7.79	**1.30**	(0.18, 9.62)	
	Tennessee	20.14	8.49	**1.10**	(0.14, 8.82)	
	Maryland	24.12	11.19	**1.39**	(0.16, 12.13)	

*OR=odds ratio; P=likelihood ratio p-value

Figure 4.1. Proportion of controls reporting exposure, by FoodNet site. FoodNet sites have been listed in order of highest average incidence (1996-2006) to lowest according to Ailes et al. (2008).[2]



CHAPTER 5. IS THERE GEOGRAPHIC VARIATION IN CLINICAL LABORATORY PRACTICES FOR THE DETECTION OF *CAMPYLOBACTER* IN THE FOODNET SITES?

Abstract

Campylobacter is a leading cause of bacterial gastroenteritis in the United States; however, rates of culture-confirmed infections vary geographically. Variation due to differences in risk exposures could help direct public health interventions; however, differences may be due to surveillance artifacts. We examined the role of regional differences in clinical laboratory detection methods. In 2005, the Foodborne Diseases Active Surveillance Network (FoodNet) conducted a survey of clinical laboratories in nine FoodNet surveillance sites to determine the procedures used to detect *Campylobacter* in clinical specimens. Multivariate Poisson regression was used to determine which laboratory procedures and characteristics were associated with the *Campylobacter* detection frequency (number of isolates/number of stools tested) and whether variation in these practices could account for the observed geographic variation in *Campylobacter* rates. The overall *Campylobacter* detection frequency among FoodNet clinical laboratories in 2004 was 1.5%. In multivariate analyses, FoodNet site (geographic location of the clinical laboratory), method and type of specimen transport, and the number of stools tested for *Campylobacter* in 2004 were the only significant predictors of the *Campylobacter* detection frequency. Variation in clinical laboratory methods do not fully explain the geographic variation in *Campylobacter* rates. Future studies should focus on surveillance artifacts, such as differences in medical care seeking, or variation in risk exposures, to determine reasons for the geographic variation in *Campylobacter* incidence in the U.S.

Introduction

Campylobacter is a leading cause of bacterial gastroenteritis in the United States (U.S.), resulting in an estimated 2 million illnesses annually.[1] Surveillance data on the incidence of culture-confirmed infections are available from selected sites participating in the Foodborne Diseases Active Surveillance Network (FoodNet).[2] Since FoodNet began in 1996, striking geographic variation in *Campylobacter* rates has been consistently observed, with the average annual incidence of culture-confirmed *Campylobacter* infections from 1996-2006 ranging from 7/100,000 in both Maryland and Tennessee to 34/100,000 in California.[3] Reasons for this geographic variation are

unclear. It is possible that regional differences are real, that is, due to regional variation in the risk factors for infection. However, differences may be due to regional variation in the surveillance steps necessary for a culture-confirmed case to be ascertained in surveillance, such as in medical care seeking, stool sample submission, or laboratory testing practices.[4, 5]

While >95% of clinical laboratories in the FoodNet sites routinely culture stool specimens for *Campylobacter*,[6] the detection and identification of *Campylobacter* from a clinical specimen is a complex process involving multiple steps. In 2003, the American Society for Microbiology (ASM) recommended a set of procedures for the detection and identification of typical thermotolerant *Campylobacter* species (e.g., *C. jejuni* and *C. coli*) from a stool specimen as follows (see Figure 5.1): use transport media (such as Cary-Blair) if the specimen transport time is $\geq$ 2 hours; process the sample immediately upon receipt in the laboratory or store the sample at 4^{o} Celsius (in transport media) upon receipt in the laboratory; incubate culture plates at 5% O_2, 10% CO_2, 85% N_2 using a method other than a candle jar to create these conditions; utilize selective media such as Campy CVA or CCDA on culture plates; and incubate plates at 42^{o} Celsius for at least 48 hours.[7] Given the complexity of *Campylobacter* detection and identification methods and the fact that *Campylobacter* bacteria are particularly sensitive to changes in temperature, humidity and oxygen concentration, differences in *Campylobacter* methods between clinical laboratories in FoodNet sites may explain the geographic variation in rates of culture-confirmed cases.

A survey of the clinical laboratories in nine FoodNet sites was conducted in 2005 to determine the methods used to detect *Campylobacter* in stool, The goal of this

analysis was to examine whether site-to-site variation in clinical laboratory practices could account for the geographic variation observed in rates of culture-confirmed campylobacteriosis in the FoodNet sites.

Methods

FoodNet

The Foodborne Diseases Active Surveillance Network (FoodNet) is a collaborative program between the Centers for Disease Control and Prevention (CDC), U.S. Department of Agriculture's Food Safety and Inspection Service, U.S. Food and Drug Administration, and participating state health departments conducted under the aegis of CDC's Emerging Infectious Diseases Program. In 2004, the FoodNet catchment area comprised 15% of the U.S. population (44.5 million people) and included the entire states of Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon and Tennessee and selected counties in California, Colorado and New York. FoodNet surveillance officers in the FoodNet sites routinely contact clinical laboratories serving the FoodNet surveillance area to ensure that all incident cases of culture-confirmed campylobacteriosis among residents of the FoodNet catchment area are ascertained.[2]

Survey Data

In 2005, surveys were mailed to the lead microbiologist at 589 clinical laboratories in nine of the ten FoodNet sites (all except New Mexico). FoodNet staff in each FoodNet site subsequently contacted survey non-responders to encourage them to

complete the survey. The survey included general questions about laboratory characteristics (e.g., type of laboratory, patient population) and specific questions about the laboratory's 2004 practices for the detection of *Campylobacter*. The lead microbiologists were asked about policies defining which stools should or should not be tested, (e.g., exclusion criteria, such as inpatients hospitalized for > 3 days), specimen transport time and use of transportation media (i.e., Cary-Blair, other, or none), and detection procedures (type of selective media, atmospheric environment, incubation temperature, and incubation time) (see Figure 5.1). Finally, laboratories were asked to report exactly, if possible, or to estimate the number of stool samples submitted for *Campylobacter* testing in 2004 and the number of samples that yielded *Campylobacter.* Completed surveys were returned to FoodNet staff in each site, entered into an Access database, and transferred to CDC for compilation, data cleaning, and analysis.

Statistical Analysis

The median and interquartile range of the *Campylobacter* detection frequency (the number of stools that tested positive for *Campylobacter* at a given laboratory in 2004 divided by the number of stools reportedly tested by that laboratory in 2004) was calculated for each FoodNet site, laboratory characteristic, and laboratory practice. Variables were categorized with cut-points assigned according to the ASM recommended procedure for the detection of *Campylobacter* (see Figure 5.1),[8] where applicable. Use of correct transportation procedures was defined as transport time < 2 hours, regardless of transport media type, or use of transport media (any type) if transport time was > 2 hours. Use of correct storage procedures was defined as storage

of specimens at 4 ºC in transport media or processing a sample immediately. Based on the incidence rates of culture-confirmed campylobacteriosis from 2004, the sites of California, Colorado and Oregon were considered as high incidence sites, Connecticut, Minnesota, and New York New Mexico were considered moderate incidence sites, and Georgia, Maryland and Tennessee were considered low incidence sites.[9]

A Poisson regression model was created to examine the factors associated with the *Campylobacter* detection frequency. Because the *Campylobacter* counts had a greater variance than would be expected under a Poisson distribution, the standard errors of the parameter estimates were scaled for Poisson overdispersion using the square root of the Pearson chi-square statistic divided by the model degrees of freedom.[10] Referent categories for categorical variables were chosen based upon the method recommended by the ASM in 2003 or, if not applicable, the category that provided the most stable parameter estimates. Georgia was chosen as the referent category in the analysis of the FoodNet sites because it had one of the lowest overall detection frequency of the FoodNet sites.

The main exposure of interest was the FoodNet site but all variables were considered candidates for the final multivariate model. Prior to consideration of confounding, interactions between FoodNet site and laboratory characteristics and procedures were also considered and the final model was screened for multicollinearity using a SAS MACRO (Cary, NC).[11] To control for potential confounding by a larger number of stools or more non-*Campylobacter* stool samples tested by the laboratory, categorical variables representing the number of stools tested and the estimated number of *Salmonella* cases ascertained at each laboratory were also considered. The rationale

behind this was that if a laboratory identified a certain number of cases of salmonellosis, it could be assumed that that laboratory's practices were at least sufficient for the identification of *Salmonella* and so that laboratory would also be more likely to isolate *Campylobacter* as well. Additionally, we thought that the more stool specimens a laboratory received, the better the laboratory might be at detecting *Campylobacter* (simply by having more practice doing so). The number of *Salmonella* cases at each laboratory was calculated indirectly by using a laboratory identification number or name to link the laboratory survey data to the *Salmonella* surveillance data for 2004 by laboratory identification number or name.[9] Categories of the number of *Salmonella* cases identified in FoodNet surveillance at a laboratory in 2004 were created by dichotomizing the number of cases at the median ($\leq$5 cases and > 5 cases). Using the laboratory survey data, categories of the number of stool specimens tested in 2004 were created (< 276 stool samples, 276-858 samples, $\geq$859 samples) based upon dividing the data from all eligible laboratories in terciles. Assessment of confounding of the association between FoodNet site and the *Campylobacter* detection frequency was assessed using the method recommended by Kleinbaum et al. (2007) by examining whether removal of a variable from the final multivariate model (containing all variables) meaningfully changed (set *a prior* at $\geq$ 10%) the rate ratio associated with any FoodNet site.[12]

All analyses were conducted using SAS v.9.2 (Cary, NC). All hypothesis tests and reported p-values were two-sided.

Results

Survey Data

Of 584 laboratories surveyed, 534 (91%) returned a completed questionnaire. Of these, 411 (77%) reported testing stools on-site for *Campylobacter,* 102 (19%) sent stools offsite for testing, and 21 (4%) did not test for *Campylobacter* (Table 5.1). These 411 laboratories were responsible for 4,127 (77%) of the 5,343 culture-confirmed cases of *Campylobacter* ascertained by the nine FoodNet sites in 2004 (83% of the 4,949 cases with the laboratory name provided). The *Campylobacter* detection frequency could be calculated for 376 (91%) of the 411 laboratories (i.e., those that reported both the number of stools tested in 2004 and the number of stools that tested positive for *Campylobacter*) (Table 5.1). Of these 376 laboratories, two were excluded because their reported detection frequencies were 100%. There were no significant differences in the reported laboratory characteristics or practices between these 374 laboratories and the 37 that tested for *Campylobacter* onsite but did not provide enough data to calculate the *Campylobacter* detection frequency.

In 2004, these 374 laboratories tested a combined total of 411,473 stool samples and detected *Campylobacter* in 6,199 (1.51%) samples (interquartile range: 0.44% to 1.93%). The number of laboratories per FoodNet site ranged from 18 in Colorado to 69 in Georgia. Most (318; 85%) clinical laboratories were hospital-based, 19 (5%) were government laboratories, and 14 (4%) were commercial reference laboratories. The remaining 23 (6%) laboratories identified themselves as clinic-based or "other" laboratories. *Campylobacter* testing procedures were performed by multiple personnel in 305 (82%) of laboratories (data not shown).

Statistical Analysis

In univariate analysis, the factors that were significantly associated with the *Campylobacter* detection frequency were FoodNet site, type of laboratory, stool testing requirements, method and type of transport media use, the percent of stools transported in transport media, type of selective media used (Campy-BAP versus other), holding plates before placing them in the incubator, method used to create appropriate atmospheric environment, and the number of stools tested in 2004 (Table 5.2).

After adjusting for all other variables, FoodNet site, type of transport media used, and the number of stool specimens tested in 2004 all remained significant predictors of the *Campylobacter* detection frequency (Table 5.3). Consideration of interactions between laboratory procedures and FoodNet site in the model containing all variables of interest was not possible due to multicollinearity (condition indices > 30). After adjusting for the various laboratory characteristics and practices, the *Campylobacter* detection frequency was twice as high in the high and moderate incidence sites such as Minnesota (adjusted rate ratio (aRR): 2.36, 95% CI: 1.56, 3.56), Oregon (aRR: 2.28, 95 % CI: 1.47, 3.56) and Colorado (aRR: 2.21, 95% CI: 1.25, 3.90) and over fifty percent higher in the high incidence site of California (aRR: 1.81, 95 % CI: 1.17, 2.80) and moderate incidence site of Connecticut (aRR: 1.60, 95 % CI: 1.04, 2.47) than in Georgia, a low incidence site. There was no significant difference in *Campylobacter* detection frequencies in New York, a moderate incidence site, and Maryland and Tennessee, both low incidence sites, compared to Georgia.

Despite multicollinearity in a model containing all variables, in a model containing only FoodNet site, type of transport media used, and the number of stools

tested in 2004 (the only three significant variables in the multivariate analyses), it was possible to examine the interaction between FoodNet site and transport media use. In this model, the interaction between FoodNet site and type of transport media used was significant (p=0.002) (Table 5.4). Four sites had significantly higher *Campylobacter* detection frequencies among laboratories that used Cary-Blair transport media as compared to laboratories in that site that did not use transport media: California, a high incidence site, Connecticut and New York, moderate incidence sites, and, Maryland, a low incidence site.

Discussion

We examined the impact of regional differences in clinical laboratory practices on the geographic variation observed in culture-confirmed rates of campylobacteriosis in the FoodNet sites. FoodNet site, the type and use of transport media, and the number of stools tested at a laboratory were the most significant predictors of the 2004 *Campylobacter* detection frequency, after adjusting for laboratory characteristics and practices. FoodNet site was the most important predictor of *Campylobacter* detection frequency, indicating that clinical laboratory practices are not the primary driver of the geographic variation in rates of culture-confirmed *Campylobacter* infections.

In this study, the overall mean *Campylobacter* detection frequency in 2004 was 1.5% among FoodNet clinical laboratories. This rate is similar to those found in previous surveys of FoodNet clinical laboratories (1.4% in 1996 and 1.2% in 1999).[6] The geographic variation in the mean *Campylobacter* detection frequencies for each of the

FoodNet sites closely mirrored the geographic variation observed in reported culture-confirmed *Campylobacter* incidence rates in 2004, with the highest *Campylobacter* detection frequency and incidence rates in California, Colorado, and Oregon, and the lowest in Georgia, Maryland, and Tennessee.[9]

Variation in *Campylobacter* detection rates among clinical laboratories has been observed in other industrialized countries. In a small 2001 survey of 18 clinical laboratories in Europe, the reported *Campylobacter* detection frequency varied by country, ranging from 2.2 to 6.2%.[5] However, differences in laboratory practices did not explain the regional difference observed in rates of campylobacteriosis. Surveys conducted among clinical laboratories in New Zealand showed that, although some changes had occurred in the *Campylobacter* detection practices over time (modifications in the type of selective media used and culturing all stools as opposed to only upon physician request), these differences did not explain the marked increase or the regional variation in rates of culture-confirmed campylobacterisosis.[13]

The use of transport media, specifically Cary-Blair transport media, was found to be associated with the *Campylobacter* detection frequency in multivariate analyses. Laboratories that used Cary-Blair transport media were found to have a *Campylobacter* detection frequency fifty percent higher than those laboratories that did not use transport media, after adjusting for other laboratory practices and characteristics, including FoodNet site. Although the impact of transport media varied by FoodNet site, given that transport of the clinical specimen to the laboratory is the first step in the *Campylobacter* detection process, it is not surprising that this factor was a significant predictor of the *Campylobacter* detection frequency. *Campylobacter* organisms are particularly sensitive

to changes in temperature, humidity and atmosphere.[14] Transport media provides a protective barrier against desiccation and allows *Campylobacter* in the stool specimen to persist longer at room temperature (25^{o} C) and colder temperatures (4^{o} C) than if it were not held in transport media.[15, 16]

The number of stools tested in 2004 was also a significant predictor of the *Campylobacter* detection frequency in our multivariate analyses. Somewhat surprisingly, there was an inverse relationship between the *Campylobacter* detection frequency and the number of stools tested. At least one previous study found that testing more stool specimens increases the likelihood of detecting a bacterial pathogen.[17] However, we have no data to determine if the laboratories that tested more stool samples were more likely to test multiple samples from the same patient. Another possibility is that laboratories that were testing more stools had less stringent guidelines on which stool samples should be tested for *Campylobacter*. For example, these laboratories might be routinely testing all stools for *Campylobacter* rather than excluding specimens from individuals who had been hospitalized for three or more days and are more likely to have nosocomial infections with other pathogens. The American College of Pathologists recommended in 1996 that routine bacterial cultures not be performed on individuals hospitalized for 3 or more days because the likelihood of detecting *Campylobacter* at this point was low.[18]

This study has several limitations. First, the variation in *Campylobacter* detection frequencies could be a result of variation in the number of stools submitted for testing due to non-*Campylobacter* enteric illnesses. In previous stool screening studies, *Campylobacter* has been identified in anywhere from two to nine percent of samples,

and no pathogen was identified in approximately half of all samples.[19-21] By including a variable for the number of culture-confirmed cases of salmonellosis identified at a given laboratory and the total number of stools tested at each laboratory, we hoped to control for potential confounding by a larger number of stools or more non-*Campylobacter* stool samples being tested. Secondly, some clinical laboratories, particularly the large referent and government laboratories serving the FoodNet catchment area also serve populations residing outside the catchment area. Therefore, the number of *Campylobacter* diagnoses reported at a given laboratory will not necessarily correspond to the number reported to FoodNet. Thirdly, laboratory practices in 2004 may not be reflective of practices from 1996-present (the time period over which geographic variation in rates of campylobacteriosis have been observed). However, we do not believe that clinical laboratory techniques changed dramatically during our period of interest. This is supported by the fact that the *Campylobacter* detection frequency has not varied substantially between the laboratory surveys conducted in 1996, 1999 and 2005.

Clinical diagnostic laboratories are an integral part of the public health response to infectious diseases in general. It is essential to understand factors that influence the ability of clinical laboratories to perform their vital functions, such as changes in staffing, outsourcing and centralization of laboratory testing, and changes in laboratory procedures. In general, the reported practices in clinical laboratories that are part of FoodNet are consistent with the procedures recommended by the ASM in 2003 for the detection of *Campylobacter*. Periodic evaluation of clinical laboratory practices will be helpful in the future to determine what effect changes in diagnostic testing are having on disease surveillance.

If variation in clinical laboratory practices is not responsible for the geographic variation observed in the incidence of campylobacteriosis, what is? Differences in the frequency of healthcare use and stool sample submission do not appear to exist (Chapter 4), though these might be expected to affect all enteric pathogens simultaneously. Differences in the population exposure to *Campylobacter* may exist, though they have yet to be identified. The principal risk factors identified for sporadic campylobacteriosis in the United States are related to consumption of poultry, untreated surface water, raw milk, exposure to animals, and foreign travel, and for infants, riding in a shopping cart with packages of meat and poultry.[22-25] A preliminary analysis of FoodNet population survey data did not identify regional variation in exposures that would explain the geographic variation.[26]

In the United States, the striking five-fold geographic variation of *Campylobacter* infections is not explained by differences in laboratory practice. This variation may reflect an unidentified variation in exposure to *Campylobacter* that is not captured by simple risk factor epidemiology, or an as yet unidentified *Campylobacter*-specific difference in health-seeking behavior. Further efforts to characterize determinants of this variation may identify more specific risk determinants and thus pathways to reduce that risk.

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Tables and Figures

Table 5.1. Frequency and rate of testing of stool specimens at clinical laboratories in the 2004 FoodNet *Campylobacter* laboratory survey

	No. of laboratories			No. of specimens		
FoodNet Site	**Surv-eyed**	**Reported testing stools for *Campylo-bacter***	**Reported the no. of specimens tested***	**Tested, median (range)**		**Total**
	No.	No. (%)	No. (%)			
California	26	24 (92%)	24 (100%)	773.5	(10 -- 12,328)	51,724
Colorado	18	18 (100%)	18 (100%)	752	(50 -- 15,500)	34,979
Connecticut	33	32 (97%)	32 (100%)	820	(7 -- 14,755)	42,857
Georgia	90	81 (90%)	73 (90%)	500	(65 -- 26,548)	78,542
Maryland	46	42 (91%)	39 (93%)	825	(10 -- 21,000)	52,818
Minnesota	112	67 (60%)	63 (94%)	241	(18 -- 4,600)	34,835
New York	56	46 (82%)	43 (93%)	495.5	(11 -- 33,863)	77,112
Oregon	41	41 (100%)	38 (93%)	277.5	(57 -- 4,694)	29,365
Tennessee	112	60 (54%)	60 (100%)	509	(4 -- 4,000)	52,105
All Sites	534	411 (77%)	390 (95%)	519	(4 -- 33,863)	454,337

* Reported number of specimens tested for *Campylobacter* in 2004.

Table 5.2. Number of laboratories, *Campylobacter* detection rate median and interquartile range, and Poisson regression model results.

