ABSTRACT

Title of Document: IMPACT OF RURALITY, BROILER OPERATIONS, AND COMMUNITY SOCIOECONOMIC FACTORS ON THE RISK OF CAMPYLOBACTERIOSIS IN MARYLAND

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The combined impact of community-level environmental and socioeconomic factors on the risk of campylobacteriosis were evaluated. *Campylobacter* case data (2002-2010, n=3,694) were obtained from the Maryland Foodborne Diseases Active Surveillance Network. Community-level socioeconomic and environmental data were obtained from the 2000 U.S. Census and the 2007 U.S. Census of Agriculture. Data were linked by zip code. Incidence rate ratios were derived by Poisson regressions. A subset of zip code-level characteristics was mapped. In zip codes that were

100% rural, incidence rates of campylobacteriosis were 6 times (IRR=6.18; 95%CI=3.19-11.97) that of urban zip codes. In zip codes with broiler chicken operations, incidence rates were 1.45 times that of zip codes without broilers (IRR=1.45, 95%CI=1.34-1.58). Higher rates were also observed for zip codes that were predominantly white and had high median incomes. Findings suggest that the risk of campylobacteriosis could be significantly influenced by the community and environment where one lives.

IMPACT OF RURALITY, BROILER OPERATIONS, AND COMMUNITY SOCIOECONOMIC FACTORS ON THE RISK OF CAMPYLOBACTERIOSIS IN MARYLAND

By

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DEDICATION

For my parents, who have remained sturdy sources of wisdom throughout.

For my daughters, whose cheerful patience and boundless appreciation have shown me the wisdom that endurance can reveal.

七転び八起き

Fall down seven times, stand up eight.

Acknowledgements

I would like to thank my parents and siblings for their long-standing support of my efforts to embark on a new path. I would also like to thank my children for waiting patiently so many evenings "while Mommy just had to finish one more thing before the bedtime story."

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CHAPTER 1: INTRODUCTION

Public Health Significance

Campylobacter is a leading cause of bacterial gastroenteritis in much of the developed and developing world (CDC, 2009; Butzler, 2004). In addition to the diarrhea and vomiting associated with gastroenteritis, infection with *Campylobacter* can lead to more serious sequelae, ranging from bloody diarrhea to reactive arthritis and Guillain-Barré syndrome, a demyelinating autoimmune disorder leading to nerve damage and sometimes death (Riddle, 2012). Scallan et al. (2011) estimated that *Campylobacter* causes approximately 845,000 domestically acquired illnesses in the United States each year, along with 8,463 hospitalizations and 76 deaths (Scallan, 2011). While the majority of these illnesses are estimated to be foodborne (Scallan, 2011), attributing specific infections to specific sources has been challenging.

Risk Factors

Common Risk Factors

The most commonly reported risk factors for *Campylobacter* outbreaks include exposure to undercooked poultry (Friedman, 2004), unpasteurized milk, (CDC, 2007; Heuvelink, 2009) and contaminated water (Carrique-Mas, 2005). Eating in restaurants (Kassenborg, 2004), not observing proper food preparation practices (van Asselt, 2009) and traveling abroad (Kassenborg, 2004; Ravel, 2011), have also been associated with both outbreaks and sporadic (non-outbreak) cases of campylobacteriosis. Common risk factors in developing countries include extensive exposure to contaminated foods through

local markets (Bodhidatta, 2013) and a potential predisposition to enteric infection arising from malnutrition (Brown, 2003; Gupta, 2011).

Environmental Risk Factors

Additional risk factors for sporadic infections include contact with pets (Friedman, 2004; Acke, 2011), contact with farm animals and livestock (Doorduyn, 2010; Potter, 2003), and contact with animal feces (Chaban, 2010), especially among ruminants. (Arsenault et al., 2012) Significant associations between living in rural areas and risk of campylobacteriosis also have been identified in Europe and Canada (Green, 2006; Spencer, 2012; Fitzenberger, 2010). Moreover, a specific feature of rural environments, animal density, has been identified as a significant predictor of *Campylobacter* incidence in Canada and New Zealand (Green, 2006; Spencer, 2012).