			Campylobacter Detection Frequency		Poisson Regression Model Bivariate		
Characteristic		**N**	**Median**	**IQR**	**RR (95% CI)**		**P**
FoodNet Site	California	23	1.67	0.84 -- 2.75	**2.59**	(1.86, 3.61)	
	Colorado	18	1.62	1.29 -- 2.40	**1.79**	(1.22, 2.61)	
	Connecticut	29	1.05	0.66 -- 2.00	**1.89**	(1.31, 2.72)	
	Georgia	69	0.75	0.33 -- 1.33	**ref**		<0.0001*
	Maryland	38	0.55	0.31 -- 0.88	**1.57**	(1.10, 2.25)	
	Minnesota	62	1.59	0.76 -- 2.38	**2.38**	(1.66, 3.41)	
	New York	41	1.27	0.59 -- 1.67	**1.54**	(1.09, 2.18)	
	Oregon	36	1.92	0.91 -- 2.74	**2.43**	(1.68, 3.52)	
	Tennessee	58	0.72	0.39 -- 1.36	**1.01**	(0.68, 1.51)	
Hospital-based laboratory	Yes	318	1.01	0.45 -- 1.76	**0.61**	(0.52, 0.72)	
	No	56	1.33	0.05 -- 2.20	**ref**		<0.0001*
Test all stools	All stools routinely tested	202	1.09	0.36 -- 2.00	**ref**		0.0030*
	No 3 day inpatient	120	1.02	0.51 -- 1.97	**0.85**	(0.70, 1.03)	
	No other day inpatient	21	0.88	0.35 -- 1.69	**0.75**	(0.51, 1.09)	
	Other	31	0.94	0.49 -- 1.88	**1.32**	(1.06, 1.66)	
Transport time ≤ 2 hours	Yes	156	1.00	0.35 -- 2.00	**0.87**	(0.71, 1.06)	
	Unknown	44	0.86	0.38 -- 1.65	**0.99**	(0.80, 1.23)	
	No	174	1.10	0.57 -- 1.93	**ref**		0.3698
Correctly transport specimens[1]	Yes	323	1.09	0.44 -- 2.00	**1.44**	(1.02, 2.04)	
	No	38	0.98	0.59 -- 1.32	**ref**		0.0303*
	Unknown	13	0.44	0.00 -- 1.11			
Type of	Cary-Blair	211	1.33	0.60 -- 2.30	**1.96**	(1.53, 2.51)	

transport	Other	47	0.89	0.25 -- 1.54	**1.30**	(0.88, 1.91)	
media	None	118	0.73	0.32 -- 1.37	**ref**		<0.0001*
Stools	0-50%	215	0.89	0.35 -- 1.66	**ref**		<0.0001*
transported	51-90%	62	1.42	0.75 -- 2.37	**1.67**	(1.33, 2.08)	
in transport	91-100%	64	1.41	0.44 -- 2.31	**1.75**	(1.44, 2.13)	
media	Unknown	35	0.95	0.67 -- 1.64			
Correctly	Yes	91	1.21	0.46 -- 2.17	**0.85**	(0.67, 1.08)	
store	No	268	1.00	0.44 -- 1.91	**ref**		0.1729
specimens[2]	Unknown	15	0.89	0.00 -- 1.30			
Use	Yes	20	0.67	0.28 -- 1.55	**0.71**	(0.44, 1.15)	
enrichment[3]	No	354	1.05	0.44 -- 1.98	**ref**		0.1376
Use filtration[4]	Yes	5	1.02	0.89 -- 1.30	**0.64**	(0.24, 1.71)	
	No	364	1.02	0.43 -- 1.91	**ref**		0.3408
	Unknown	5	2.46	0.44 -- 2.76			
Use Campy-	Yes	189	0.87	0.39 -- 1.56	**0.72**	(0.61, 0.84)	
Bap selective media	No	174	1.31	0.47 -- 2.30	**ref**		0.0001*
	Unknown	11	1.02	0.36 -- 2.00			
Hold plates	No	279	1.00	0.41 -- 1.88	**0.68**	(0.57, 0.81)	
before	Unknown	52	0.98	0.28 -- 1.76	**0.99**	(0.75, 1.31)	
incubating	Yes	43	1.39	0.84 -- 2.37	**ref**		<0.0001*
Atmospheric	BBL Biobag	84	0.96	0.24 -- 2.00	**ref**		0.0067*
conditions	CFJ						
	BBL Campy Pouch	76	0.85	0.35 -- 1.52	**0.83**	(0.55, 1.23)	
	BBL Campy Pak	83	1.10	0.48 -- 1.98	**1.24**	(0.89, 1.73)	
	Evacuation & Replacement	23	0.85	0.45 -- 1.16	**1.24**	(0.76, 2.02)	
	Mitsubishi CampyGen	29	1.21	0.70 -- 2.26	**1.36**	(0.93, 1.99)	
	Other	68	1.31	0.58 -- 2.12	**1.43**	(1.03, 1.99)	
	Unknown	11	0.98	0.05 -- 1.60			

Incubation temperature	37 degrees Celsius	5	0.67	0.48 -- 0.79	**0.45**	(0.09, 2.34)	
	37 and 42 degrees Celsius	11	1.32	0.60 -- 2.37	**0.92**	(0.37, 2.28)	
	Other	7	1.02	0.05 -- 2.12	**1.14**	(0.55, 2.37)	
	42 degrees Celsius	346	1.02	0.42 -- 1.93	**ref**		0.7109
	Unknown	5	1.59	0.00 -- 3.33			
Last check plates @ 72 hours	Yes	165	1.05	0.44 -- 1.93	**0.99**	(0.84, 1.17)	
	No	203	1.02	0.45 -- 2.00	**ref**		0.9012
	Unknown	6	0.60	0.00 -- 1.33			
Number of stools tested in 2004	< 276 samples	123	1.33	0.00 -- 2.08	**ref**		0.0302 *
	276-<859 samples	128	0.91	0.52 -- 1.82	**0.60**	(0.41, 0.87)	
	> 859 samples	125	1.05	0.46 -- 1.64	**0.72**	(0.51, 1.00)	
***Salmonella* cases 2004**	> 5 cases	179	1.09	0.59 -- 2.00	**1.06**	(0.89, 1.26)	
	≤ 5 cases	197	0.93	0.31 -- 1.88	**ref**		0.5216

IQR=Interquartile Range; RR=Rate Ratio; aRR=adjusted Rate Ratio; CI=Confidence Interval; P=likelihood ratio test p-value; ref=referent value for Poisson model

* $P < 0.05$

[1] Transport time < 2 hours, regardless of transport media use type, or use of transport media if transport time > 2 hours

[2] Storage of specimens at 4 ºC in transport media or processed immediately

[3] Routinely use enrichment broths

[4] Routinely use membrane filtration

Table 5.3. Poisson multivariate regression model results.

		Multivariate**				
Characteristic		**aRR (95% CI)**			**P**	
FoodNet Site	California	**1.81**	(1.17,	2.80)		
	Colorado	**2.21**	(1.25,	3.90)		
	Connecticut	**1.60**	(1.04,	2.47)		
	Georgia	**ref**			<0.0001	*
	Maryland	**1.11**	(0.71,	1.74)		
	Minnesota	**2.36**	(1.56,	3.56)		
	New York	**1.36**	(0.88,	2.11)		
	Oregon	**2.28**	(1.47,	3.56)		
	Tennessee	**0.98**	(0.63,	1.53)		
Type of transport media	Cary-Blair	**1.52**	(1.01,	2.30)		
	Other	**1.05**	(0.63,	1.77)		
	None	**ref**			0.0291	*
Number of stools tested in 2004	< 276 samples	**ref**			0.0157	*
	276-<859 samples	**0.57**	(0.37,	0.86)		
	> 859 samples	**0.52**	(0.34,	0.80)		

aRR=adjusted Rate Ratio (adjusted for all other characteristics listed in Table 5.2); CI=Confidence Interval; P=likelihood ratio test p-value; ref=referent value for Poisson model

* $P < 0.05$

** N=324 (50 observations excluded due to missing values)

Table 5.4. Poisson interaction model including FoodNet site and transport media use type, adjusted for number of stools tested in 2004.

FoodNet site and transport media type	Rate Ratio (95% CI)		P	
California				
No Trans Media	**Referent**			
Cary-Blair	**5.33**	(1.94, 14.63)	0.0012	*
Other transport media	**6.11**	(0.95, 39.30)	0.0567	
Colorado				
No Trans Media	**Referent**			
Cary-Blair	**1.10**	(0.46, 2.65)	0.8310	
Other transport media	**1.17**	(0.18, 7.38)	0.8687	
Connecticut				
No Trans Media	**Referent**			
Cary-Blair	**1.85**	(1.08, 3.15)	0.0246	*
Other transport media	**1.45**	(0.64, 3.26)	0.3694	
Georgia				
No Trans Media	**Referent**			
Cary-Blair	**0.97**	(0.56, 1.67)	0.9096	
Other transport media	**0.77**	(0.37, 1.62)	0.4929	
Maryland				
No Trans Media	**Referent**			
Cary-Blair	**3.74**	(2.10, 6.65)	<.0001	*
Other transport media	**0.83**	(0.18, 3.84)	0.8088	
Minnesota				
No Trans Media	**Referent**			
Cary-Blair	**0.69**	(0.41, 1.15)	0.1555	
Other transport media	**0.52**	(0.18, 1.49)	0.2266	
New York^				
No Trans Media	**Referent**			
Cary-Blair	**2.72**	(1.16, 6.39)	0.0211	*

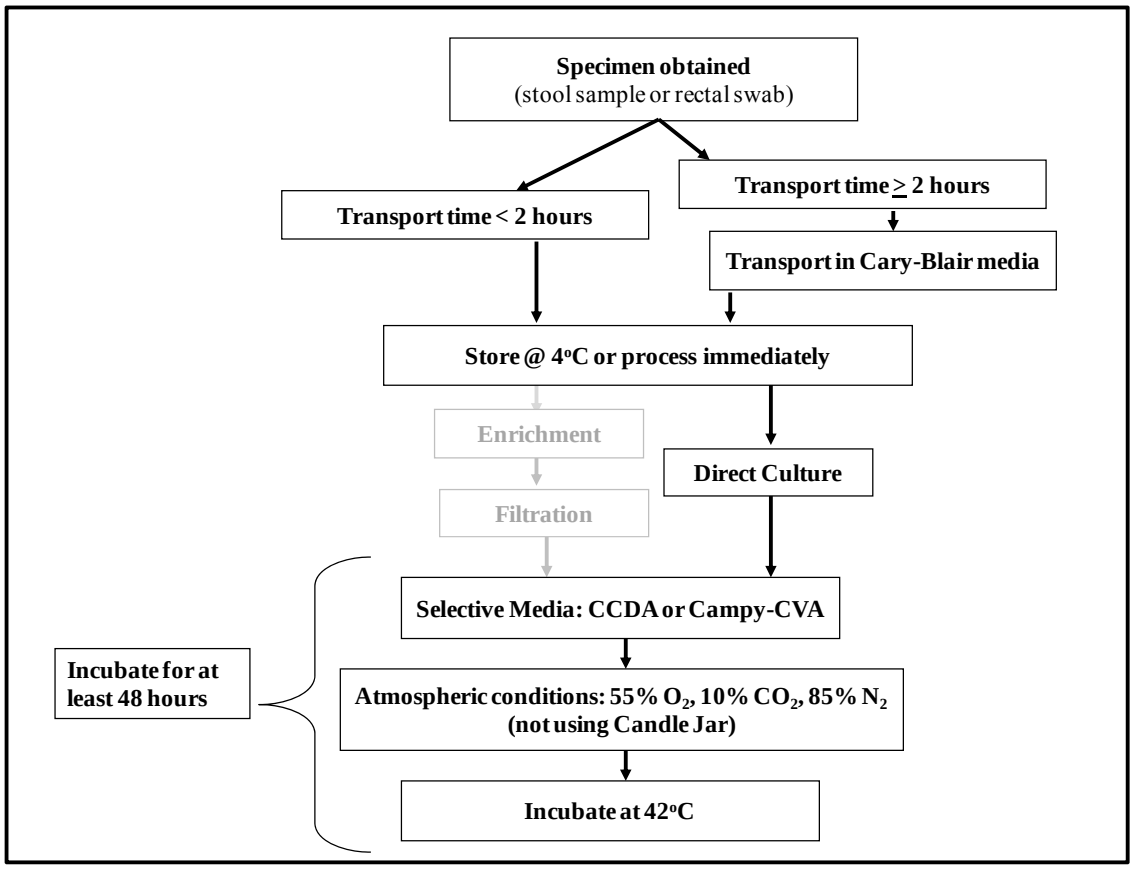
Oregon			
No Trans Media	**Referent**		
Cary-Blair	**2.41**	(0.58, 9.91)	0.2238
Other transport media	**2.05**	(0.47, 8.90)	0.3362
Tennessee			
No Trans Media	**Referent**		
Cary-Blair	**1.31**	(0.75, 2.30)	0.3387
Other transport media	**1.18**	(0.55, 2.56)	0.6690

Adjusted for category of number of stools tested in 2004

* $P < 0.05$

^ Not possible to estimate the effect of other transport media due only one response

Figure 5.1. ASM recommended procedures for the detection of *Campylobacter* from stool. Items in gray have not been recommended by the ASM for routine use.



Appendix 5A. Additional Analyses

Frequency of laboratory characteristics by FoodNet site

The frequency of the various laboratory characteristics and procedures by FoodNet site was also performed. In order to determine if there was any significant difference in the proportion of laboratories from each FoodNet site that fell into the various categories, an exact test was used because most factors under consideration had counts of < 5 for at least one FoodNet site. A Monte Carlo simulation procedure (the MC option in the PROC FREQ procedure in SAS v. 9.2) of Fisher's exact test p-values was used to estimate exact p-values for these comparisons because Fisher's exact test was too computationally intensive.[‡]

There were significant differences between the proportion of laboratories that routinely tested all stools as compared to those that exclude stools from individuals hospitalized for three or more days or for other reasons (p-value 0.012) (Table 5A.1). The proportion of laboratories that tested all stools ranged from 31% in Connecticut to 67% in Oregon. Significant site-to-site variation was also observed in the proportion of laboratories that routinely used transport media (p-value: 0.0026). The proportion of laboratories that used transport media ranged from 45% in Maryland to 86% in Oregon. There was also significant variation in the proportion of stools that were transported in transport media (p-value: <0.001). The proportion of laboratories that reported transporting less than 50% of stool samples in transport media ranged from 39% in

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[‡] Agresti A. A Survey of Exact Inference for Contingency Tables. Statistical Science 1992;7(1):131-53.

Minnesota to 85% in Tennessee. The proportion of laboratories that reported holding plates before incubating also varied significantly by FoodNet site (p-value <0.001) and ranged from 0% in Oregon to 6% in California. The use of filtration (passing the stool suspension through a filter to remove non-*Campylobacter* organisms) also varied by FoodNet site (p-value: 0.0246) and, although not reported by most sites, was used by two percent, four percent, and 11% of laboratories in Tennessee, New York and Colorado, respectively. Use of Campy-BAP selective media showed site-to-site variation (p-value: <0.001) and ranged from 6% in Oregon to 68% in Maryland. Sites varied in their use of BBL Biobag CFJ to create an appropriate microaerobic environment for *Campylobacter* as well (p-value: <0.001). Only four percent of laboratories in California used this method compared to 31% of laboratories in Georgia. There was variation by site in the proportion of laboratories that last read plates for *Campylobacter* at 72 hours (p-value: 0.0053), ranging from 22% in New York to 65% in California. FoodNet sites also showed variation in the two variables created to help potentially control for unmeasured variables. The proportion of laboratories that tested less than 276 samples during 2004 varied significantly by site (p-value: <0.001) and ranged from 11% in both Colorado and Maryland to 55% in Minnesota. The proportion of laboratories that detected greater than 5 cases of *Salmonella* in FoodNet surveillance also varied significantly (p-value: <0.001), ranging from 31% in Connecticut to 72% in Oregon. No other significant differences in the frequency of laboratory characteristics or procedures by FoodNet site was observed.

Laboratory profile analyses

As a sub-analysis, a composite variable based upon the recommended procedures used to detect *Campylobacter* was also created. This variable was a tally of the number of the suggested procedures a laboratory used to detect *Campylobacter*, based upon five recommended by the ASM (2003).[§] A laboratory was given one point for each of the following that the laboratory reported performing: use of correct transportation procedure (transport time less than 2 hours, regardless of transport media use type, or use transport media if transport > 2 hours), use of correct storage procedure (store @ 4 ºC in transport media or process immediately), use of suggested selective media (Campy CVA alone), use of suggested incubation temperature (incubate plates at 42 ºC), and use of suggested incubation time (incubate plates for at least 72 hours before last read of plate). Laboratories that performed all of these five procedures as suggested by the ASM received a value of five; those that performed four out of the five correctly received a four, etc… If the information on a procedure was missing for a laboratory, it was classified as not performing the specific procedure. A Poisson regression model accounting for overdispersion was created to determine whether there was any difference in the *Campylobacter* detection rate between the levels of the composite variable. To test whether there was a significant linear trend in categories of the profile variable, a Poisson regression model was developed using an ordinal version of the profile variable.

[§] Nachamkin I. *Campylobacter* and *Arcobacter*. In: Murray PR, ed. Manual of Clinical Microbiology. 8th ed: American Society for Microbiology; 2003:902-14.

In the laboratory profile sub-analysis 11 (2.9%) of the 372 laboratories used all five procedures recommended by the ASM, 79 (21.1%) used four, 153 (40.9%) used three, 108 (28.9) used two, 20 (5.4%) used one, and 3 (0.8%) used none. The association between the categorical laboratory profile variable and the *Campylobacter* detection rate was significant (p-value=0.0298). As laboratories used fewer of the ASM-recommended procedures, the *Campylobacter* detection rate decreased (p-value for trend: 0.0081) (Table 5A.2, Figure 5A.1). However, when FoodNet site was included in the model along with the profile variable, the only significant predictor of the *Campylobacter* detection rate was the FoodNet site.

To determine whether the use of correct transport procedures was the main driver of the significance of the profile variable, a modified profile variable was created that excluded information about the correct transport of the specimen. When this variable alone was included in the Poisson model, it was not significant ($p<0.05$). When it was included in a model with a variable for the correct transport of the specimen, only the correct transport variable was significant ($p=.0058$). In order to determine if these was effect modification of the modified profile variable by the use of correct transport procedures, a model that also included the interaction between the modified profile variable and the correct transport variable was created. No significant interaction ($p<0.05$) was found.

Tables and Figures

Table 5A.1 Frequency of laboratory practices and characteristics by FoodNet site

	CA N(%)	CO N(%)	CT N(%)	GA N(%)	MD N(%)	MN N(%)	NY N(%)	OR N(%)	TN N(%)	P *
Test all stools	15(65)	7(39)	9(31)	43(62)	13(34)	36(58)	24(59)	24(67)	31(53)	*
Hospital-based lab	18(78)	14(78)	26(90)	61(88)	31(82)	52(84)	36(88)	26(72)	54(93)	
Transport time ≤ 2 hours	8(42)	7(44)	11(42)	25(42)	22(61)	25(46)	12(33)	14(45)	32(60)	
Use transport media	18(78)	14(78)	21(72)	42(61)	17(45)	47(76)	32(78)	31(86)	35(60)	*
Stools in transport media (<50)	11(50)	11(69)	19(76)	46(74)	30(81)	23(39)	16(42)	17(53)	41(85)	*
Correctly transport specimens	21(95)	15(88)	26(90)	53(83)	29(81)	56(92)	37(90)	33(94)	53(95)	
Correctly store specimens	3(14)	8(47)	6(21)	20(30)	9(25)	16(27)	5(12)	9(26)	15(28)	
Hold plates	6(29)	5(29)	11(39)	3(6)	3(9)	7(15)	5(13)	0(0)	3(6)	*
Enrichment	0(0)	1(6)	1(3)	6(9)	2(5)	3(5)	3(7)	1(3)	3(5)	
Filtration	0(0)	2(11)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)	2(4)	*
Campy-Bap media	3(13)	2(12)	15(54)	38(58)	26(68)	41(67)	26(63)	2(6)	36(67)	*
BBL Biobag CFJ	1(4)	2(13)	1(3)	21(31)	5(14)	28(47)	4(10)	8(23)	14(25)	*
Incubate 42°C	23(100)	17(94)	26(90)	65(94)	35(92)	54(93)	38(93)	34(94)	54(95)	
Read plates @ 72 hours	15(65)	13(72)	16(55)	30(45)	14(38)	29(48)	9(22)	17(49)	22(39)	*
< 276 stools	4(17)	2(11)	4(14)	23(33)	4(11)	34(55)	12(29)	17(47)	21(36)	*
***Salmonella* cases (> 5)**	10(43)	7(39)	9(31)	22(32)	17(45)	44(71)	26(63)	26(72)	35(60)	*

* Monte Carlo simulation exact $P < 0.05$

Table 5A.2 Frequency of laboratory profiles and Poisson model results showing the association of the laboratory profile with the Campylobacter detection rate. The "Profile" column shows the total number of "correct" procedures used by the laboratory based on the ASM (2000) guidelines.

Profile*	N (%)	Rate Ratio (95% Confidence Interval)	P
5 (all) correct	11 (3)	**ref**	
4 vs 5 correct	79 (21)	**1.30** (0.78, 2.16)	
3 vs 5 correct	153 (41)	**1.06** (0.65, 1.75)	0.0298
2 vs 5 correct	108 (29)	**1.02** (0.61, 1.70)	
1 vs 5 correct	20 (5)	**0.73** (0.36, 1.49)	
0 vs 5 correct	3 (1)	**0.16** (0.01, 2.87)	

** Profiles are based on the number of laboratory procedures done correctly, based on ASM (2003) recommended procedures*:

Correct transportation procedure: Transport time less than 2 hours (regardless of transport media use type) or use transport media if transport > 2 hours

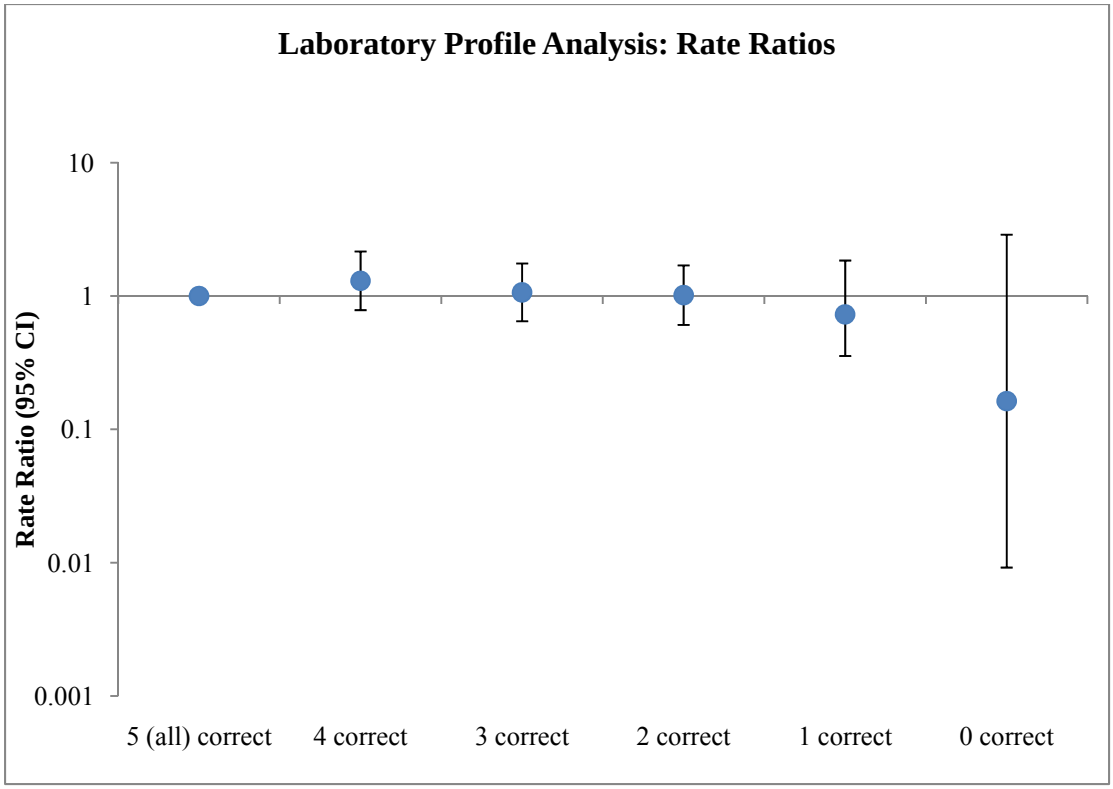
Correct storage procedure: Store @ 4 degrees Celsius in transport media or process immediately

Use suggested selective media: Campy-CVA alone

Use suggested incubation temperature: Incubate plates at 42 degrees Celsius

Use suggested incubation time: Incubate plates for at least 72 hours before last read of plate

Figure 5A.1 Rate Ratios and associated 95% confidence intervals of laboratory profiles. The "Profile" column shows the total number of "correct" procedures used by the laboratory based on the ASM (2003) guidelines.*



** Profiles are based on the number of laboratory procedures done correctly, based on ASM (2003) recommended procedures*:

Correct transportation procedure: Transport time less than 2 hours (regardless of transport media use type) or use transport media if transport > 2 hours

Correct storage procedure: Store @ 4 degrees Celsius in transport media or process immediately

Use suggested selective media: Campy-CVA alone

Use suggested incubation temperature: Incubate plates at 42 degrees Celsius

Use suggested incubation time: Incubate plates for at least 72 hours before last read of plate

Appendix 5B. Rationale for Use of Salmonella Data

Isolating the Impact of Laboratory Practices

The goal of the laboratory survey analysis was to determine whether differences in clinical laboratory detection methods could explain the geographic variation observed the rates of culture-confirmed campylobacteriosis. However, differences in the *Campylobacter* detection rate (the proportion of stools that tested positive for *Campylobacter* at a laboratory in 2004) could be due to factors unrelated to *Campylobacter* (i.e., more stool samples from non-*Campylobacter* infected individuals at one laboratory compared to another). Therefore, the method proposed to isolate any differences in the *Campylobacter* detection rate to those related to laboratory practices was to involve the *Salmonella* surveillance data. Because the detection of *Salmonella* from a stool sample is simpler than the detection of *Campylobacter*, the *Salmonella* detection rate was used as a proxy of laboratory competence. Geographic areas with a stable and similar *Salmonella* incidence may have a similar true *Campylobacter* incidence as well. Furthermore, it was hypothesized that any differences in the *Campylobacter* detection rate among laboratories within the same *Salmonella*-defined area may be due to differences in laboratory methods.

Using the FoodNet active surveillance data for *Salmonella* from 2004, the number of *Salmonella* cases identified in FoodNet surveillance from each laboratory was calculated. To determine whether this approach was feasible, the 23 laboratories that responded to the survey in California were first examined. Overall, there was significant heterogeneity in the *Salmonella* detection rate (number of cases of salmonellosis

captured in FoodNet surveillance in 2004 from a laboratory divided by number of stools tested) even within the three-county FoodNet catchment area of California. The California data was further subdivided based upon the counties that laboratories each served: Alameda alone, San Francisco alone, Contra Costa alone, or any combination of the three (e.g., Contra Costa and Alameda but not San Francisco, etc…). Within the groupings of counties there still remained significant variation in the *Salmonella* detection rate.

Since the creation of geographic categories based on *Salmonella* rates was difficult and the *Salmonella* detection rates were not homogenous even within these categories in the three-county area of California, we examined whether adjusting for the number of cases of salmonellosis identified at a given laboratory and categories of the number of stool specimens tested could be utilized. If a laboratory had identified a certain number of cases of salmonellosis, it could be assumed that that laboratory's practices were at least sufficient for the identification of *Salmonella* and so perhaps that laboratory would be more likely to be better at *Campylobacter* detection as well. Additionally, perhaps the more stool specimens a laboratory received, the better the laboratory was at detecting *Salmonella* (simply by having more practice doing so).

In order to determine if this approach was plausible, we conducted a comparison of the average annual rates of salmenollosis and campylobacteriosis in the FoodNet sites (Table 5B.1, Figure 5B.1). No laboratory survey data was used for this analysis. Instead, using *Campylobacter* and *Salmonella* active surveillance data from 1996-2006 and annual census population estimates, the annual rate of salmonellosis and campylobacteriosis by FoodNet site and county was calculated. The average annual rate

of these infections by county was then calculated and Pearson correlation coefficient (and two-sided p-values) between the rate of salmonellosis and campylobacteriosis were calculated. Scatter plots of the relationship between the average annual rates of campylobacteriosis and salmonellosis were also created. For the FoodNet sites of California, Colorado, Connecticut, New Mexico and Tennessee there was no clear relationship between the rates of salmonellosis and campylobacteriosis. For the sites of Georgia, Maryland, Minnesota, New York and Oregon, however, there was significant ($P < 0.05$) positive correlation. This suggests that our assumption that counties with higher rates of salmonellosis also have higher rates of campylobacteriosis is plausible. Therefore, laboratories that are better at detecting *Salmonella* may also be better at detecting *Campylobacter*.

Tables and Figures

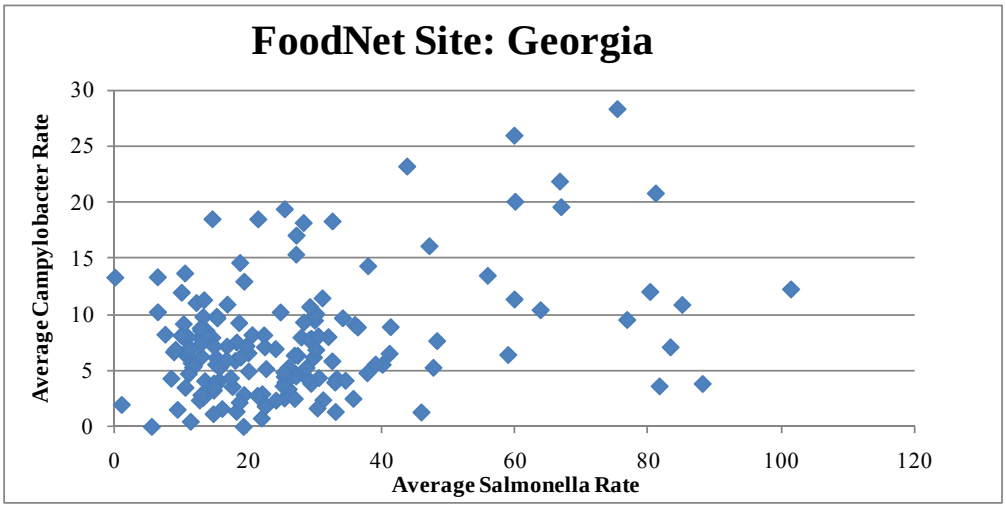
Table 5B.1 Average rates of campylobacteriosis and salmonellosis by FoodNet site and correlation between campylobacteriosis and salmonellosis rates by county.

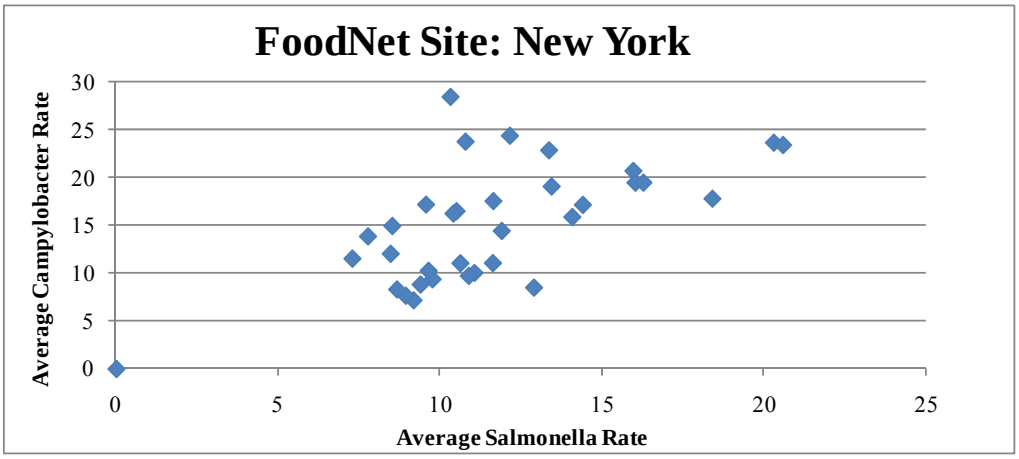
			Annual Rate* per 100,000 persons				Pearson
Site	Pathogen	N (counties)	Mean	Standard Deviation	Minimum	Maximum	correlation coefficient
CA	*Campylobacter*	3	35.19	16.65	23.42	54.24	0.94225
	Salmonella	3	15.22	3.87	11.46	19.20	
CO	*Campylobacter*	7	17.48	4.60	14.48	27.48	0.55835
	Salmonella	7	13.42	1.63	11.69	15.97	
CT	*Campylobacter*	8	16.18	2.42	13.87	21.29	0.17577
	Salmonella	8	13.32	2.31	8.65	16.00	
GA	*Campylobacter*	159	7.69	5.23	0.00	28.34	0.36068*
	Salmonella	159	27.16	19.10	0.00	101.41	
MD	*Campylobacter*	24	7.56	3.61	1.86	17.88	0.47141*
	Salmonella	24	19.23	10.26	9.42	54.69	
MN	*Campylobacter*	87	19.69	9.79	1.64	45.68	0.68786*
	Salmonella	87	11.62	4.78	2.13	25.81	
NM	*Campylobacter*	33	17.91	17.74	0.00	74.09	0.32937
	Salmonella	33	13.42	9.00	0.00	45.23	
NY	*Campylobacter*	34	15.02	6.24	0.00	28.33	0.65244*
	Salmonella	34	11.60	3.96	0.00	20.57	
OR	*Campylobacter*	36	18.72	7.61	4.89	41.82	0.4657*
	Salmonella	36	8.52	3.36	0.00	14.94	
TN	*Campylobacter*	95	7.47	4.51	0.00	20.51	0.13409
	Salmonella	95	12.95	5.54	0.00	29.78	

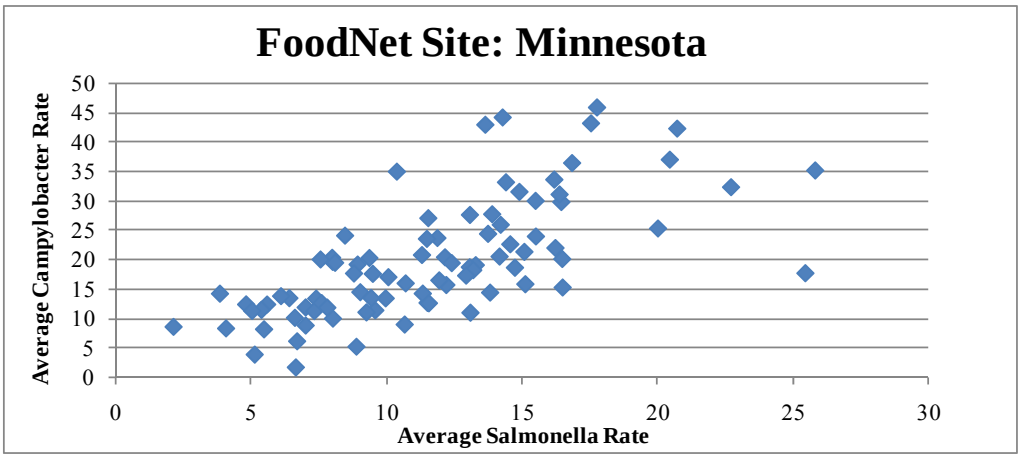
*1996-2006 (CA, CT, GA, MN, OR), 1998-2006 (MD, NY), 2000-2006 (TN), 2001-2006 (CO), 2004-2006 (NM)

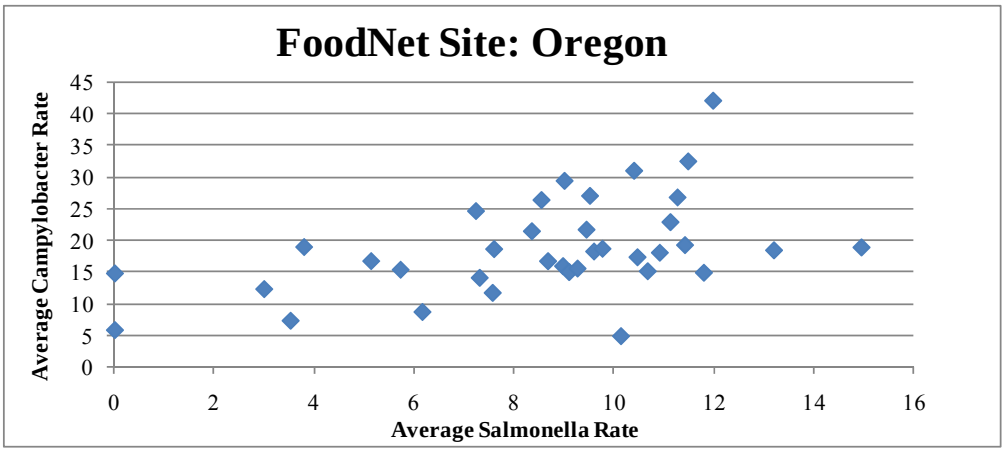
* $P < .05$

Figure 5B.1. Scatter plots of average rates of campylobacteriosis by average rates of salmonellosis for each county, select FoodNet sites 1996-2006.








FoodNet Site: Oregon
Average Campylobacter Rate
45
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
Average Salmonella Rate

CHAPTER 6. WHAT IS THE ASSOCIATION BETWEEN RAINFALL AND TEMPERATURE AND *CAMPYLOBACTER* INFECTIONS IN THE FOODNET SITES FROM 1996-2006?

Abstract

In the United States, seasonal variation in rates of *Campylobacter* infections has been consistently observed, with the highest incidence occurring in the summer months. As *Campylobacter* is not thought to multiply readily outside a human or animal host, reasons for this seasonal variation are unclear although some studies have suggested a role for climatic factors such as temperature and precipitation. We used active surveillance data from the Foodborne Diseases Active Surveillance Network and meteorological data to assess the relationship between climatic factors and culture-confirmed infections with *Campylobacter*. Using a case-crossover study design, we found a modest, but significant, and independent positive association between both minimum temperature and precipitation and an increased odds of campylobacteriosis. The odds of campylobacteriosis increased by six percent for every ten degree (Fahrenheit) increase in the weighted average of minimum temperature three to twelve days prior to specimen collection (adjusted odds ratio of 1.060 and 95% confidence interval of 1.020, 1.090) and the odds of campylobacteriosis were eight percent higher during periods of extreme precipitation compared to those without extreme precipitation (adjusted odds ratio of 1.081 and 95% confidence interval of 1.018, 1.0147). We found no evidence of effect modification of these effects by geographic location or age. Future studies are needed, however, to further elucidate the pathway between these meteorological factors and campylobacteriosis.

Introduction

The incidence of culture-confirmed *Campylobacter* infections in the United States (U.S.) has shown consistent seasonal fluctuations, with a higher incidence often observed during summer months.[1-3] Campylobacteriosis is not alone in its seasonal periodicity; many other infectious diseases, including those caused by other enteric (e.g., salmonellosis and cholera) and respiratory (e.g., legionellosis, measles) microorganisms, also exhibit seasonal patterns.[4] Mechanisms leading to this seasonal variation are often multifaceted and complex and may include: changes in environmental conditions that favor the growth and spread of pathogenic microorganisms (e.g., changing precipitation

or temperature); and changes in human behavior (e.g., congregation of school children during the beginning of the school year, or increased outdoor recreational activities during warmer summer temperatures).[4, 5]

The seasonality of *Campylobacter* closely mirrors general fluctuations in temperature with the highest incidence of infection during the warmer months of the year. However, *Campylobacter*, unlike many other foodborne bacteria, do not multiply outside a human or animal host.[6] This suggests that the impact of climatic factors is indirect. A number of identified risk factors for campylobacteriosis, such as eating meat from a barbecue,[7, 8] and swimming in natural water sources,[9] are more likely to occur in the warm summer months. The occurrence and concentration of *Campylobacter* in poultry, livestock and water sources has also been shown to change throughout the year,[10, 11] leading some investigators to suggest that this influences the seasonality in human cases.[11-15] The presence of suspected vectors of *Campylobacter* transmission, such as flies and other insects, are also varies by season.[16-19]

Precipitation may also play a role in the seasonality of campylobacteriosis as above-average periods of precipitation have been linked to waterborne disease outbreaks,[20] including *Campylobacter*-associated outbreaks.[21] Extreme precipitation events are thought to overwhelm water and wastewater treatment plans, allowing pathogenic microorganisms to enter the drinking water supply and leading to increased water turbidity levels. The efficacy of typical disinfection procedures designed to inactivate *Campylobacter* in drinking water is decreased with increasing turbidity levels.[22] Previous studies have found lags of one to three weeks between increased water turbidity at treatment plants and increased rates of gastroenteritis.[23-25] During a

waterborne outbreak of campylobacteriosis in Canada, heavy rainfall occurred approximately one week before the peak in cases.[21] A study of waterborne diseases outbreaks in the U.S. from 1948-1994 found that over half of outbreaks were proceeded by "extreme" precipitation in the month prior to the outbreak.[20]

While studies have been conducted in a number of countries to better understand these associations,[26, 27] few studies have yet been conducted in North America.[28, 29] An examination and quantification of the relationship between climatic factors and *Campylobacter* incidence is in the U.S. is needed. The purpose of this study is to examine seasonal patterns in campylobacter rates and whether climatic factors, such as rainfall and temperature, are associated with an increased risk of campylobacteriosis.

Methods

Data Sources

Foodborne Diseases Active Surveillance Network (FoodNet). Active, population-based surveillance data on culture-confirmed *Campylobacter* infections occurring between 1996 and 2006 was obtained from FoodNet. When first established in 1996, the FoodNet surveillance area (hereafter referred to as the "FoodNet sites") was comprised of five sites: select counties in California, Connecticut, and Georgia and the entire states of Minnesota and Oregon (14.2 million persons, five percent of the U.S. population). By 2006, the FoodNet surveillance are had increased to included 10 sites: select counties in California, Colorado, and New York and the states of Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon and Tennessee (44.9 million persons, 15.2% of the U.S. population) and encompassed 486 counties.

Personnel at each FoodNet site have established procedures for contacting all the clinical laboratories (> 650 as of 2006) serving their respective surveillance areas at least monthly to collect information on culture-confirmed cases of *Campylobacter*. All records of culture-confirmed *Campylobacter* infection, including information on specimen collection date, are sent to a central database at CDC. Demographic information including age, gender, race/ethnicity, and county of residence are collected.

National Climatic Data Center. Daily weather station-level precipitation and temperature (degrees Fahrenheit) data was obtained from the National Climatic Data Center (NCDC) of the National Oceanic and Atmospheric Administration (NOAA) for all counties within the FoodNet surveillance area (486 counties as of 2006) that had at least one weather station. This database includes information on the minimum, maximum, and mean temperature and the amount of precipitation that fell within a 24-hour period for every weather station within the FoodNet surveillance area from 1996 to 2006. These data were then aggregated by day and county. In the event that a county had more than one weather station, the data was averaged across weather stations.

Data Analysis

We described the seasonal variation of culture-confirmed *Campylobacter* infections in FoodNet from 1996-2006 and examined whether the seasonal pattern was similar across the FoodNet sites and age groups. In order to calculate rates of campylobacteriosis, annual census estimates were obtained (U.S. Census Bureau 2006). The week during which each case occurred was calculated by including the first seven days in a year as week one, days eight to 14 as week two and so on. A main-effect, log-

linear negative binomial regression model was used to estimate the weekly rates of infection, adjusted for changing surveillance area and calendar year.[30] To determine whether changes in rates were similar across various factors, the analysis was stratified by FoodNet site and age group. Age groups were defined as follows: <1 year, 1-4 years, 5-14 years, 15-24 years, 25-34, 35-54 years, 55-74 years, and ≥75 years.

The association between meteorological factors, precipitation and minimum temperature, and the occurrence of a culture-confirmed case of campylobacteriosis was examined using a case-crossover study design. Case-crossover studies are similar to case-control studies but each case serves as his or her own control. This type of study design has been used in other studies of the relationship between meteorological factors and infectious diseases.[31] The date of specimen collection for all culture-confirmed cases of campylobacteriosis in FoodNet surveillance was each considered a "case day". Each case day was matched to two "control days" which represented the case's *Campylobacter*-free days during the same county, year, week group, and day of the week as the case. Week groups, or "strata", were calculated by dividing each calendar year into 17 three-week long segments, beginning with the first day of the year (the 17th segment contained 29-30 days). Control day selection was bi-directional in that, within strata, case days could be matched to control days that fell either before, after, or both before and after, the date of the case. For example, a case that fell on the Monday in the 5th week of the year could then be matched to a control day that was the 4th Monday of the year or the 6th Monday of the year. Similarly, a case that fell on a Tuesday during the first week of the year could be matched to a control day that was the Tuesday in the 2nd week of the year and a Tuesday that was during the 3rd week of the year. Matching on

these characteristics controlled for a number of factors: annual and seasonal trends in campylobacteriosis, day-of-the-week effects, geographic variation in *Campylobacter* incidence, and long-term effects of meteorological variables.

A conditional logistic regression model that accounted for the matching between the case and control days was employed to examine the effect of temperature and precipitation. In order to mitigate against multiple testing, climatic factor exposure periods of interest were defined *a priori*. For the temperature analysis, the effect of minimum temperature at the estimated time of exposure to *Campylobacter* was chosen based upon the assumption of a possible threshold effect of temperature. Based on the data from a case-control study of risk factors for campylobacteriosis in the FoodNet sites,[2] the median length of time from onset of symptoms to specimen collection was 3 days (interquartile range: 2-5 days). The reported incubation period of campylobacteriosis is three days (range: one to seven days).[32] Thus, plausible lag periods between exposure to *Campylobacter* and specimen collection date range from three to 12 days, with the most likely exposure occurring six days prior to specimen collection date. A weighted average of minimum temperature variable was created with weights half the weight assigned to the most likely day of exposure (day six) assigned to the extreme values (days three, 11, and 12) (see Table 6.1).

For the precipitation analysis, the average precipitation 7-14 days prior to specimen collection date (each day given equal weight) was examined, in order to allow sufficient time for *Campylobacter* to travel into the drinking water supply. Both temperature and precipitation categories were created by dividing the distribution into

percentiles. Extreme precipitation was defined as >95th percentile and compared to periods of non-extreme precipitation ($\leq$ 95th percentile).

The relationship between climatic factors and campylobacteriosis was modeled in two ways: linearly and categorically. In order to more fully control for seasonality not related to temperature or precipitation, adjusted models also included terms for the day of year (1 through 365; in a leap year, February 29th was assigned the same day of the year as February 28th), day of year squared, and day of year cubed. Additionally, terms for the interaction between FoodNet site and the day of year effects, to account for differences in seasonality by FoodNet site, were included. The final multivariate model was constructed by examining the effects of those found significant in earlier analyses as well as interactions between climatic factors (temperature, precipitation) and demographic factors (FoodNet site and age group).