Sociodemographic Risk Factors

Several sociodemographic risk factors for campylobacteriosis have also been identified, the two most consistent being gender (males) and age (less than 5 yr) (Carrique-Mas, 2005; Green, 2006; Spencer, 2012; Fitzenberger, 2010). Previous studies have also evaluated socioeconomic factors associated with the incidence of *Campylobacter* infection and the findings suggest that these infections may occur more frequently among individuals characterized by higher socioeconomic status (Green, 2006; Simonsen, 2008). Moreover, Samuel et al. (2004) reported that the incidence of campylobacteriosis among African-Americans was lower than that among other ethnic groups across multiple sites in the United States, although hospitalization rates for this

group were higher. (Samuel, 2004) Although these findings could be influenced by differentials in illness reporting among varying races and ethnic groups, they do shed light on the complex interplay of multiple risk factors for campylobacteriosis among varied population groups.

Research Rationale

The overall goal of my Master's thesis research project was to examine how the interplay between community-level environmental and sociodemographic factors influence the risk of campylobacteriosis. A wide body of research exists regarding the various risk factors contributing to infection from *Campylobacter*, but previous reports have largely resulted from population-based case-control studies focused on individual-level data. To my knowledge, no U.S. study has yet to examine the combined effect of community-level environmental and socioeconomic risk factors on the risk of campylobacteriosis.

My Master's thesis linked Maryland Foodborne Diseases Active Surveillance (FoodNet) data to U.S. Census data and USDA Census of Agriculture data at the zip code level to evaluate associations between community-level environmental and socioeconomic risk factors and the incidence of *Campylobacter* infections in Maryland. My central hypothesis was that community-level factors—notably rurality—play a principal role in the risk of infection from exposure to *Campylobacter*. After extensive examination of the data, I posited that residence in a rural zip code might be a predictor of campylobacteriosis incidence. As a consequence, risk of campylobacteriosis might

derive less from individual behaviors or sociodemographic backgrounds than from geographic location of an individual's residence.

The specific aims of this study were to examine, at the zip-code level:

1. the impact of community-level socioeconomic factors on *Campylobacter* incidence;
2. the degree to which rurality influences the risk of campylobacteriosis; and
3. whether the presence of animal operations impacts risk of campylobacteriosis.

My findings will help elucidate the complex interplay of sociodemographic and environmental risk factors contributing to risk of campylobacteriosis, specifically at the community (zip-code) level. These findings will also assist in identifying, and potentially predicting "hot-spot" communities that bear high burdens of this illness. This research will also address significant research gaps concerning potential health disparities in the risk of infectious disease (Abara, 2012) insofar as geographic variables are concerned.

The remainder of this thesis is organized into three additional Chapters. In Chapter 2, I will briefly describe the organism *Campylobacter,* its clinical presentation, list some of its more prevalent species, and mention some key growth and survival characteristics as they relate to *Campylobacter's* impact on public health. Then I will explain where *Campylobacter* can be commonly found, such as avian and mammal reservoirs and water sources. After briefly discussing issues of seasonality and antimicrobial susceptibility, I will expand upon the common risk factors for campylobacteriosis in humans – notably poultry and contaminated water consumption. In

a more detailed discussion on environmental risk factors, I will review rurality, animal husbandry and animal density. After this, in Chapter 3, I will discuss sociodemographic risk factors for infection such as age, gender, race and ethnicity, socioeconomic status and level of education. Chapter 4, which follows, is the manuscript detailing the research study, and Chapter 5 encompasses a discussion and overall conclusions about the public health significance of this research.