As an exploratory analysis to check the model specification, a model using distributed lags of minimum temperature and precipitation were considered. Distributed lag models include measurements of the exposure of interest at multiple time points and allow one to consider the effect of an exposure at one time point adjusted for the effect at other time points. The distributed lag models included minimum temperature and/or precipitation at various time points, ranging from twelve days *before* the specimen collection date to one day *after* the specimen collection date (i.e., they included the time period plausibly associated with campylobacteriosis in the primary analysis). Presumably, temperature or precipitation on the day of and day after specimen collection are not plausibly associated with the campylobacteriosis and thus may indicate that the model was specified incorrectly. As in the primary analyses, a conditional logistic

regression model accounting for the matching between the case day and its two control days was employed to examine the effect of the temperature and precipitation and analyses were adjusted for day of year effects (day of year, day of year^2, and day of year^3) as well as the interaction between FoodNet site and day of year effects. In addition to the climatic factor that was the exposure of interest (e.g., temperature, precipitation), multivariate (adjusted) models also included the climatic factor that was not the exposure of interest in a specific analysis (e.g., precipitation, temperature).All p-values were two sided and the significance level was 0.05. All analyses were conducted in SAS v9.2 (Cary, NC).

Results

Rates of culture-confirmed campylobacteriosis closely mirrored the overall trend in temperature in most FoodNet sites, with the exception of California (Figure 6.1). The peak in campylobacteriosis in summer months occurred during periods of minimal precipitation in the California and Oregon, whereas both precipitation and rates of campylobacteriosis peaked in the summer in Minnesota, and precipitation was distributed evenly throughout the year in the remaining FoodNet sites (Figure 6.2). Compared to other age groups, the largest seasonal peaks appeared in those aged 5-<15 years of age and in those ≥ 75 years (Figure 6.3).

From 1996-2006, a total of 52,124 culture-confirmed cases of *Campylobacter* infection were ascertained; daily temperature and precipitation data from NCDC were available for 45,556 (87%) and 48,072 (92%) case days, respectively. Of the 104,248

control days (two for every case day), daily temperature and precipitation data from NCDC were available for and 91,118 (87%) and 96,165 (92%), respectively. A significant positive relationship between increasing categories of minimum temperature (degrees Fahrenheit) and risk of campylobacteriosis during the three to twelve days prior to specimen collection was observed (Table 6.2), after matching on state, county, day of week, week strata, and year, and adjusting for day of year effects and their interactions with FoodNet site. No linear relationship was observed between an increased risk of campylobacteriosis and increasing categories of precipitation; however, there was a modest but significant effect of extreme precipitation (>95th percentile). If the average precipitation was greater than the 95th percentile during the seven to fourteen days prior to specimen collection, the odds of campylobacteriosis increased by approximately seven percent (adjusted odds ratio [OR]: 1.066, 95% confidence interval [CI]: 1.007, 1.128).

Because analyses using nominal categorical variables for the levels of minimum temperature were supportive of a linear relationship, a continuous variable for minimum temperature was used in further analyses. For every ten degree increase in minimum temperature (Fahrenheit), the odds of campylobacteriosis increased by six percent (aOR: 1.060, 95% CI: 1.030, 1.090), after adjusting for day of year effects (Table 6.3). Similar associations were observed with weighted maximum temperature and weighted mean temperature (Table 6.3).

The final multivariate model included the main effects of minimum temperature (continuous) and extreme precipitation (>95th percentile) (Table 6.4). Interactions between these two climatic factors and FoodNet site and age group were considered

using a likelihood ratio test and were not significant. In this model, the odds of campylobacteriosis increased by six percent for every ten degree (Fahrenheit) increase in the weighted average of minimum temperature three to twelve days prior to specimen collection (aOR: 1.060, 95% 1.020, 1.090) and the odds of campylobacteriosis were eight percent higher during periods of extreme precipitation compared to those without extreme precipitation (aOR: 1.081, 95% 1.018, 1.0147), after adjusting for day of year effects, the interaction between the day of year effects and FoodNet site.

When all time points were considered simultaneously, the mean minimum temperature the day before specimen collection was the strongest and only significant predictor of campylobacteriosis (Table 6A.1). This association persisted after adjusting for day of year effects and the occurrence of extreme precipitation (>95th percentile of rainfall) events on the day of interest. Model results were similar when mean precipitation was used instead of extreme precipitation (data not shown). However, the association between the minimum temperature on the day after the specimen collection date was restricted to one FoodNet site. When the minimum temperature distributed lag analysis was stratified by FoodNet site, the strongest association between minimum temperature in the day after specimen collection and campylobacteriosis occurred in Colorado, whereas in other sites this association was not observed (Table 6A.2). When extreme precipitation was considered as the primary exposure of interest, although none of the lagged precipitation measurements were significantly associated with campylobacteriosis, the strongest association was observed at four and five days prior to the specimen collection date in the unadjusted analysis. After adjusting for day of year effects and minimum temperature, this effect was diminished (Table 6A.3).

Discussion

A modest, but significant, and independent positive association between both minimum temperature and precipitation and campylobacteriosis was observed in the case-crossover study, after matching on state, county, year, and three-week time period and controlling for day of year effects. The association between minimum temperature and campylobacteriosis was approximately linear, with a six percent increase the odds of campylobacteriosis for every ten degree Fahrenheit increase in weighted average of minimum temperature during the three to twelve days prior to specimen collection. Periods of extreme precipitation during the seven to fourteen days prior to specimen collection were also associated with increased odds of campylobacteriosis. Weekly temperature fluctuations closely mirrored the weekly rates of campylobacteriosis in nine of 10 FoodNet sites. The association between weekly rates of campylobacteriosis and Weekly precipitation exhibited more variation; in Minnesota as the peak in rainfall occurred during the summer peak in campylobacteriosis, whereas in Oregon and the California site, the peak in rainfall occurred in winter or fall, when rates of campylobacteriosis are generally lowest; the pattern of campylobacteriosis and precipitation fluctuated throughout the year for the remaining sites. The largest differences in rates of campylobacteriosis by season occurred in those aged 15-30 and over 75 years or age.

The differences in weekly rates of campylobacteriosis by age group is worth noting and suggests that seasonal risk factors may differ by age group; a finding which is supported by other studies. For example, a recent study of bacterial infections, including those of campylobacteriosis, by Denno et al. in persons <19 years of age found an

increased risk from recreational water exposure and noted that this association was not as strong in studies conducted among all age groups.[33] Furthermore, a study by Miller et al. (2005) found that there were serotype differences in *Campylobacter* by the age of the infected person, suggesting that there are strain differences by age.

A positive association between increased temperature and *Campylobacter* incidence has been found in other studies, although the use of temperature measurements (mean, minimum), lag periods, and study methodologies has varied.[26, 34] In a study of two Canadian provinces , Fleury and colleagues (2006) found that the weekly incidence of campylobacteriosis increased by between two and four percent for every degree Celsius increase in mean weekly temperature.[29] A significant association was found for each week between zero and six weeks prior to infection, suggesting that temperature may play a role at many points along the pathway from food production to consumption. In Denmark, maximum temperature four weeks prior to the date of diagnosis (which coincided with the approximate time of exposure), was found to be the best predictor of weekly *Campylobacter* rates.[12] Given the additional association with hours of sunlight, the authors hypothesized the pathway could be related to behavioral factors.[12] A study in England found that, given a lag of six weeks prior to specimen collection date, mean weekly temperature to be related to weekly incidence of campylobacteriosis, with a one degree (Celsius) increase in temperature related to a five percent increase in campylobacteriosis notifications, but only up to temperatures of 14 degrees C.[27] However, a more recent examination of similar data in England by Lake et al. (2009) found a positive association between increased temperature in the week of and one week prior to specimen collection in a recent study conducted.[35] In the U.S., Naumova et al.

(2007) found that the peak in daily *Campylobacter* incidence occurred on the same day as the peak in maximum temperature, although this study was limited to one state.[28]

As *Campylobacter* has not been shown to multiply outside of a human or animal host, the relationship between temperature and campylobacteriosis is likely to be complex. In this study, an association was found between temperature at the approximate time of exposure (estimated to be 3-12 days prior to specimen collection) and campylobacteriosis. Higher temperature may be correlated with human behaviors that put individuals at greater risk for exposure to *Campylobacter*. One might speculate that some identified risk factors for campylobacteriosis, such as eating chicken from a barbecue, and swimming in natural water sources, are likely to occur much more frequently in the summer, however these do not appear to be large contributing factors to the seasonality of campylobacteriosis in the FoodNet sites (Chapter 7). In addition, the occurrence and concentration of *Campylobacter* in food and the environment has also been shown to change throughout the year. For example, the proportion of poultry flocks that test positive for *Campylobacter*, and the occurrence of *Campylobacter* in natural water sources, varies seasonally.[10, 11]

Another potential pathway suggested by Nichols (2005),[18] as well as others,[36-38] is that flies contribute to *Campylobacter* transmission and seasonality. Housefly (*Musca domestica*) development from larvae to adult is largely dependent on temperature, as well as moisture content.[39] At warmer temperatures, housefly development can range from days to weeks and is fastest at warmer temperatures. Flies are most active in the northern hemisphere from May-October.[37] Nichols reported an association between an excess number of *Campylobacter* cases (> 170 per week) and ambient temperatures

during which flies would more likely have the shortest development time (< 3 weeks) of the housefly. We are not aware of evidence suggesting direct fly-human transmission occurs. Fly densities in henhouses may affect poultry contamination. A study in Iceland of risk factors for *Campylobacter* contamination of boiler chickens found a link between fly activity and poultry contamination, mediated by temperature[16] and the implementation of henhouse fly screens was found to reduce the proportion of *Campylobacter*-positive poultry flocks in Denmark.[40]

In this study, an association between extreme precipitation and campylobacteriosis was found. Waterborne outbreaks of campylobacteriosis have occurred after heavy precipitation events,[21, 41] and case-control studies have identified improperly treated drinking water as a risk factor for infection.[42] In addition, *Campylobacter* was found more often in the environment on rainy days as opposed to sunny days in a study conducted in 2007 by Hansson et al.[43] Despite this, an association between precipitation and campylobacteriosis has rarely been found in other studies, although it is unclear whether many studies examined events of extreme precipitation.[12, 26, 27] Reasons why our study found an effect of precipitation and others did not could include differences in the estimated period of exposure (i.e., lag times), or differences in the geographic scale of the precipitation measurement. We examined the relationship of the average precipitation for a specific county lagged seven to fourteen days prior to specimen collection date. This exposure period was chosen because it was presumed to allow sufficient time for the precipitation to affect water treatment systems and for that water to travel to consumers. and was consistent with the lag time found in other studies.[41, 44]

Despite the substantial differences in rainfall patterns across the FoodNet sites, no differences in the effect of precipitaiton across the FoodNet FoodNet sites were found. While temperature patterns tend to track over counties and even larger geographic areas, precipitation events can occur at a much more localized scale. For example, in general, the surface areas of counties in FoodNet are smaller than the two geographic regions examined in the U.K. study that found no association between campylobacter and precipitation.[27] Differences in the source of drinking water (e.g., groundwater vs. surface water) could also modify the impact of precipitation on the role of drinking water as a cause of campylobacteriosis. Rainfall effects may be more important in areas where drinking water is derived from surface water sources than groundwater surfaces, and the importance of the timing of the rainfall may vary with the type of water system used, as was found in a study by Curriero et al. (2001).[20] In their study of the relationship between extreme precipitation events and waterborne disease outbreaks due to multiple pathogens, rainfall during the month of the outbreak was a significant predictor of outbreaks in areas using surface water as the source of drinking water. However, rainfall two months prior to the outbreak was a predictor in areas using groundwater as the source of drinking water. Their analyses were more localized to the area likely to be impacted by the precipitation events, as it was conducted at the watershed level as compared to the county level in our analyses. A similar study in the FoodNet sites might be useful.

This study has several limitations. First, international travel history has only been collected in FoodNet since 2004; therefore we were unable to exclude cases likely to have acquired their *Campylobacter* infections abroad. Data from a 1998-1999 case-

control study of risk factors for campylobacteriosis conducted in the FoodNet sites indicated that approximately 13% of cases traveled internationally in the seven days prior to their illness.[2] It has been suggested that some of the summer peak in campylobacteriosis in other countries, particularly the Netherlands and Denmark, may be due to international travel.[26] The authors felt this was a more likely cause than domestic exposure because the peak in temperature in these countries occurred prior to that in cases. In most FoodNet sites, however, the peak in campylobacteriosis occurs concomitantly or after the peak in minimum temperature, so the contribution of travel-associated cases to the summer peak in infections may be less in the FoodNet sites. Secondly, the temperature and precipitation experienced by each case of campylobacteriosis was assumed to be that of their county of residence. Additionally, the impact of attributing the county average precipitation and temperature measurements to each case in a county may vary somewhat by state, given that the counties in the FoodNet sites are not of a uniform area. Counties involved in FoodNet surveillance range from ninety-two square miles (Baltimore City County, Maryland) to ten thousand square miles (Harney County, Oregon).[45] Third, the results of our distributor lag analysis suggests that it is possible that our conditional logistic regression models may not have been specified correctly. However, the main association of concern, that of an association between campylobacteriosis and minimum temperature the day after specimen collection was only found in Colorado. *Campylobacter* are known to be sensitive to changes in humidity and temperature, and thus temperature may affect the ability of *Campylobacter* to survive transport to the clinical laboratory. Alternately, the

association between campylobacteriosis and temperature that was observed could be due to chance.

Future studies should consider more refined measurements of temperature and precipitation in examining the effect of these climatic factors on campylobacteriosis, particularly in measuring the precipitation and temperature effects at relevant areas of exposure. For instance, given the high proportion of cases attributable to poultry consumption,[2] it is possible that a more relative exposure of temperature and precipitation is in the locations where the poultry are reared. Conversely, temperature and precipitation at a person's home may be more relevant as it impacts cooking practices and animal exposures, other established risk factors for infection.[2, 46] Additionally, studies that elucidate the pathway between meteorological factors and campylobacteriosis and which clarify the reasons for the seasonal distribution in campylobacteriosis are needed.

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Tables and Figures

Table 6.1. Weights assigned to minimum temperature on each day prior to specimen collection date during possible exposure period.

Day prior to specimen collection	**Weight**
3, 11, and 12 days	1/(1+1.33+1.66+2+2*(1.66)+2(1.33)+2(1)=13.97)
4, 9 and 10 days	1.33/13.97
5, 7 and 8 days	1.66/13.97
6 days	2/13.97

Table 6.2. Association between climatic factors (temperature and precipitation as categorical variables) during a priori specified lag periods and the probability of the occurrence of a culture-confirmed case of campylobacteriosis.

Categories of Climate Variables	**aOR (95% CI)†**	**P**	**P for trend**
Categories of Weighted Average* of Minimum Temperature (degrees Fahrenheit) (days 3-12 prior to specimen collection)			
< 37^{o} Fahrenheit	Ref	0.0337	0.0044
37-48^{o} Fahrenheit	**1.063** (0.996, 1.133)		
48-56^{o} Fahrenheit	**1.074** (0.987, 1.168)		
57-66^{o} Fahrenheit	**1.148** (1.039, 1.269)		
>67^{o} Fahrenheit	**1.209** (1.057, 1.383)		
Categories of Average Precipitation (inches) (days 7-14 prior to specimen collection)			

No Rain		Ref		0.1566	0.0978
<0.17 inches	**1.023**	(0.974,	1.075)		
0.17-0.60 inches	**1.001**	(0.950,	1.055)		
0.60-1.36 inches	**1.029**	(0.975,	1.086)		
1.36-3.20 inches	**1.026**	(0.970,	1.086)		
>3.20 inches	**1.091**	(1.010,	1.178)		
Extreme Precipitation					
Extreme precipitation (>3.2 inches)	**1.066**	(1.007,	1.128)	0.0275	
Non-extreme precipitation (≤ 3.2 inches)		Ref			

* see Methods for a description of weights

† Case days and controls days were matched on day of the week (i.e., Monday, Tuesday, etc…), and 3-week strata, and year. Odds ratios represent increase in odds comparing minimum temperature or precipitation in a given range compared to minimum temperatures < 25% of the distribution or precipitation of 0 inches. **aOR**=Odds Ratio adjusted for day of year effects (day, day squared, day cubed) and interaction between day of year effects and FoodNet site

Table 6.3. Association between climatic factors (temperature and precipitation as categorical variables) during a priori specified lag periods and the probability of the occurrence of a culture-confirmed case of campylobacteriosis.

Temperature Variable	**aOR (95% CI)**			**P**
Weighted Average* of Minimum Temperature (degrees Fahrenheit) (days 3-12 prior to specimen collection)	**1.060**	(1.030,	1.090)	0.0006
Sensitivity analyses examining other temperature measurements				
Weighted Average* of Mean Temperature (degrees Fahrenheit) (days 3-12 prior to specimen collection)	**1.050**	(1.020,	1.080)	0.0032

Weighted Average* of Maximum Temperature (degrees Fahrenheit) (days 3-12 prior to specimen collection)	**1.030**	(1.000,	1.060)	0.0328

* see Methods for a description of weights

† Case days and controls days were matched on day of the week (i.e., Monday, Tuesday, etc…), and 3-week strata, and year. Odds ratios represent increase in odds comparing minimum temperature or precipitation in a given range compared to minimum temperatures < 25% of the distribution or precipitation of 0 inches.

aOR=Odds Ratio adjusted for day of year effects (day, day squared, day cubed) and interaction between day of year effects and FoodNet site

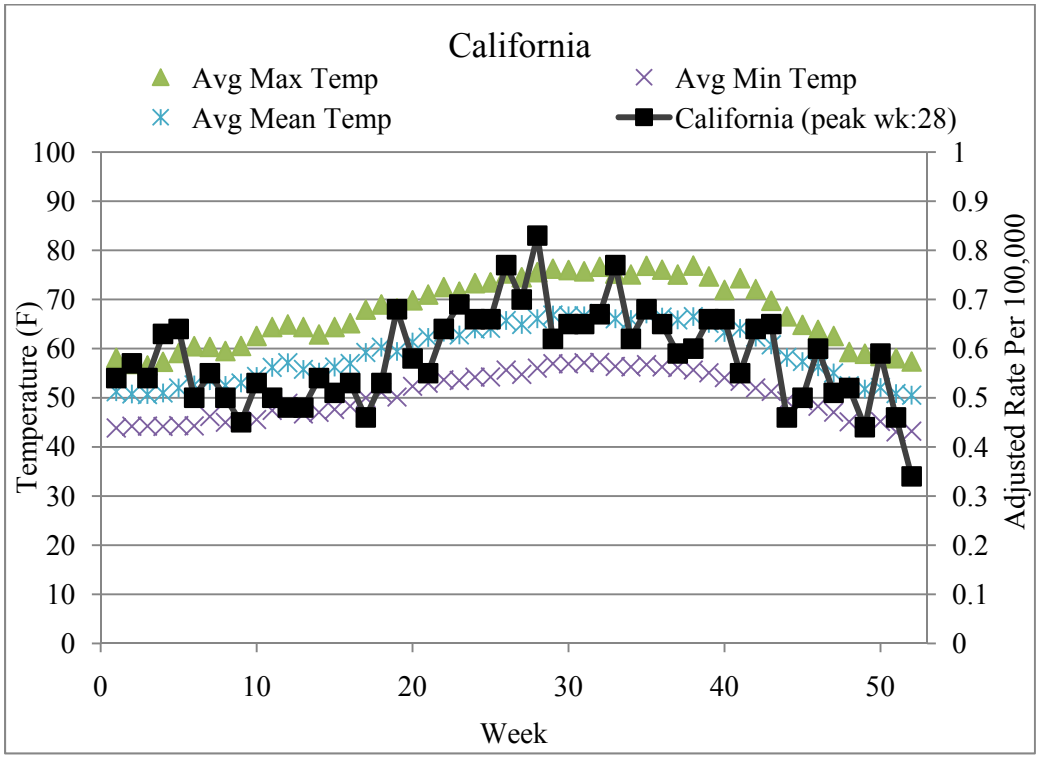
Table 6.4. Multivariate Model: Association between climatic factors during a priori specified lag periods and the probability of the occurrence of a culture-confirmed case of campylobacteriosis. Case days and controls days were matched on day of the week (i.e., Monday, Tuesday, etc…), and 3-week strata, and year. For temperature, odds ratios represent increase in odds per 10 degree (Fahrenheit) increase in temperature.

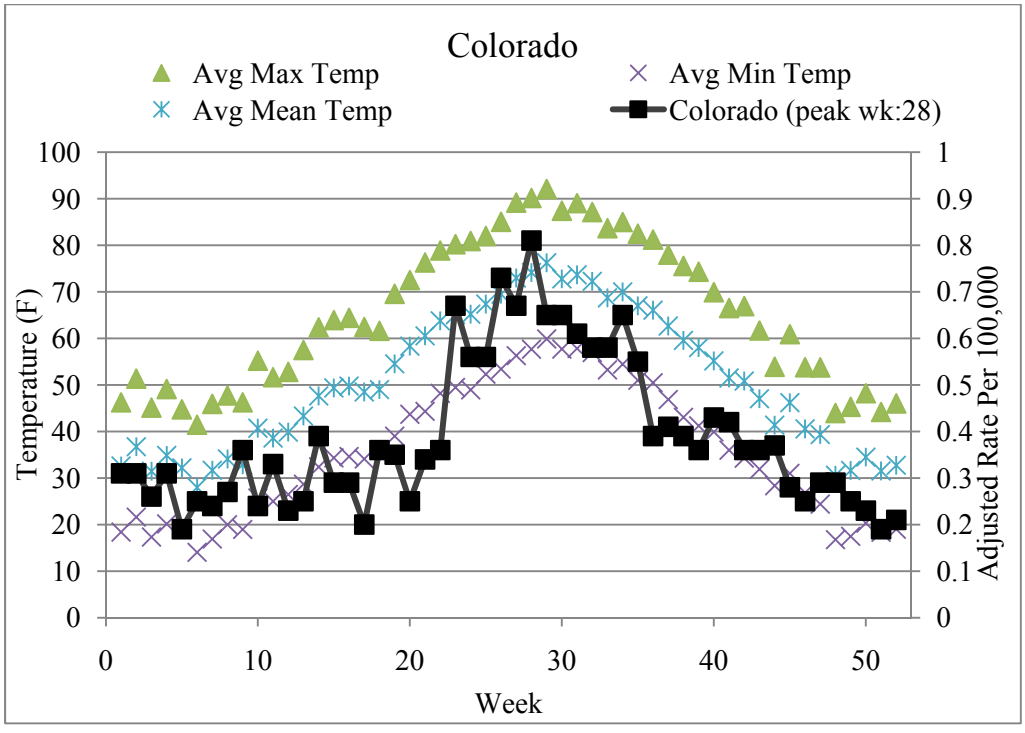
Climatic Factor	**aOR (95% CI)**			**P**
Weighted Average* of Minimum Temperature (degrees Fahrenheit) (days 3-12 prior to specimen collection)	**1.060**	(1.020,	1.090)	0.0009
Extreme precipitation (> 3.20 inches) during the 7-14 days prior to specimen collection	**1.081**	(1.018,	1.147)	0.011

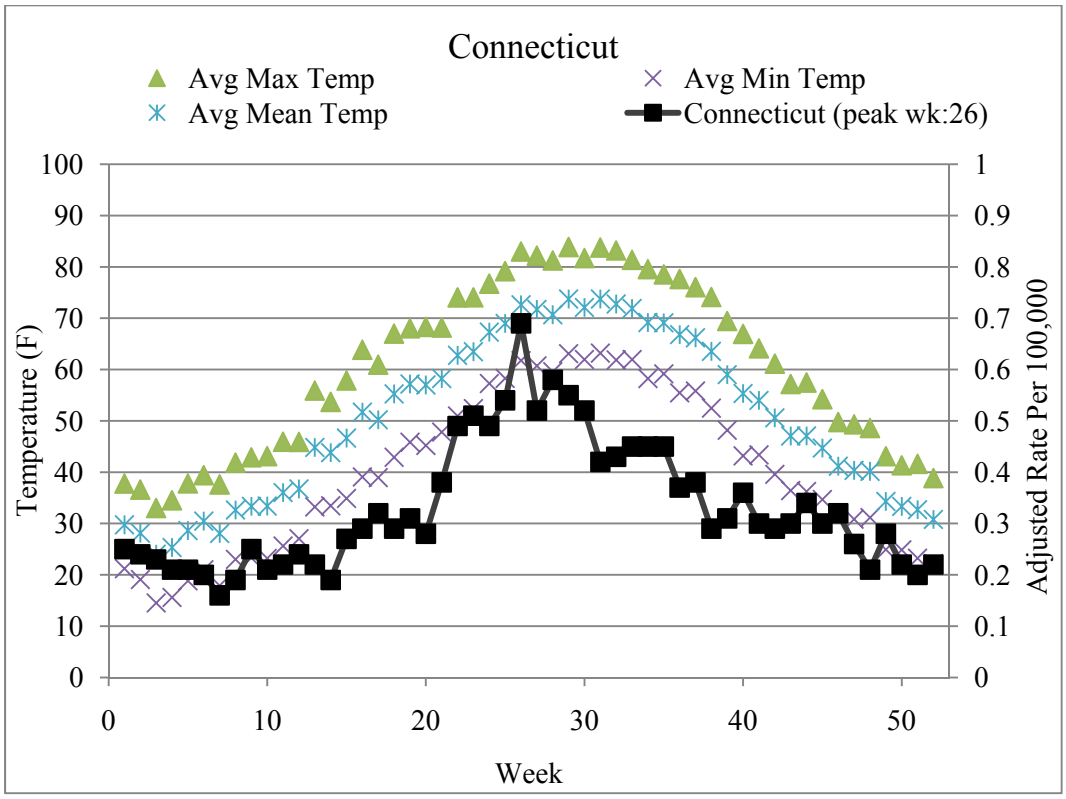
* see Methods for a description of weights

aOR=Odds Ratio adjusted for day of year effects (day, day squared, day cubed) and interaction between day of year effects and FoodNet site, as well as main effects of weighted temperature and precipitation

Figure 6.1. Average weekly rates of campylobacteriosis (adjusted for FoodNet site and year) and temperature, FoodNet sites 1996-2006






Connecticut
Avg Max Temp
Avg Min Temp
Avg Mean Temp
Connecticut (peak wk:26)
Temperature (F)
Adjusted Rate Per 100,000
Week
0
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30
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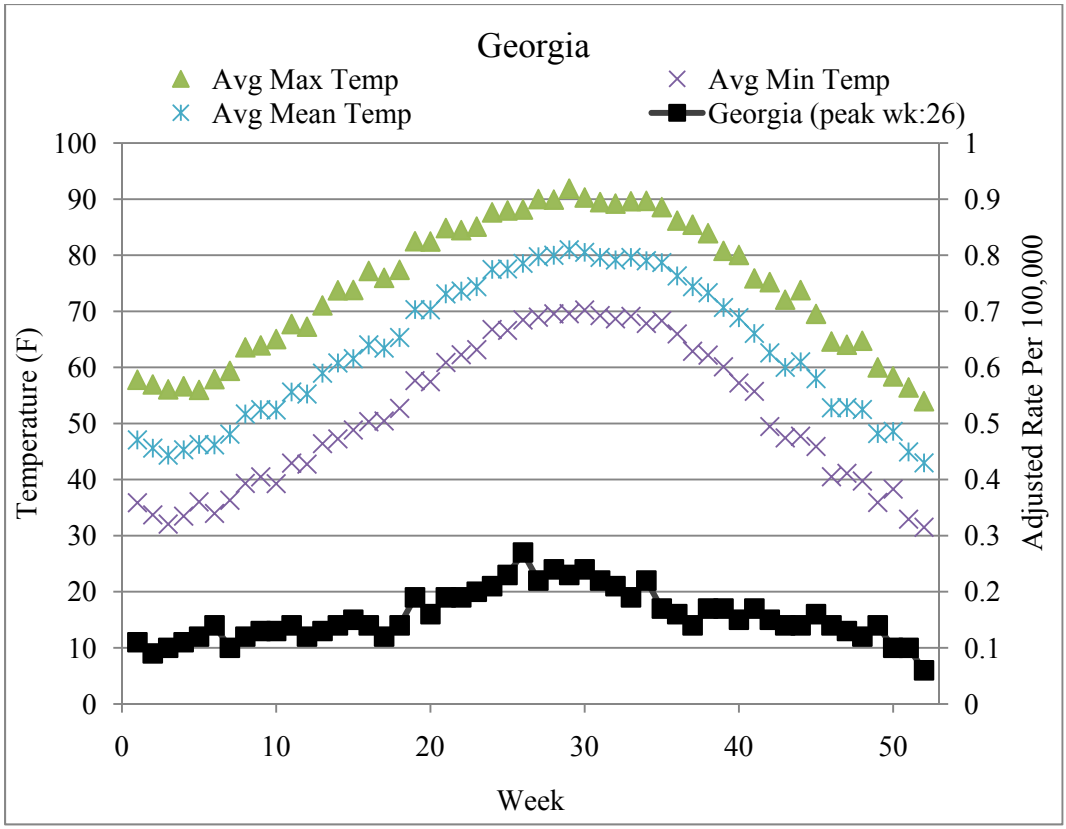
0.9
1


Georgia
Avg Max Temp
Avg Min Temp
Avg Mean Temp
Georgia (peak wk:26)
Temperature (F)
Adjusted Rate Per 100,000
Week
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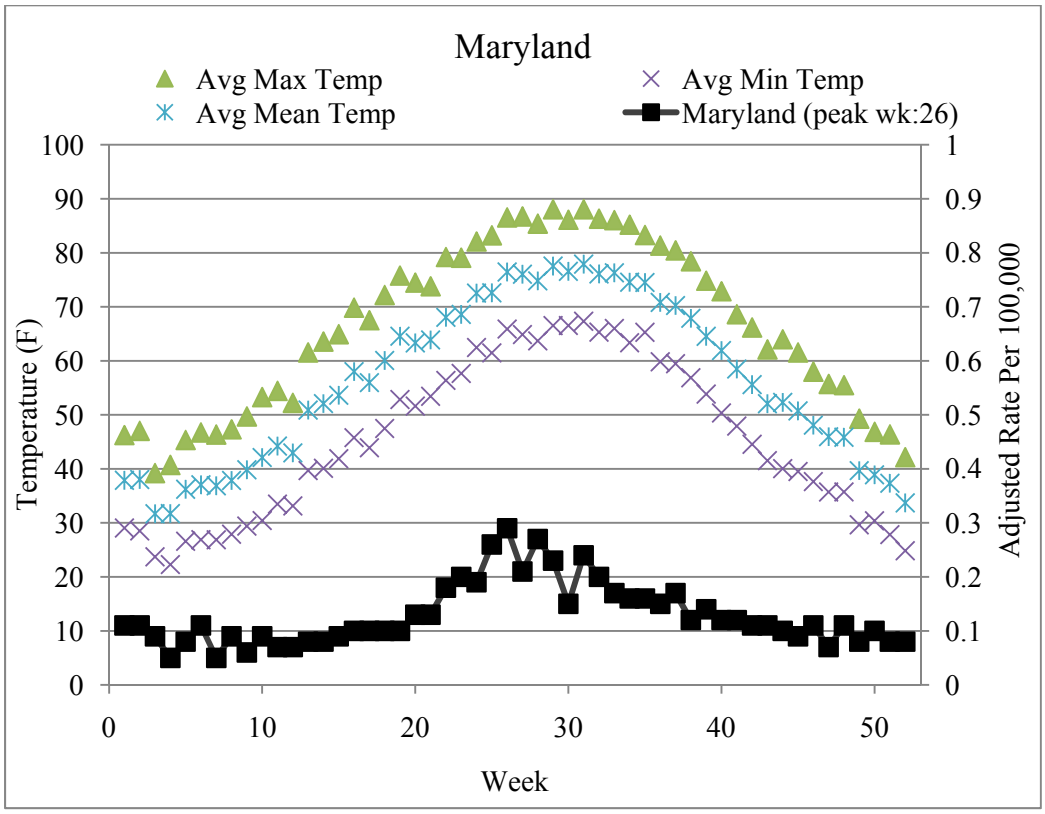
Maryland
Avg Max Temp
Avg Min Temp
Avg Mean Temp
Maryland (peak wk:26)
Temperature (F)
Adjusted Rate Per 100,000
Week
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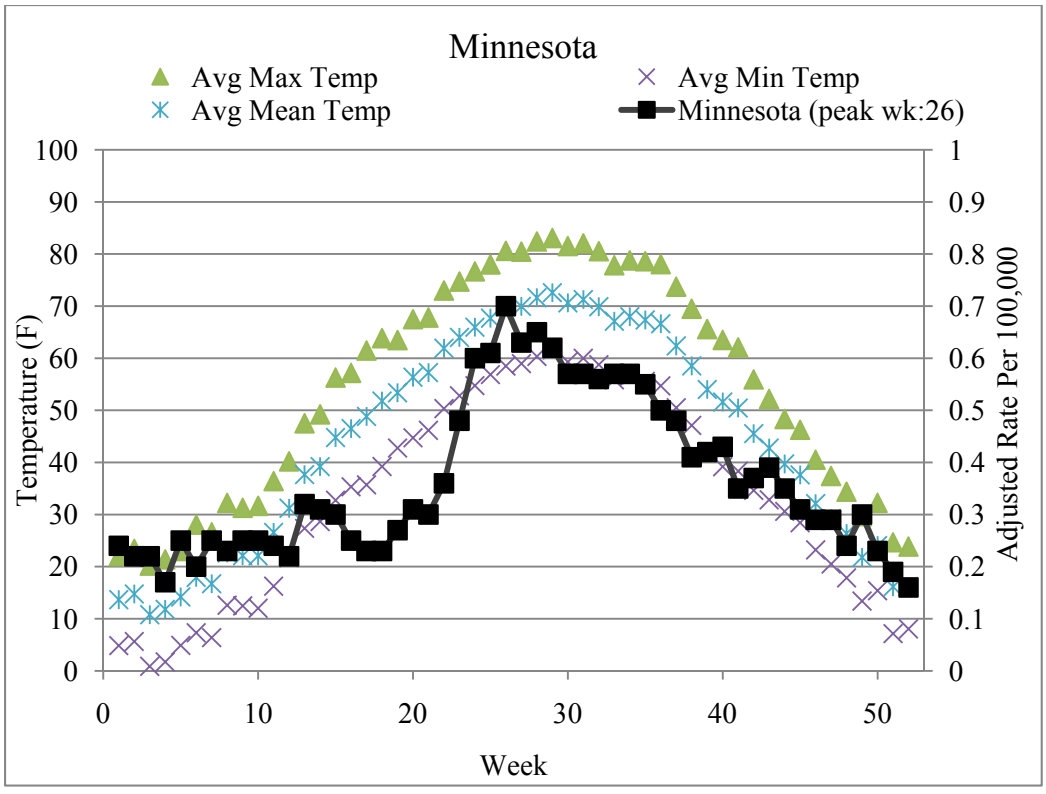
Minnesota
Avg Max Temp
Avg Min Temp
Avg Mean Temp
Minnesota (peak wk:26)
Temperature (F)
Adjusted Rate Per 100,000
Week
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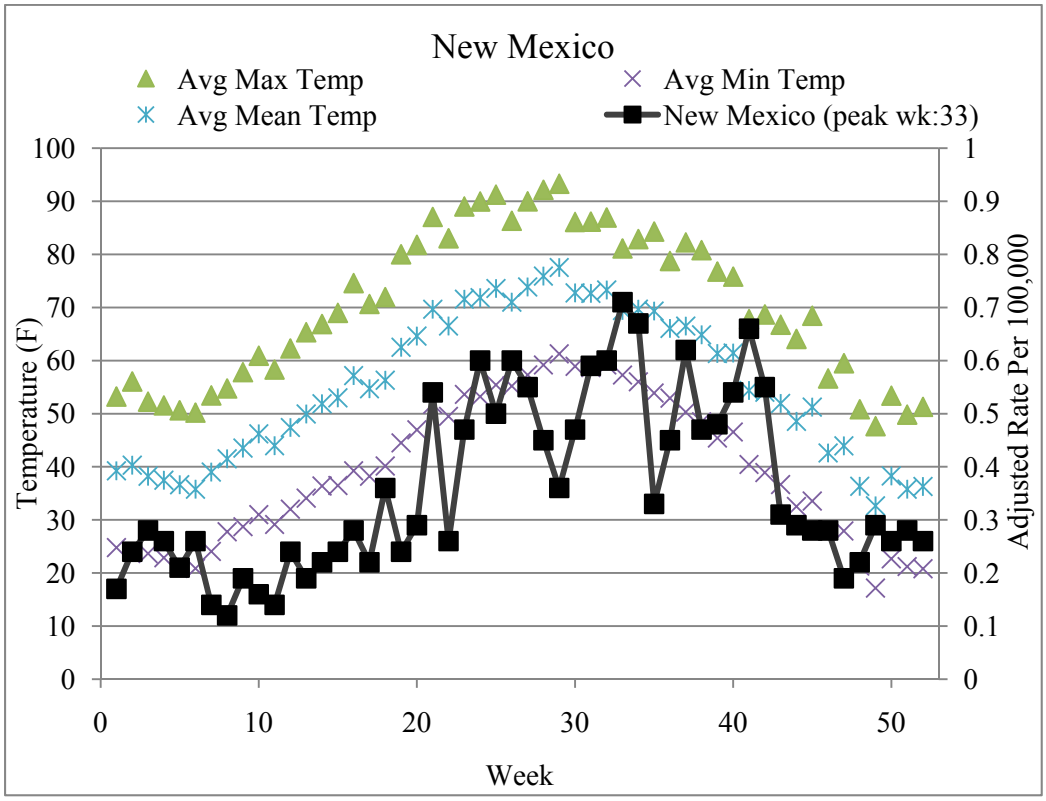
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Avg Max Temp
Avg Min Temp
Avg Mean Temp
New Mexico (peak wk:33)
Temperature (F)
Adjusted Rate Per 100,000
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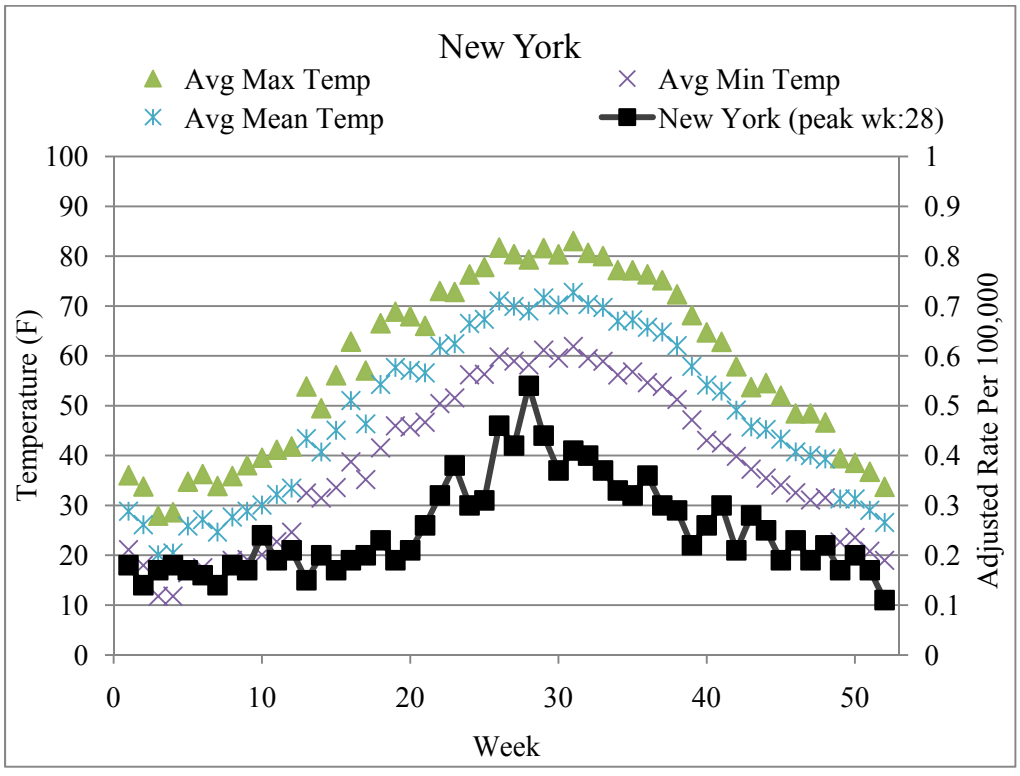
New York
Avg Max Temp
Avg Min Temp
Avg Mean Temp
New York (peak wk:28)
Temperature (F)
Adjusted Rate Per 100,000
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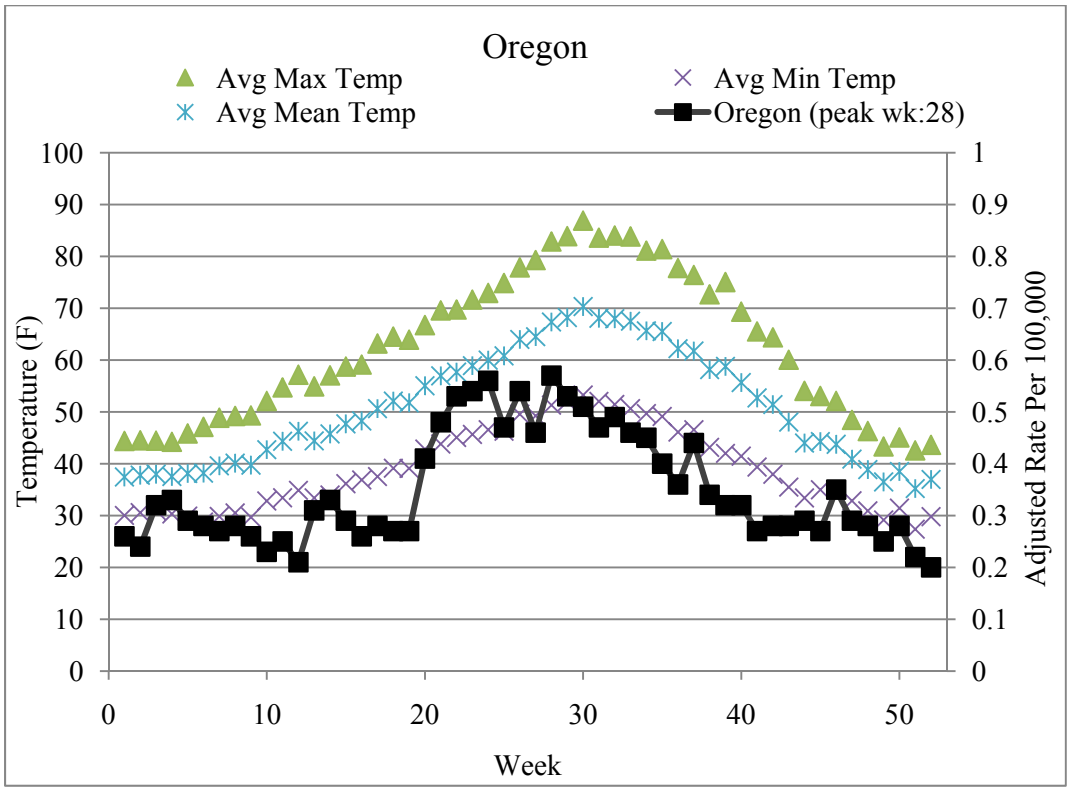
Oregon
Avg Max Temp
Avg Min Temp
Avg Mean Temp
Oregon (peak wk:28)
Temperature (F)
Adjusted Rate Per 100,000
Week
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1