CHAPTER 2: BACKGROUND ON CAMPYLOBACTER

Overview

Campylobacter is a Gram-negative, spiral-shaped motile bacteria most often associated with gastroenteritis and recognized as one of the leading causes of bacterial foodborne illness in the U.S. and worldwide (Samuel, 2004). Scallan et al. (2011) have estimated that *Campylobacter* causes approximately 845,000 domestically acquired illnesses in the United States each year, along with 8,463 hospitalizations and 76 deaths (Scallan, 2004). Recent Morbidity and Mortality Weekly Reports (MMWR) issued by the Centers for Disease Control and Prevention (CDC) indicate that *Campylobacter* is the second most common source of infection in the United States after *Salmonella*. (MMWR, 2011) Similarly, the World Health Organization (WHO) reports that *Campylobacter* causes more cases of diarrhea in the developing and developed nations than *Salmonella*. (WHO, 2011, Fact Sheet) Overall, the CDC estimates that 2.4 million people worldwide are affected annually by *Campylobacter*, resulting in 124 deaths. (CDC, 2011)

Clinical Presentation

Clinical presentation of campylobacteriosis is similar to that of enteritis stemming from other common foodborne pathogens such as *Salmonella*: diarrhea, bloody diarrhea, abdominal pain, nausea and sometimes vomiting (Heymann, 2008). These symptoms may follow a brief period of headache, fever, malaise or myalgia occurring 12-24 hours prior to the onset of diarrhea. (Allos, 2009) Some cases of campylobacteriosis mimic symptoms of appendicitis or inflammatory bowel disease (Heymann, 2008) while others

remain asymptomatic. Illness resolves itself within two to five days in most cases, though prolonged illness or relapses may occur. (Heymann, 2008; Allos, 2009; Galanis, 2007). Immunocompromised individuals are susceptible to prolonged cases of intestinal infection. Although complications are rare, they may include cholecystitis (gallbladder infection), pancreatitis and gastrointestinal hemorrhage (Allo, 2001). Immunoreactive complications include reactive arthritis and Guillain-Barré syndrome, a demyelinating autoimmune disorder leading to nerve damage and sometimes death (Riddle, 2012).

Genus and Species Description

Although *Campylobacter* is believed to have been first identified by Theodore Escherich in the late 19th century, it has only been recognized as a cause of human illness since the 1980's (Silva, 2011). Since then, it has become increasingly significant in foodborne illness disease attribution. Currently, Friedman et al. (2001) estimate that infections from campylobacters are the most widely reported causes of acute gastroenteritis infections in the developed world.

The genus *Campylobacter* is comprised of an estimated 14 to 20 species (Debruyne, 2005; Fernandez, 2008) and of these species *Campylobacter jejuni and Campylobacter coli* are most frequently associated with cases of campylobacteriosis. Until the mid-1990s, *Campylobacter jejuni* was taken to be the predominant species in the etiology of campylobacteriosis and indeed, of all diarrheal illness, identified as causing more cases of diarrheal illness than *Salmonella*, species of *Shigella* and *Escherichia coli* O157:H7 combined. (Blaser, 1983; MacDonald et al., 1988). Since that

time, however, the importance of *Campylobacter coli* in disease attribution has become more widely recognized (Gillespie, 2002). Case-control studies have increasingly sought to differentiate between *Campylobacter* species (Gillespie, 2002; Sopwith, 2003) pointing to higher rates of *C. coli* infection than previously presumed (Gürtler, 2005). To address possible underreporting of campylobacteriosis, current research is focusing on isolating additional species of *Campylobacter* which act as human gastrointestinal pathogens (Lastovica, 2000; Fernandez, 2002). The link between species such as *C. lardis* and *C. upsaliensis* and human gastrointestinal disease, especially in the developing world, is being examined (Matsuda and Moore, 2004). Nonetheless, the two species of *Campylobacter* that play the largest role in foodborne illness remain *C. jejuni* and *C. coli* (Humphrey et al., 2007) and are therefore the focus of this thesis.

Growth and Survival Characteristics

In the *Campylobacter* genus, it is the thermophilic species which are most often associated with gastrointestinal disease in humans. (Snelling, 2005) It is suggested that since thermophilic *Campylobacter* species grow best between 37°C and 42°C, but not below 30°C that they be called thermotolerant (Levin, 2007). This is significant insofar as it relates to survival rates of *Campylobacter* on food contact surfaces. Despite isolation of some *C. jejuni* on surfaces at temperatures below 30°C (De Cesare et al., 2003), most *Campylobacters* do not survive or multiply well outside of animal hosts or in food during processing and storage (Park, 2002), unlike other common foodborne pathogens such as *Salmonella* (Hong, 2004). Moreover, these species are easily inactivated by heating and

freezing (Silva, 2011). It is therefore significant that despite this fragility, *Campylobacter spp.* are nonetheless responsible for the highest percentage of bacterial diarrhea in the developed world, affecting 1.1% and 1% of the populations in the UK and USA, respectively, every year (Snelling, 2005) and contributing to worldwide financial losses of $1.3–6.2 billion annually in the USA (Forsythe, 2000).