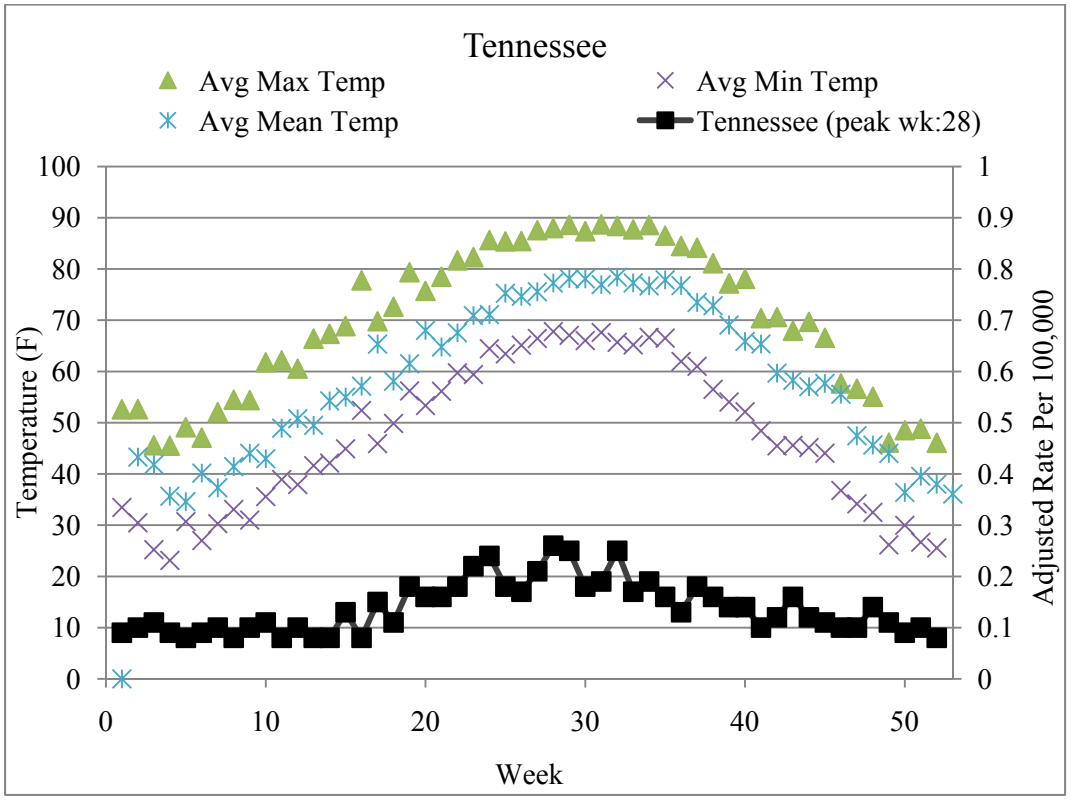
Tennessee
Avg Max Temp
Avg Min Temp
Avg Mean Temp
Tennessee (peak wk:28)
Temperature (F)
Adjusted Rate Per 100,000
Week
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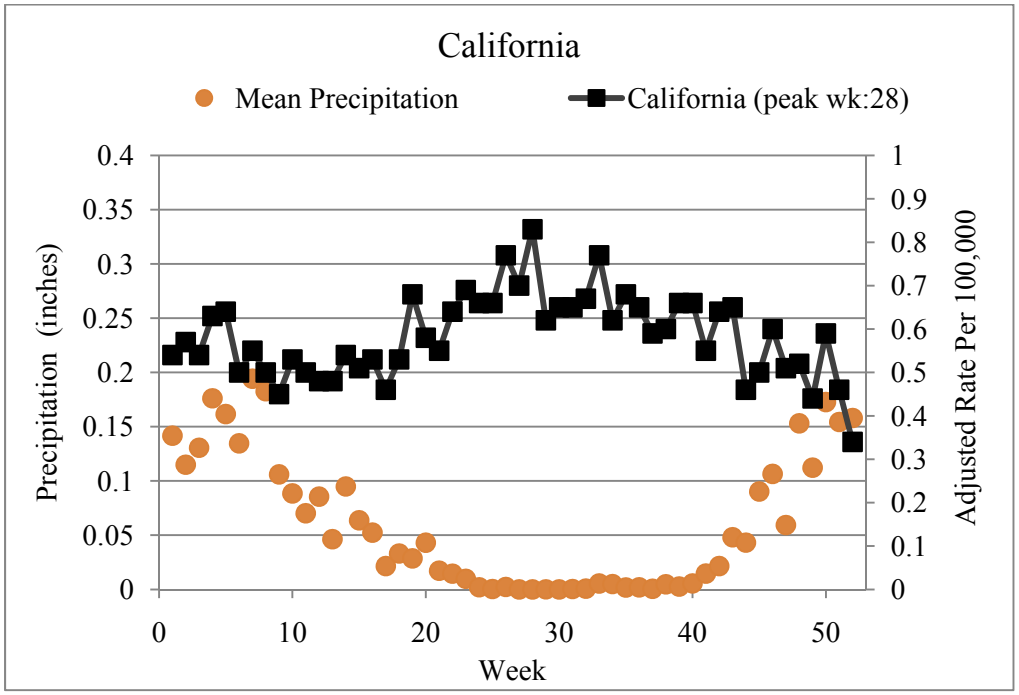
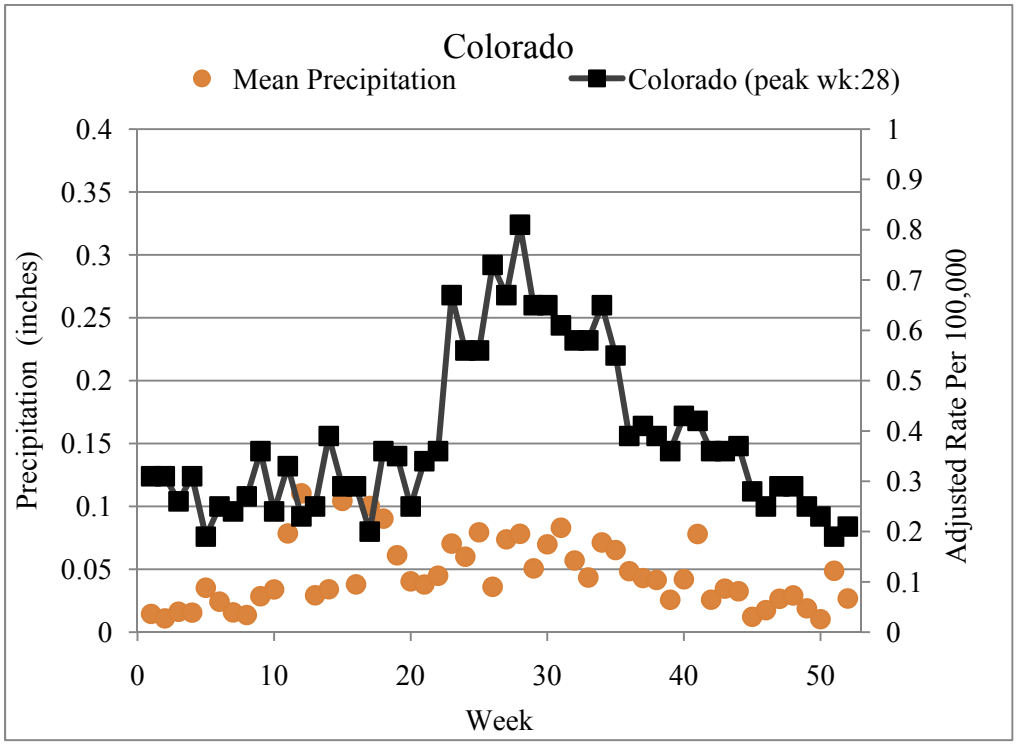
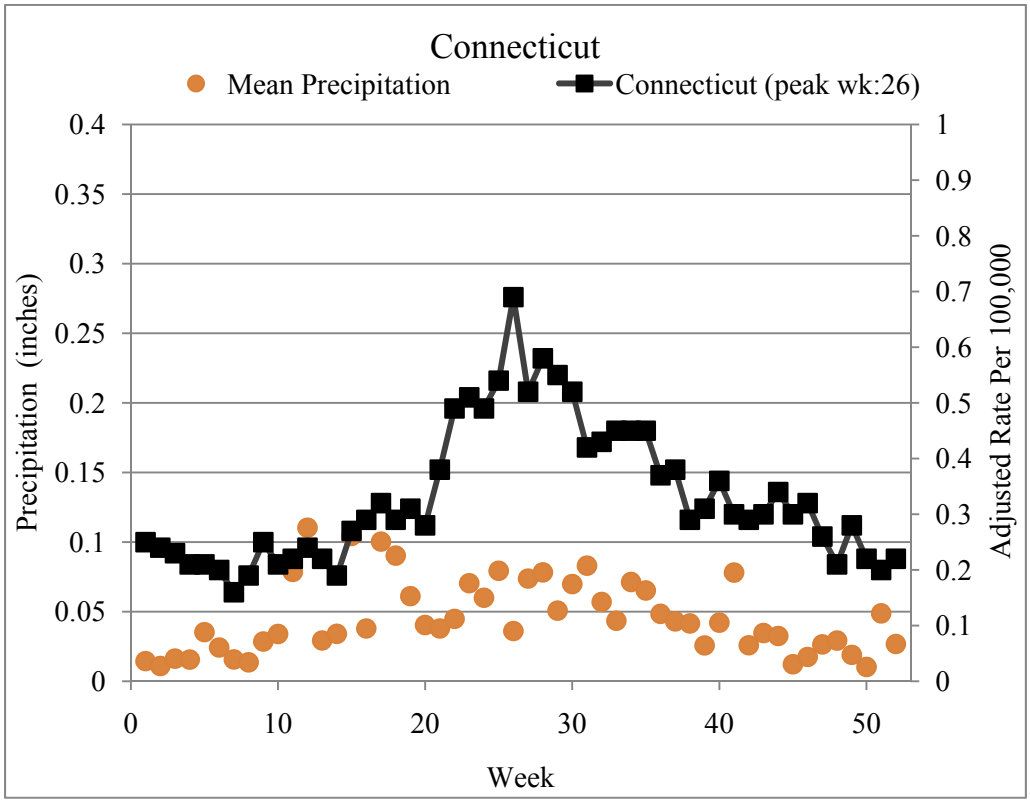
Figure 6.2. Average weekly rates of campylobacteriosis (adjusted for FoodNet site and year) and precipitation, FoodNet sites 1996-2006






Connecticut
Mean Precipitation
Connecticut (peak wk:26)
Precipitation (inches)
Adjusted Rate Per 100,000
Week
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0.05
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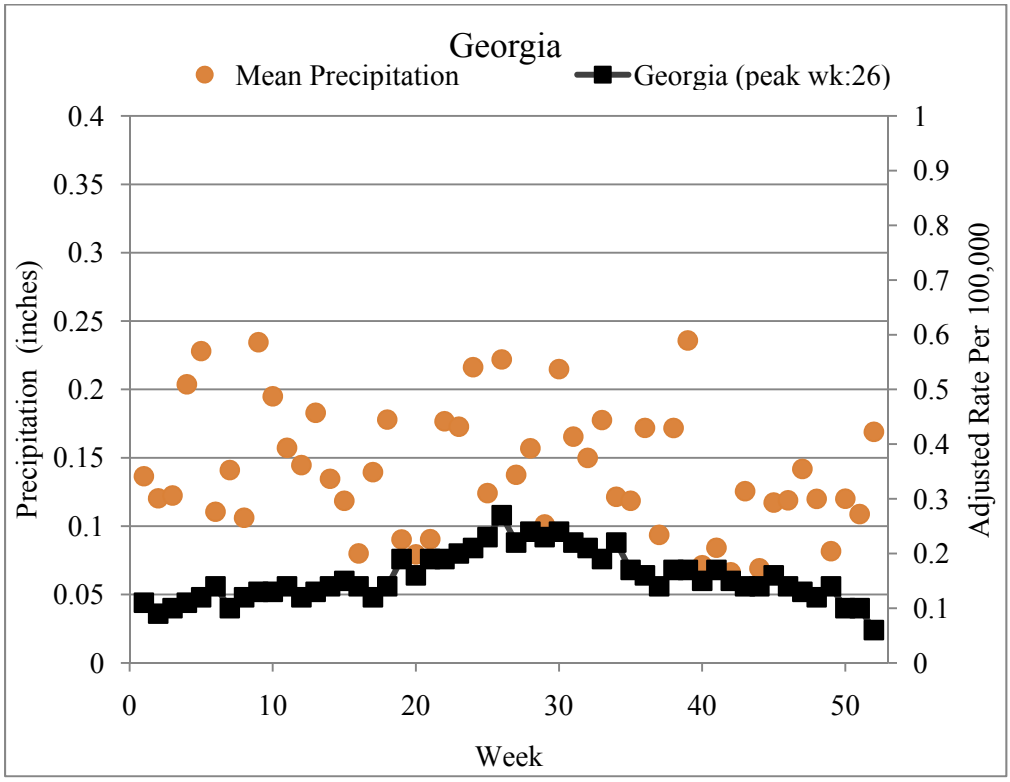
Georgia
Mean Precipitation
Georgia (peak wk:26)
Precipitation (inches)
Adjusted Rate Per 100,000
Week
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0.05
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0.35
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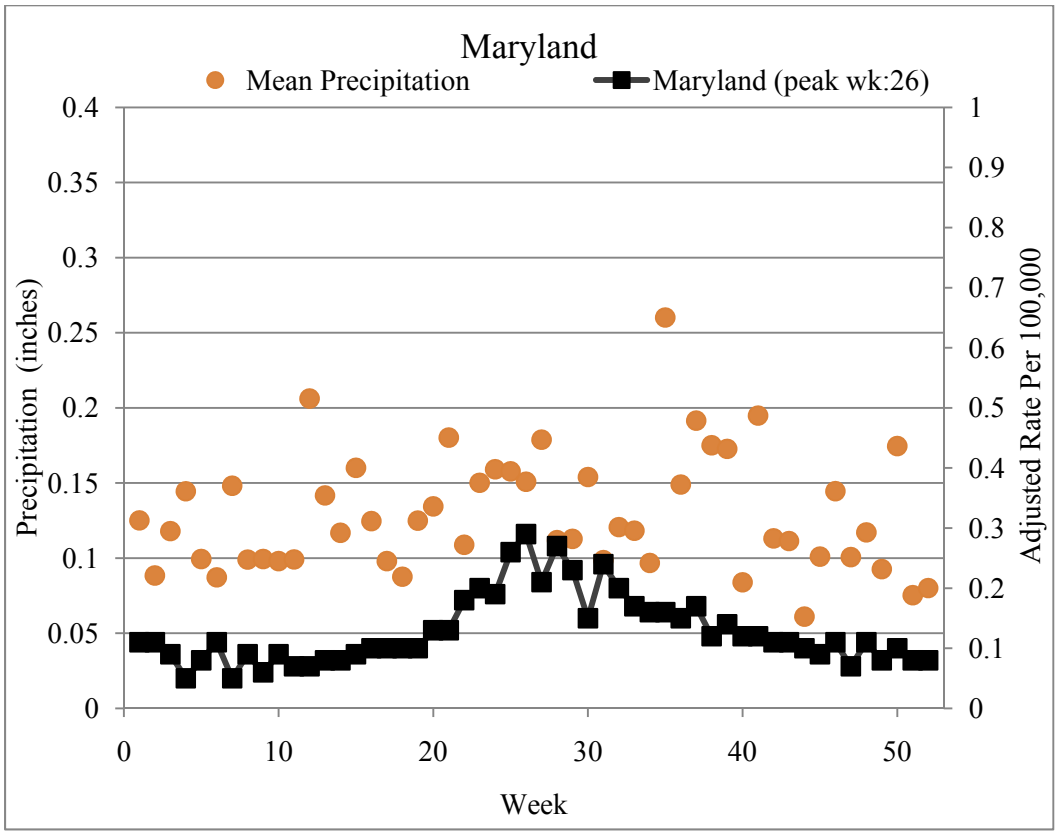
Maryland
Mean Precipitation
Maryland (peak wk:26)
Precipitation (inches)
Adjusted Rate Per 100,000
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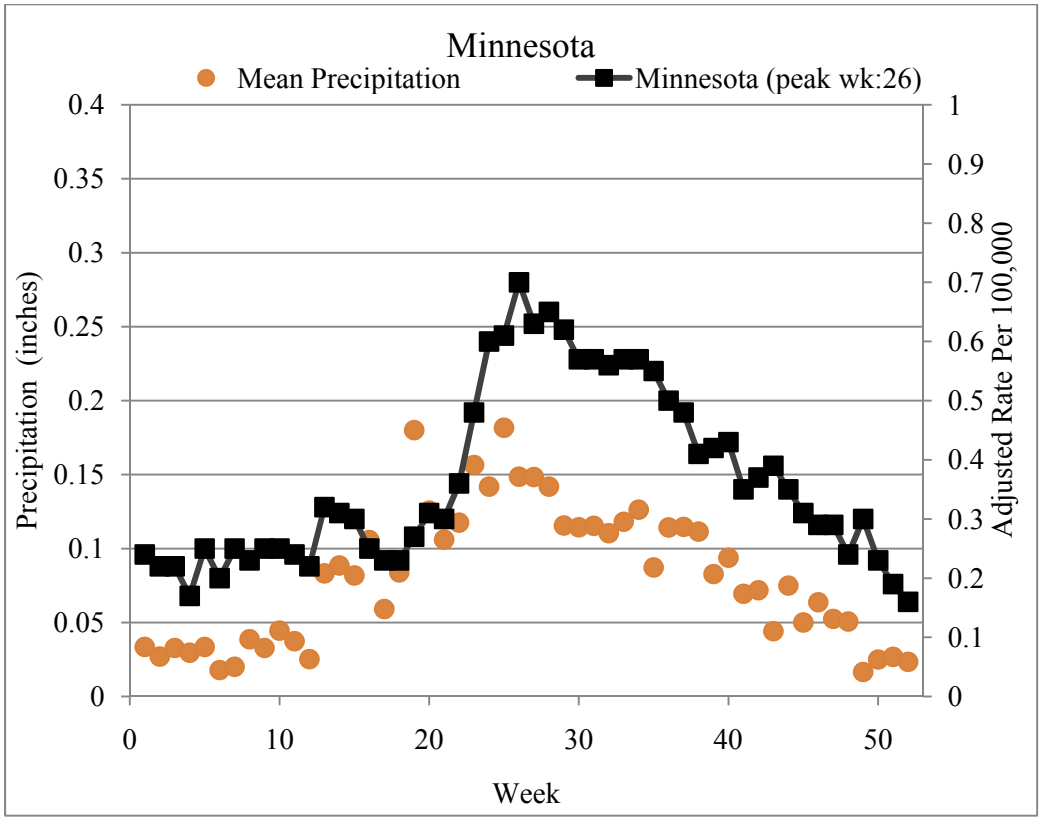
Minnesota
Mean Precipitation
Minnesota (peak wk:26)
Precipitation (inches)
Adjusted Rate Per 100,000
Week
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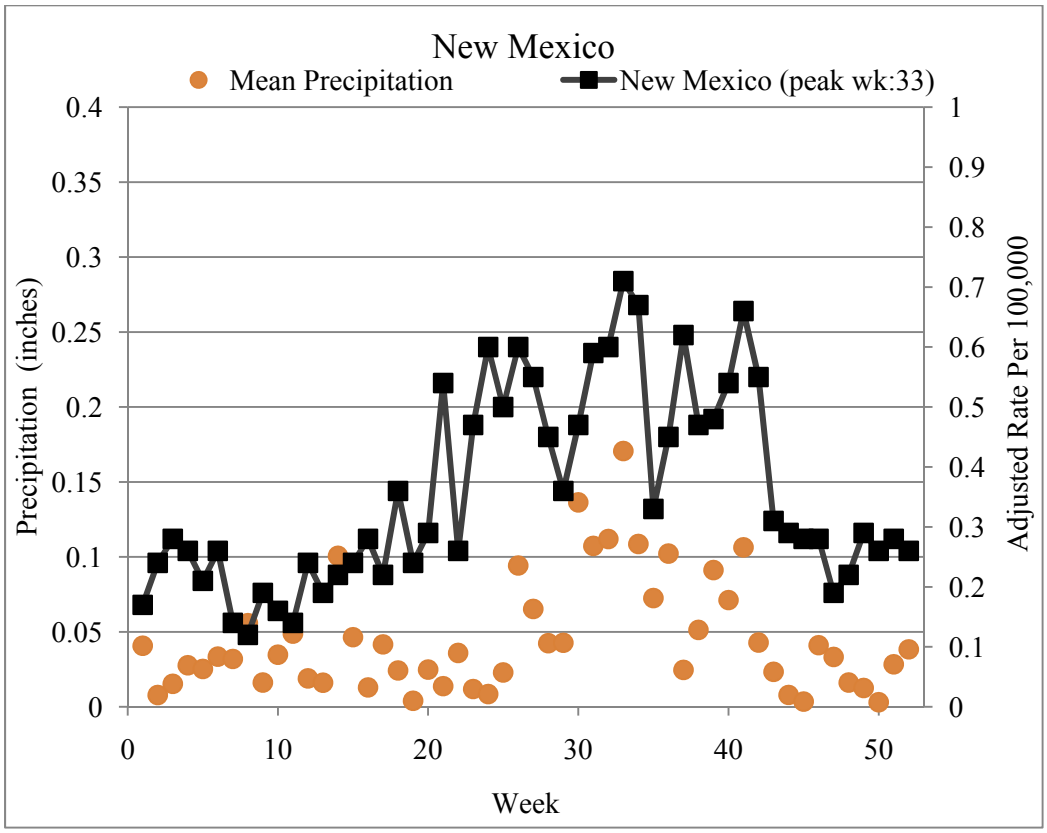
New Mexico
Mean Precipitation
New Mexico (peak wk:33)
Precipitation (inches)
Adjusted Rate Per 100,000
Week
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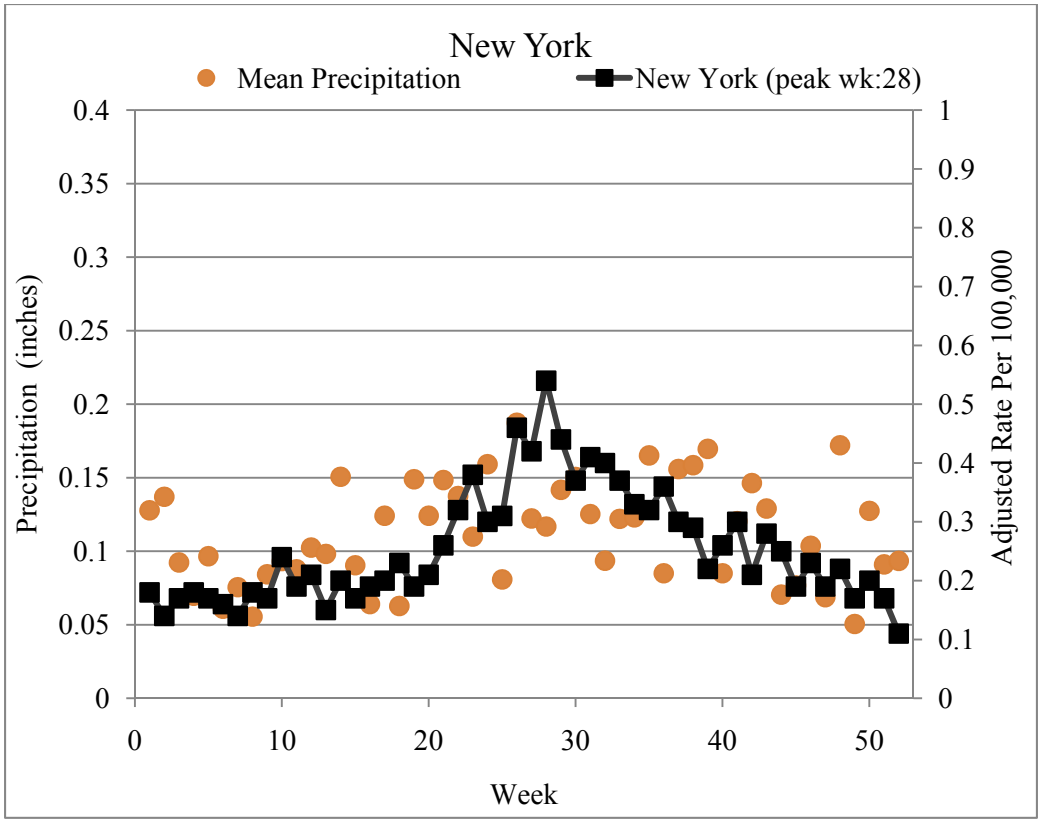
New York
Mean Precipitation
New York (peak wk:28)
Precipitation (inches)
Adjusted Rate Per 100,000
Week
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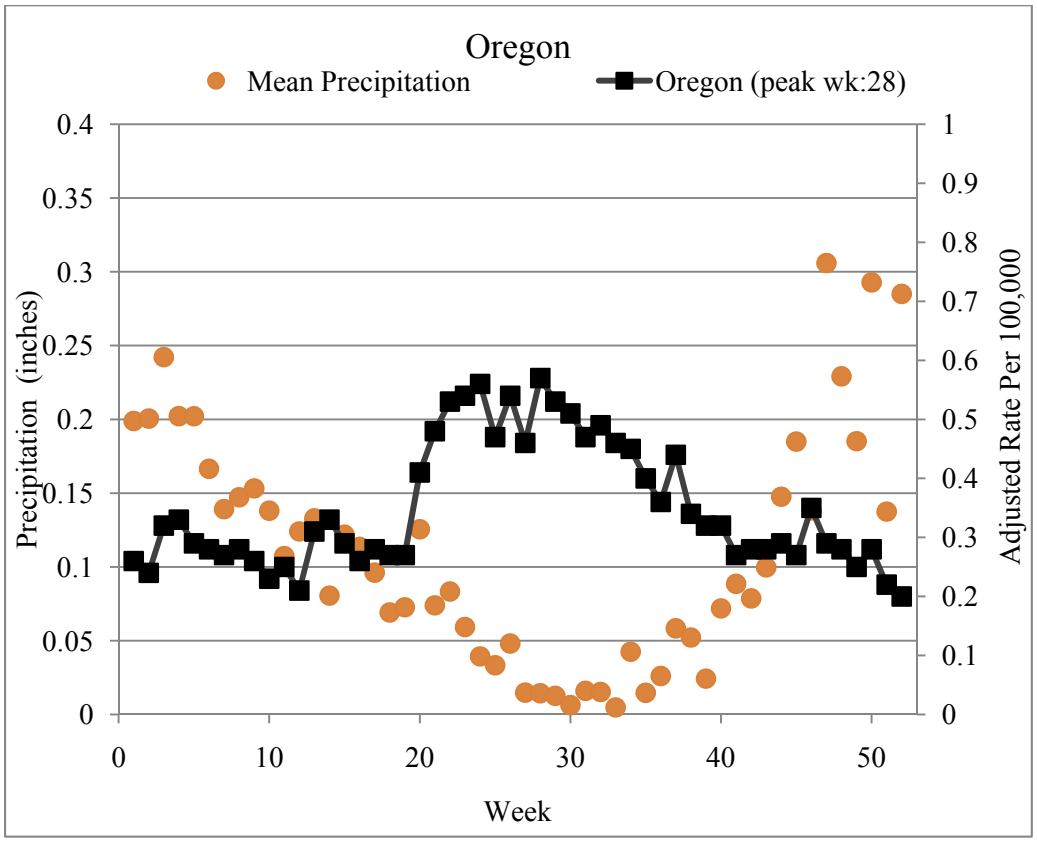
Oregon
Mean Precipitation
Oregon (peak wk:28)
Precipitation (inches)
Adjusted Rate Per 100,000
Week
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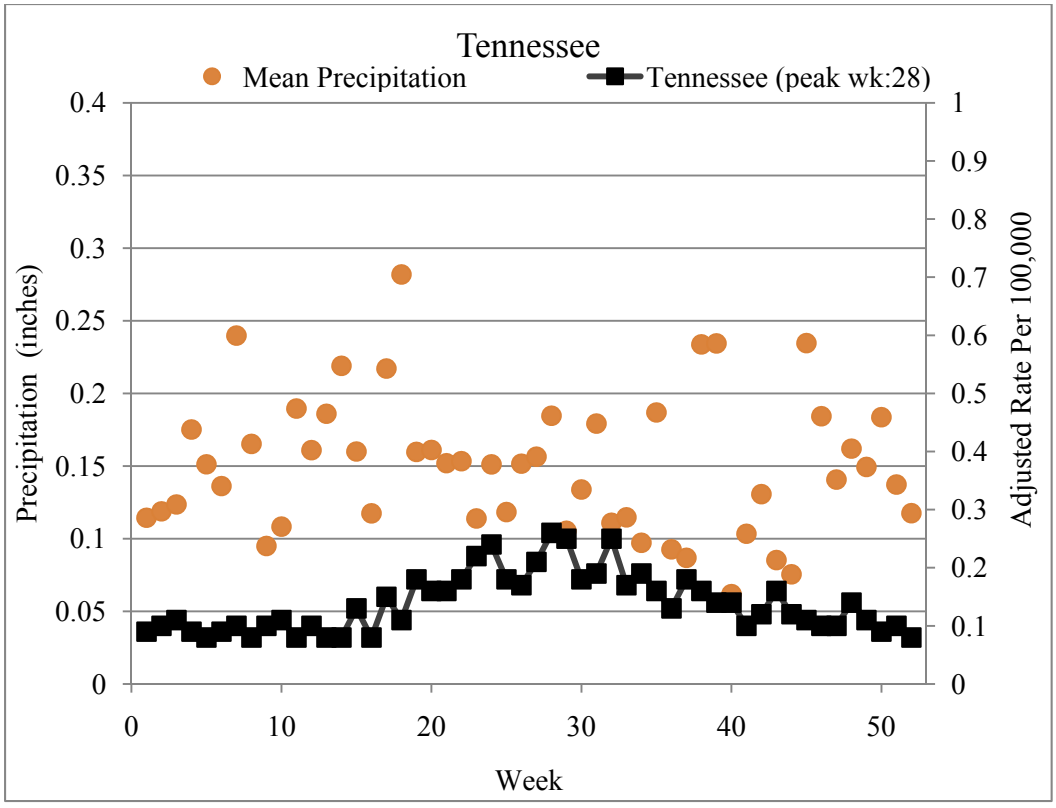
Tennessee
Mean Precipitation
Tennessee (peak wk:28)
Precipitation (inches)
Adjusted Rate Per 100,000
Week
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0.05
0.1
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0.25
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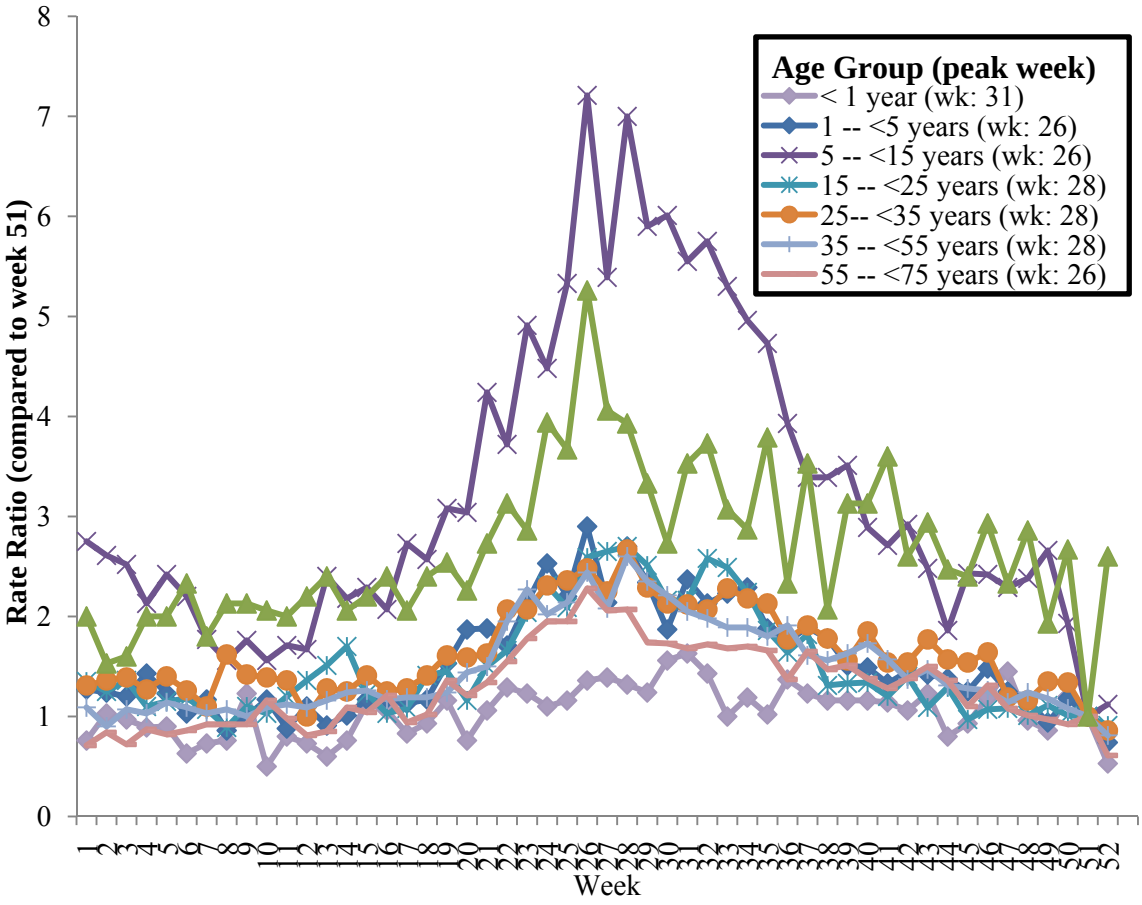
Figure 6.3. Rate ratios (adjusted for calendar year and changing FoodNet site and comparing the rate of campylobacteriosis for a given week compared to the rate in the 51st week) of campylobacteriosis by age group and week.



Appendix 6A:Distributed Lags

Table 6A.1. Minimum Temperature Distributed Lag, All FoodNet Sites

Lag (day before specimen collection)	OR (95% CI)			P	Adjusted OR (95% CI)*			P
-1	1.003	(1.000,	1.006)	0.0401	1.003	(1.001,	1.006)	0.0204
0	1.001	(0.998,	1.004)	0.5890	1.001	(0.997,	1.004)	0.7464
1	1.000	(0.996,	1.003)	0.9061	1.000	(0.997,	1.004)	0.9764
2	1.000	(0.997,	1.004)	0.9847	1.001	(0.997,	1.004)	0.7008
3	1.000	(0.997,	1.004)	0.9739	1.001	(0.997,	1.004)	0.7525
4	1.000	(0.997,	1.004)	0.9589	1.000	(0.996,	1.003)	0.9355
5	1.002	(0.999,	1.006)	0.1558	1.002	(0.999,	1.006)	0.2014
6	1.001	(0.998,	1.004)	0.6002	1.002	(0.998,	1.005)	0.3649
7	1.001	(0.998,	1.005)	0.5203	1.001	(0.998,	1.005)	0.4633
8	1.002	(0.998,	1.005)	0.2717	1.002	(0.999,	1.006)	0.2370
9	0.999	(0.996,	1.003)	0.7050	1.000	(0.996,	1.003)	0.8315
10	0.999	(0.995,	1.002)	0.4224	0.998	(0.995,	1.002)	0.3885
11	0.999	(0.995,	1.002)	0.3967	0.999	(0.995,	1.002)	0.4304
12	1.001	(0.999,	1.004)	0.3012	1.002	(0.999,	1.005)	0.1193

* Adjusted for day of year effects (day of year, day of year^2, day of year^3), interaction between FoodNet site and day of year effects, and extreme precipitation

Table 6A.2. Minimum Temperature Distributed Lag, Adjusted for Day of Year Effects and Extreme Precipitation, by FoodNet site

	Colorado Alone				All Sites Except Colorado			
Lag (day before specimen collection)	**Adjusted OR (95% CI)***			**P**	**Adjusted OR (95% CI)***			**P**
-1	1.018	(1.006,	1.030)	0.0032	1.002	(0.999,	1.005)	0.1067
0	0.995	(0.980,	1.010)	0.5123	1.001	(0.997,	1.004)	0.6481
1	0.998	(0.983,	1.013)	0.7728	1.000	(0.997,	1.004)	0.8900
2	1.007	(0.992,	1.021)	0.3858	1.000	(0.997,	1.004)	0.8024
3	0.994	(0.980,	1.009)	0.4507	1.001	(0.997,	1.004)	0.6431
4	0.987	(0.973,	1.002)	0.0875	1.000	(0.997,	1.004)	0.8036
5	1.008	(0.993,	1.023)	0.2849	1.002	(0.998,	1.006)	0.2876
6	1.016	(1.001,	1.032)	0.0354	1.001	(0.997,	1.005)	0.5972
7	0.988	(0.973,	1.003)	0.1289	1.002	(0.998,	1.006)	0.2697
8	1.004	(0.989,	1.019)	0.6151	1.002	(0.998,	1.006)	0.2597
9	1.007	(0.992,	1.022)	0.3763	0.999	(0.996,	1.003)	0.6411
10	0.995	(0.981,	1.010)	0.5018	0.999	(0.995,	1.002)	0.4423
11	1.001	(0.986,	1.015)	0.9357	0.998	(0.995,	1.002)	0.3970
12	1.001	(0.990,	1.013)	0.8010	1.002	(0.999,	1.005)	0.1171

* Adjusted for day of year effects (day of year, day of year^2, day of year^3), interaction between FoodNet site and day of year effects, and extreme precipitation

Table 6A.3. Extreme Precipitation Distributed Lag, All FoodNet Sites

Lag (day before specimen collection)	OR (95% CI)			P	Adjusted OR (95% CI)*			P
-1	1.039	(0.980,	1.101)	0.2016	1.029	(0.968,	1.095)	0.3608
0	0.997	(0.940,	1.058)	0.9252	0.990	(0.930,	1.054)	0.7549
1	0.961	(0.906,	1.020)	0.1904	0.943	(0.885,	1.004)	0.0654
2	0.949	(0.894,	1.007)	0.0864	0.949	(0.891,	1.011)	0.1039
3	1.007	(0.950,	1.069)	0.8054	0.989	(0.929,	1.054)	0.7380
4	1.030	(0.971,	1.093)	0.3224	1.038	(0.975,	1.105)	0.2444
5	1.032	(0.973,	1.095)	0.2922	1.028	(0.965,	1.095)	0.3895
6	0.990	(0.933,	1.051)	0.7457	0.962	(0.904,	1.025)	0.2335
7	1.012	(0.954,	1.074)	0.6874	1.009	(0.947,	1.075)	0.7817
8	1.006	(0.948,	1.068)	0.8418	0.992	(0.931,	1.056)	0.7966
9	1.022	(0.963,	1.084)	0.4699	1.024	(0.962,	1.091)	0.4538
10	0.997	(0.939,	1.058)	0.9197	0.992	(0.931,	1.057)	0.8089
11	0.981	(0.924,	1.042)	0.5341	1.016	(0.954,	1.082)	0.6204
12	1.016	(0.958,	1.078)	0.5949	0.993	(0.933,	1.057)	0.8279

* Adjusted for day of year effects (day of year, day of year^2, day of year^3), interaction between FoodNet site and day of year effects, and minimum temperature

CHAPTER 7. DO DIFFERENCES IN RISK FACTORS EXPLAIN THE SEASONAL VARIATION IN CAMPYLOBACTERIOSIS?

Abstract

Campylobacter is a leading cause of bacterial gastroenteritis in the United States and many other countries where seasonal variation in rates of culture-confirmed infections has been consistently observed. The reasons for the seasonal pattern in campylobacteriosis are unclear, but climatic factors and seasonal variation in exposures have been suggested as possible contributors. We used a case-control study conducted by the Foodborne Diseases Active Surveillance Network to examine whether the risk for *Campylobacter* infection from various exposures, and the prevalence of these exposures (proportion of individuals reporting exposure), varied seasonally. We found no evidence of seasonal effect modification of select previously-identified (consumption of chicken at commercial eating establishments, raw milk, and untreated water, and exposures to farm animals and animal stool) or plausible seasonally-varying (eating chicken cooked outdoors, and swimming in a river, lake or stream) risk factors for *Campylobacter* infection. The reasons for the seasonal periodicity observed in rates of campylobacteriosis remain unexplained.

Introduction

Campylobacter is a leading cause of bacterial gastroenteritis in the United States (U.S.) and many other countries where seasonal variation in rates of culture-confirmed infections has been consistently observed.[1, 2] It has been suggested that seasonal differences in risk factors for *Campylobacter* infection, including the consumption of raw or undercooked chicken, unpasteurized milk, and untreated water[3-7] and exposure to dogs, farm animals, and foreign travel,[3, 5, 7] contribute to the summer peak in campylobacteriosis. The occurrence and concentration of *Campylobacter* in the environment and in food sources has been shown to vary throughout the year. For example, the proportion of poultry flocks that test positive for *Campylobacter*, and the occurrence of *Campylobacter* in natural water sources, both were highest in the summer.[4,8] Even if the risk from a specific exposure remains constant across seasons, the prevalence of the exposure may vary seasonally. For example, some exposures, such as

eating undercooked chicken from a barbecue, are more likely to occur in the warm summer months.

Campylobacteriosis is not a nationally-notifiable disease in the U.S.; however, the Foodborne Diseases Active Surveillance Network (FoodNet) has conducted active, population-based surveillance for culture-confirmed cases of campylobacteriosis in select states since 1996. FoodNet also acts as a platform for conducting epidemiologic studies to better understand the problem of foodborne disease and conducted a 12-month case-control study during 1998-1999 to determine risk factors for campylobacteriosis.[3] The aims of this analysis were to investigate the seasonality of select risk factors identified in the original analysis (consumption of chicken at commercial eating establishments or at a barbecue, raw milk, and untreated water, and exposures to farm animals and animal stool, and swimming in a lake, pond, or stream)[3] and to determine if the prevalence of these risk factors varied by season.

Methods

FoodNet is a collaborative program between the Centers for Disease Control and Prevention (CDC), U.S. Department of Agriculture's Food Safety and Inspection Service, U.S. Food and Drug Administration, and participating state health departments in the FoodNet sites. FoodNet surveillance officers in the FoodNet sites have established reporting procedures and routinely contact clinical laboratories serving the FoodNet surveillance area to ensure that all incident cases of culture-confirmed campylobacteriosis are ascertained.

Over a 12-month period from 1998-1999, a case-control study of culture-confirmed *Campylobacter* infections was conducted in the FoodNet sites. In 1998, the FoodNet surveillance area encompassed seven percent of the U.S. population (21 million persons) and included the entire states of Connecticut, Minnesota, and Oregon and selected counties in California, Georgia, Maryland and New York. The case-control study methodology has been described previously.[3] Briefly, cases were drawn from the sample of residents of the FoodNet sites with culture-confirmed campylobacteriosis that were not associated with a recognized outbreak (i.e., sporadic) during the 12-month study period. Each FoodNet site attempted to enroll approximately 200 cases selected randomly from those that were reported throughout the study period. Controls were selected by sequential digit dialing based off of the telephone number of the case. Controls and cases were matched based on age (in strata of 2 to <6 years of age; 6 to <12 years of age; 12 to <18 years of age; 18 to <40 years of age; 40 to <60 years of age; 60 or older years of age) and the county of residence of the case. Informed consent was obtained for all participants prior to interview. Information on cases or controls <12 years of age was collected from a parent or guardian; all other cases were interviewed directly. Case patient interviews were conducted within 21 days of their specimen collection date, and controls were interviewed within seven days of their matched case's interview. Cases and controls were asked about exposures during the seven days prior to the case's onset of illness. Information was collected on symptoms experienced by the case, demographic and socioeconomic characteristics, as well as food consumption patterns and animal and water exposures. Season was assigned based upon the specimen collection date of the case and was categorized as follows: spring (March, April and

May), summer (June, July, and August), fall (September, October and November), and winter (December, January, and February).

The study protocol was approved by the Centers for Disease Control and Prevention Institutional Review Board (IRB) as well as the IRBs in the different FoodNet sites.

Statistical Methods

Exposures of interest (consumption of chicken outside the home at commercial eating establishments, raw milk, and untreated water, and exposures to farm animals and animal stool) were chosen because they were previously found to be associated with *Campylobacter* infection and because the prevalence of exposure was posited to vary by season. In addition, while they were not found to be independently significant risk factors in the original analysis, we included an examination of eating chicken cooked outdoors (at home and away from home) and swimming in a river, lake or stream in our analysis because they had been suggested in the literature as potentially associated with the seasonality of campylobacteriosis. Children $\leq$2 years of age and individuals reporting international travel were excluded from the analysis due to the evidence that risk factors for infants are different from those for older ages[9] and the likelihood that travelers were infected outside the U.S.

Conditional logistic regression models, which took into account the age group and county-of-residence-matching between cases and controls, were used. Case-control pairs with the same age group and county of residence were pooled into the same strata.[10] In univariate analyses, each exposure was consider alone in a model restricted to

each season, and again in a model including data from all seasons along with an interaction term between the exposure of interest and season. The likelihood ratio test was used to determine if there was significant interaction between season and the exposure of interest. Although season was not considered as a separate factor in the multivariate model, we were able to include it as part of the interaction term because the seasonal effects were incorporated into the model as components of the matching factors.[10]

To determine if there were differences in the risk due to a specific exposure after accounting for other known risk factors for infection (identified in the initial analysis of these data[3]), multivariate models including other known risk and protective factors for infection were created. These risk and protective factors included male sex, having a pet puppy and consumption of the following: any chicken cooked at home, any pink chicken, fried chicken, any turkey meat at home, any turkey at commercial eating establishment, any meat at home, any meat at commercial eating establishment, any raw seafood, and any berries bought in a store. Multivariate models were created using the entire dataset and included an interaction term between the exposure of interest and season.

Because the controls, if selected appropriately, represent the exposure pattern of the general population, the frequency of various exposures among the controls in each season was calculated. A chi-square test was used to determine whether the frequency of each exposure varied significantly by season. If the number of exposed controls was less than five, a Monte Carlo simulation procedure (the MC option in the PROC FREQ procedure in SAS v. 9.2) of Fisher's exact test p-values was used to estimate exact p-

values for these comparisons because Fisher's exact test was too computationally intensive. Multiple comparisons were done by a SAS macro using a Tukey type multiple comparison procedure after an overall chi-square indicated all comparisons were significant.[11, 12]

All statistical analyses were conducted using SAS v.9.2 (Cary, NC) and all hypothesis tests and p-values were two sided (alpha of 0.05).

Results

A total of 2,093 cases were contacted. However, 780 either met one of the exclusion criteria or refused to participate, 75 were $\leq$ 2 years of age, 178 reported traveling internationally in seven days prior to illness onset, 18 had missing county information, and 14 had a missing specimen collection date. A total of 1,028 case-control pairs were included in the analysis with the following breakdown by season: spring (171), summer (446), fall (255) and winter (156).

The seasonal distribution of cases showed a strong seasonal peak, with the greatest number occurring in the summer (Figure 7.1). When stratified by season, drinking unpasteurized milk, eating chicken cooked outdoors away from home, and swimming in a lake, river or stream were not significantly associated with an increased odds of campylobacteriosis during any season (Table 7.1). Eating chicken cooked outdoors at home was significantly associated with decreased odds of campylobacteriosis in summer and fall (data not shown). There was no effect modification of any risk (or protective, in the case of eating chicken cooked outside at home) factors by season when the risk factors were considered individually (Table 7.1)

or in models adjusted for other known risk factors for campylobacteriosis (data not shown).

Consuming chicken at a commercial eating establishment was reported more frequently during all seasons than the majority of the other risk factors studied (Table 7.1, Figure 7.2). Across all seasons, a small proportion of controls reported drinking raw milk or drinking untreated water from a lake, river, or stream. The proportion of controls that reported contact with animal stool was significantly higher in summer than in fall (p-value=0.031) (Figure 7.2). The proportion of controls who reported swimming in a lake, river or stream was significantly higher in summer than in any of the other three seasons, and was higher in spring than in winter (Fisher p-value <0.001). There was no difference in the prevalence of exposure across seasons for the other risk factors (data not shown).

Discussion

The goal of this study was to examine whether there were seasonal differences in the risk for campylobacteriosis from select exposures and whether the prevalence of these exposures varied by FoodNet site. We found no evidence that the odds of campylobacteriosis associated with eating chicken at commercial eating establishment, having contact with animal stool, drinking unpasteurized milk, drinking untreated water from lake, river or stream, having contact with a farm animal, eating chicken cooked outdoors, or swimming in a lake, river or stream vary seasonally. We did find seasonal differences in the proportion of controls that reported having contact with animal stool and swimming in a lake, river or stream. However, swimming was not significantly

associated with campylobacteriosis in any season (nor was it in the original analysis[3]) and contact with animal stool was only a significant risk factor in spring and fall. Still, these potential risk factors may be more important in summer as compared to other seasons because of the increased prevalence of exposure in summer. Thus, it does not appear that the seasonal changes in *Campylobacter* incidence are explained by seasonal variation in risk associated with these exposures.

Case-control studies conducted in other countries have also found some exposures to be more common in the summer months, when campylobacteriosis rates are highest. For instance, a recent case-control study in Finland was the first to identify swimming in natural sources of water as a risk factor for *Campylobacter* infection.[13] This risk factor may have only been identified because the study was limited to the summer season (July 1st -- September 30th) when Finland experiences their highest incidence of campylobacteriosis and swimming is most common. Another case-control study conducted in Sweden found the proportion of individuals reporting various exposures, particularly eating meat that had been grilled or consuming water from a stream or a lake, was greater during the summer months.[14]

Although we did not find evidence to suggest that the magnitude of association between the exposures examined in this study vary by season, there could be other, yet-unidentified risk factors which contribute to the burden of campylobacteriosis only in one season and thus have yet to be identified as risk factors. A few studies have examined the genetic profiles of *Campylobacter* isolates and found that some strains predominated during the summer peak in incidence. Hudson et al. (1999) conducted a study in New Zealand, where incidence also shows marked seasonality, and found

differences in the strains of *Campylobacter* that were prevalent during winter months as compared to summer months.[15] A Scandinavian study found similar results, with certain *Campylobacter* strains more likely to occur during summer months as compared to others and also found differences in strains between children and adults.[16]

Because of these changes in the prevalence or strains of *Campylobacter* in foodborne and environmental risk factors for *Campylobacter* infection, it is possible that the actual risks for illness from these exposures change seasonally as well, despite the lack of evidence from this study. Effect measure modification by season of specific *Campylobacter* risk factors has been observed in a handful of case-control studies. A study conducted in Denmark by Neimann et al. (2003) found that certain risk factors, principally barbecuing, drinking unpasteurized milk, and consumption of apples or pears, showed effect modification by season.[17] While barbecuing "in season" (June-October) was a strong significant risk factor for *Campylobacter* infection, barbecuing "off-season" (November-May) was found to be protective (although not statistically significant). Drinking unpasteurized milk in-season was protective, although not statistically significant, while drinking unpasteurized milk off-season was a significant risk factor for infection.

Other potential explanations for the seasonality of *Campylobacter* rates include complex, as-yet-unidentified interactions between age, season, and risk factors, or a role for climatic factors such as temperature and precipitation in the spread of *Campylobacter*. Preliminary analyses of FoodNet surveillance data have indicated that the seasonal peak in incidence appeared to be greatest in teenagers and older age groups (Chapter 6), and thus may be related to different risk factors by age. A recent study by

Denno et al. (2009) conducted in children (≤ 18 years of age), for instance, found a strong association between recreational water exposure and campylobacteriosis, with a population attributable fraction of over 20%.[18] This was not identified in our study as a risk factor for infection or one that varied significantly by season, although further stratification of our data by age group would have been useful had we had a large enough sample size. Climatic factors may also play a role in the seasonal distribution of campylobacteriosis. A number of studies, mostly conducted in other countries, have found a small, but significant, relationship between climatic factors such as temperature and campylobacteriosis,[19, 20] and the general pattern of culture-confirmed rates of campylobacteriosis tends to mirror that of temperature (Chapter 6).

This study has several limitations. When stratified by season, the number of cases and controls reporting certain exposures became quite small, as the original study was not designed to examine risk factors separately by season. As with most case-control studies, recall bias is a potential issue. Because of their illness, cases may have been more likely to recall certain exposures than controls, particularly those they may have associated with their illness. Additionally, the cases included in this study only represent a sample of those culture-confirmed cases that occurred in the FoodNet surveillance area and an even smaller fraction of all cases of campylobacteriosis.[21] Previous analyses have suggested that the culture-confirmed cases included in this study are similar to those culture-confirmed cases that were not included with regard to demographic and socioeconomic factors.[3] The culture-confirmed cases included in this study are likely to be more ill than those cases who did not seek care or submit a stool sample for *Campylobacter* testing.[22, 23] We have no reason to believe that the risk factors

for culture-confirmed cases are different from those that do not seek care or submit a stool sample for testing, although we have no evidence to this effect.

The reasons for the seasonal periodicity observed in rates of campylobacteriosis remain unexplained. Studies that examine whether differences in risk factors by season and age group exist would be useful. Additionally, the seasonality should be considered when investigators use molecular techniques to attribute specific *Campylobacter* strains to various exposures.

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Tables and Figures

Table 7.1. Association of risk factors with campylobacteriosis, stratified by season (spring: March, April and May; summer: June, July, and August; fall: September, October and November; and winter: December, January, and February; S). Conditional logistic regression models (adjusting for FoodNet site, county and age group) were used. Where indicated, exact models were used if case or control counts were less than 5.

Risk Factor	Season	Exposed Cases N (%)	Exposed Controls N (%)	Odds Ratio (95% Confidence Interval)	P†
Ate any chicken at commercial eating establishment (excluding deli meat, pot pie, salad)	Spring	74 (43.3%)	50 (29.2%)	**1.82** (1.14, 2.91)*	0.6756
	Summer	191 (42.8%)	105 (23.5%)	**2.68** (1.96, 3.66)*	
	Fall	121 (47.5%)	68 (26.7%)	**2.74** (1.82, 4.12)*	
	Winter	69 (44.2%)	42 (26.9%)	**2.35** (1.40, 3.92)*	
Contact with any animal stool	Spring	48 (28.1%)	31 (18.1%)	**1.79** (1.06, 3.03)*	0.7185
	Summer	104 (23.3%)	84 (18.8%)	**1.38** (0.98, 1.93)	
	Fall	47 (18.4%)	28 (11.0%)	**1.79** (1.05, 3.04)*	
	Winter	33 (21.2%)	21 (13.5%)	**1.67** (0.89, 3.11)	
Drank any unpasteurized (raw) milk	Spring	5 (3.0%)	1 (0.6%)	**5.00** (0.56, 236.49)^	0.8274
	Summer	6 (1.4%)	3 (0.7%)	**2.16** (0.45, 13.60)^	
	Fall	6 (2.4%)	1 (0.4%)	**6.00** (0.73, 275.99)^	
	Winter	1 (0.6%)	0 (0.0%)	**1.00** (0.03, ∞)^	
Drank untreated water from lake, river or stream	Spring	3 (1.8%)	1 (0.6%)	**3.00** (0.24, 157.49)^	0.4194
	Summer	23 (5.4%)	8 (1.8%)	**3.43** (1.41, 8.37)*	
	Fall	11 (4.4%)	4 (1.6%)	**4.27** (1.08, 24.67)*^	
	Winter	2 (1.3%)	3 (1.9%)	**0.66** (0.05, 5.89)^	
Had contact with a farm animal (chicken, turkey,	Spring	18 (10.7%)	9 (5.3%)	**2.85** (1.01, 8.03)*	0.4657
	Summer	52 (11.8%)	26 (5.9%)	**2.39** (1.37, 4.18)*	
	Fall	23 (9.1%)	20 (8.0%)	**1.29** (0.62, 2.70)	

cow, goat, horse or pig)	Winter	11 (7.1%)	3 (2.0%)	**3.51** (0.79,	21.83)^	
Chicken cooked outdoors away from home	Spring	7 (4.2%)	2 (1.2%)	**3.42** (0.65,	33.75)^	0.5643
	Summer	18 (4.2%)	13 (3.0%)	**1.47** (0.71,	3.07)	
	Fall	9 (3.8%)	6 (2.5%)	**1.47** (0.52,	4.21)	
	Winter					
Swim in lake, river or stream	Spring	6 (3.5%)	4 (2.4%)	**1.67** (0.32,	10.73)^	0.879
	Summer	49 (11.1%)	39 (8.8%)	**1.33** (0.83,	2.12)	
	Fall	6 (2.4%)	8 (3.2%)	**0.59** (0.16,	2.11)	
	Winter	3 (1.9%)	0 (0.0%)	**3.18** (0.33,	∞)^	

*Significant at $p<0.05$

^ Exact conditional logistic regression used

† Likelihood ratio p-value from test of interaction

Figure 7.1. Number of cases of culture-confirmed campylobacteriosis by week and season (N=1,028).

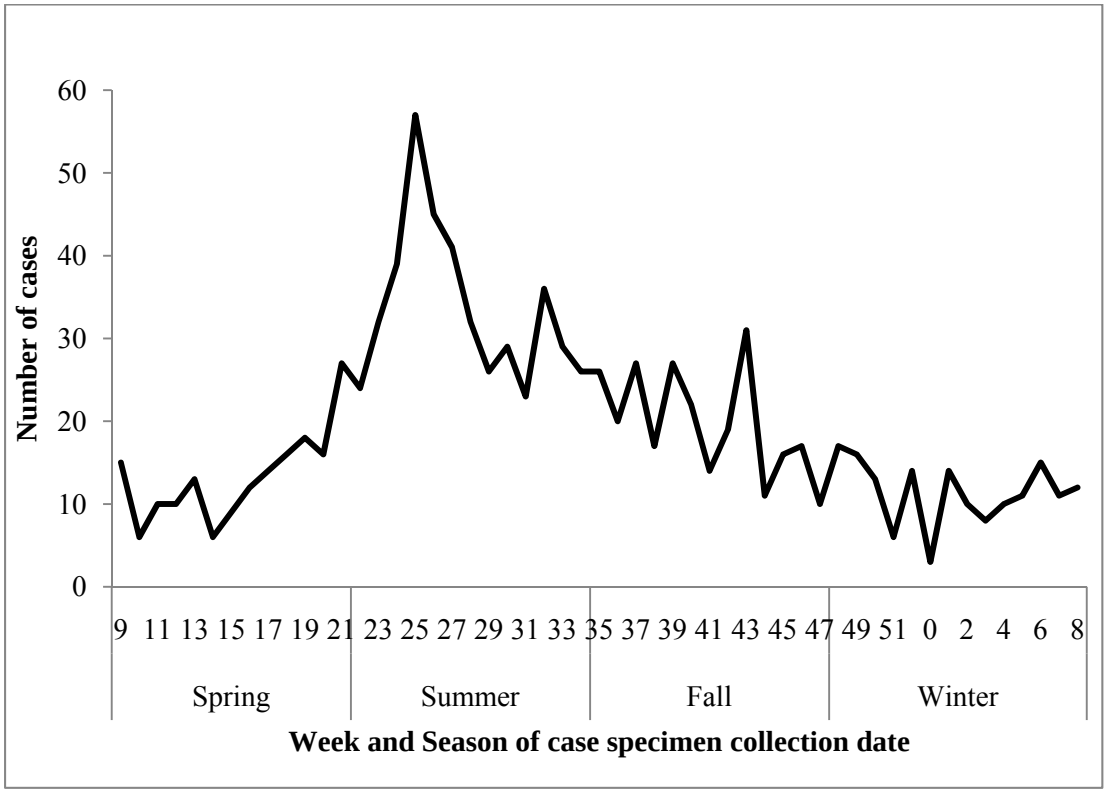
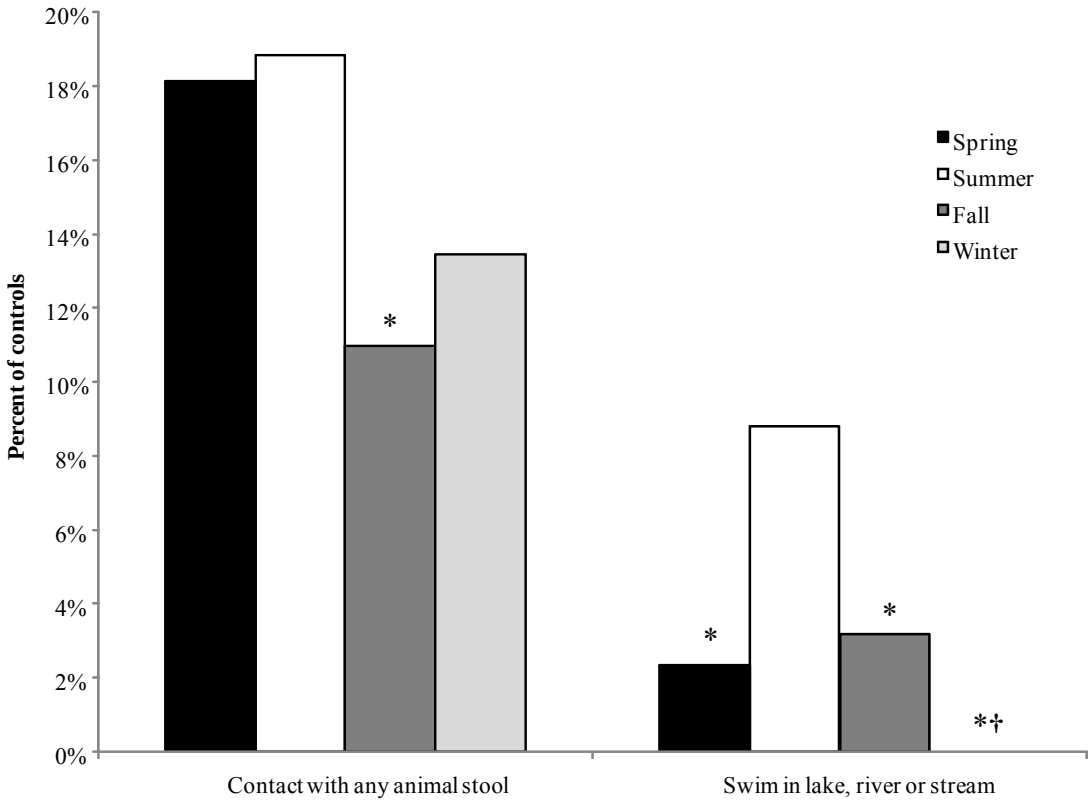


Figure 7.2. Proportion of controls reporting exposure to risk factor, by season, for risk factors with a prevalence of exposure that varied significantly by season



* P<0.05 compared to Summer † P<0.05 compared to Spring

CHAPTER 8. DISCUSSION

Conclusion

Campylobacteriosis is one of the most common causes of bacterial gastroenteritis in the United States. As campylobacteriosis is not a nationally-notifiable disease, information on this important pathogen is captured through the Foodborne Diseases Surveillance Network (FoodNet), a collaboration among select state health departments, the Food and Drug Administration, the Department of Agriculture, and the Centers for Disease Control and Prevention. Since FoodNet began conducting active surveillance for culture-confirmed infections in 1996, geographic and seasonal variation in the rates of campylobacteriosis has been consistently observed. Reasons for the geographic and seasonal variation in campylobacteriosis remain elusive.

The number of culture-confirmed cases of campylobacteriosis only represents a fraction of those that occur in the community. Samuel et al. (2004) estimated that there are 34 cases in the community for every one case of culture-confirmed *Campylobacter* that was captured in FoodNet surveillance.[1] Not every case of campylobacteriosis will seek health care as a result of his/her illness or have a stool sample submitted for testing. Differences at any of the *surveillance steps*[2] in the process from an individual becoming exposed to *Campylobacter,* to health care utilization as a result of his/her illness, clinical laboratory testing procedures, and reporting of cases to public health authorities may result in overall differences in the rates of campylobacteriosis detected by FoodNet. This study found no evidence to suggest that surveillance artifacts explain the observed geographic variation in campylobacteriosis. Differences in health care utilization or stool sample submission practices did not appear to be important in explaining the geographic

differences, though these might be expected to impact reported rates of all enteric pathogens similarly. Furthermore, despite the some variation in the process used to detect *Campylobacter* in clinical specimens, geographic differences in clinical laboratory practices appear to explain little of the geographic differences in campylobacteriosis. As the goal of FoodNet is to capture every instance of a culture-confirmed case of campylobacteriosis in surveillance, and this is confirmed by laboratory audits, reporting differences are not likely to exist.

The principal risk factors identified for sporadic campylobacteriosis in the U.S. are related to consumption of poultry, untreated surface water, raw milk, exposure to animals, and foreign travel.[3-5] Geographic differences in risk factors for campylobacteriosis, as a result of variation in the population exposure to *Campylobacter*, have not been identified. No significant differences in the risk for campylobacteriosis from selected risk factors (thought to have the largest impact and to vary geographically) were identified in this study. Some differences in the frequency of reported exposures were found across the FoodNet sites, suggesting that the impact of these exposures could vary due to the differing frequencies of exposure. Interestingly, those in California reported some of the highest frequency of chicken consumption, thought to be a major risk factor for *Campylobacter* infection.

Additionally, although temperature and precipitation were associated with campylobacteriosis, they do not explain much of the seasonality of campylobacteriosis. A modest, but significant, association was found between both a weighted average of the minimum temperature in the three to twelve days prior to specimen collection and "extreme" precipitation during the seven to fourteen days prior to specimen collection.

Although a handful of studies in other countries have found an association between temperature and campylobacteriosis, this is the first study in the U.S. to identify this association in multiple states. Furthermore, the finding of an association between sporadic cases of campylobacteriosis and extreme precipitation is novel. However, the effects were small and do not appear to explain the striking overall seasonal variation.

Future Directions

Given these findings, what factors are likely to explain the geographic variation observed in rates of campylobacteriosis? One factor that has yet to be fully explored is that of immunity. A recent study by Havelaar et al. (2009) has suggested that population immunity to *Campylobacter* in epidemiological studies may contribute to observed epidemiological features.[6] For instance, a recent case-control study of campylobacteriosis in the U.K. found that individuals who were habitual consumers of chicken had a lower risk of infection with *Campylobacter* than those who consumed chicken less often, suggesting that this risk for illness might be modified by immunity, related to frequently previous exposure to the risky food .[7] Other risk factors for campylobacteriosis, such as owning a pet dog, were also found to vary by duration of exposure. In the same study, recent dog owners had an increased risk of *Campylobacter* infection whereas those who had owned dogs for a longer period of time did not have the same risk. Furthermore, a British study suggested that variation in strain-specific immunity may help explain the epidemiology of campylobacteriosis. Miller et al. (2005) found that older age groups were more likely to be infected with "rare" serotypes of *Campylobacter*, in comparison to younger individuals, who were infected with more

common serotypes. They suggested that this pattern indicates that acquired immunity plays a role in the epidemiology of campylobacteriosis. It would be helpful to include direct or proxy measures of immunity to *Campylobacter* in future epidemiological studies.

As it is a major identified risk factor for infection that is consistently identified in case control studies, future studies should continue to better quantify the association between chicken consumption and campylobacteriosis. In the study conducted in the FoodNet sites, chicken eaten outside the home was a risk for infection, while chicken eaten at home was protective.[5] Whether this discrepancy is due to differences in food handling practices or another factor is unclear. It is still possible that part of the reason for California's high rates of campylobacteriosis is related to California's law prohibiting of labeling previously-frozen chicken as "fresh".[8] In other countries, national *Campylobacter* control programs have focused on reduction of the quantity of *Campylobacter* that occurs when poultry meat is frozen. In Iceland, for example, recent policies dictate that poultry from *Campylobacter* positive-flocks is required to be frozen before being sold to consumers. These policies seem to have led to a reduction in the reported rates of campylobacteriosis.[9] Therefore, future studies of the geographic variation in campylobacteriosis should consider the quantity of *Campylobacter* present on poultry.

The seasonality of campylobacteriosis is also still largely unexplained. This study found some differences in the frequency of exposures to factors known to be associated with the risk for campylobacteriosis, although these did not appear to fully explain the seasonality of campylobacteriosis. As we noted a stronger seasonal peak in

children and the elderly, risk factors for these age groups may have a stronger seasonally-varying component that should be explored in future studies. The evidence of modest associations between both temperature and precipitation and campylobacteriosis in this study do not appear to fully explain the seasonality of campylobacteriosis. It remains likely that the pathway between these meteorological factors, particularly temperature, and campylobacteriosis is indirect. Due to its specific growth requirements for lower than atmospheric levels of oxygen and warmer temperatures, *Campylobacter* is not thought to readily multiply in the open environment. It is possible that increasing temperature may lead to increases in vectors for *Campylobacter*, such as flies, and the role for this exposure pathway with regard to human illness has not fully been explored. In poultry operations, studies have found that using fly screens significantly reduces the proportion of poultry flocks that become colonized with *Campylobacter*.[10]

This study used multiple data sources that were collected for public health surveillance purposes. While this study did not find any striking evidence to suggest that differences in risk factors for campylobacteriosis or surveillance artifacts explain the geographic variation in campylobacteriosis or that seasonal variation in risk factors explain the seasonal peak in campylobacteriosis, this study provides a model of how one can use public health surveillance data to better understand key factors that could influence geographic and seasonal differences in reported rates of illness.

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