Animals as a reservoir for *Campylobacter*

Wild Avian Species

C. jejuni and C. coli commonly colonize the intestinal tracts of most mammals and birds (Van Damme, 2004) in what has been presumed to be a harmless commensal relationship (Hendrixson and DiRita, 2004). A variety of *Campylobacter* species have been found in many types of wild birds, especially among raptors and opportunistic feeders that forage at ground level, as documented in studies from Sweden (Waldenström, 2002). Wild geese in the U.K. (Colles, 2008) have been shown to carry *Campylobacter* and migratory geese may be responsible for fecal contamination of water supplies in Norway (Varslot, 1996). Starlings were also found to carry strains of *Campylobacter* (Colles, 2008). Similarly, a study in England pointed to ducks as a source of freshwater *Campylobacter* contamination at human bathing sites (Obiri-Danso, 1999). It should be kept in mind, however, that the *C. jejuni* strains found in the geese studied by Colles et al. (2008) did not represent a high proportion of strains sampled from human disease cases. Although researchers do not rule out the possibility that such strains could cause human disease or waterborne outbreaks, Colles et al. (2008) posit that wild and/or

migratory birds are not a significant source of camplybacteriosis in humans (Colles, 2008). Indeed, Pulsed Field Gel Electrophoresis (PFGE) analyses have indicated that strains in wild birds are largely different from those in humans interesting!, but that strain similarities occur in birds associated with human activities (Broman et al., 2004). Similarly, many species of gulls contain novel *Campylobacter* species in their intestinal flora unrelated to human infections, but the fact that gulls feed in recreational waters contaminated by human refuse could make them vectors of *Campylobacter* infection to humans (Ramos, 2010; Lu, 2011).

Domesticated Avian Species, notably poultry

Ubiquity of Campylobacter in chicken

The primary avian species associated most frequently with campylobacteriosis in humans is chickens. It is commonly thought that *Campylobacter* is endemic to chickens (Humphrey, 2007). Although the source of infection to young chicks has not yet been clearly identified, flocks tend to become infected after about 3 weeks. Caecal contents have been measured at high levels (106±107 cfu/g) at this early stage. (Corry, et al., 2001). However, there is little evidence of vertical transmission of *Campylobacter* in chickens (Callicott, 2006). Most studies in which *Campylobacter* has been measured in eggs have postulated that this transmission is due to fecal contamination (Newell and Fearnley, 2003). Cox et al. (2012) suggest that transmission from hen to egg is possible, not from vertical, transovarian transmission, rather from contamination of shell membranes and albumen through fecal exposure (Cox et al., 2012). Further research is needed in this area.

It is likely that the bulk of transmission of *Campylobacter* to young chicks after hatching occurs through other means. Possible sources of thermophilic *Campylobacter* transmission to young chickens include unchlorinated drinking water, wild birds, rodents, flies, cross-contamination from farm boots and clothing, or cross-contamination during the "thinning" process preceding slaughter (Corry, et al., 2001; Bahrndorff, 2013). Transmission through rodents has also been suggested (Meerburg, 2007). Once a broiler flock becomes infected, *Campylobacter* spreads rapidly. Some studies indicate that flock infection reaches more than 40% of flocks by the time the chicks are 4 weeks old and greater than 90% by 7 weeks (Evans and Sayers, 2000). Other studies suggest infection rates reach nearly 100% within two to three weeks (Newell and Wagenaar, 2000; Newell, et al., 2001; Newell and Fearnley, 2003). Therefore, the intestinal tract of poultry, especially the cecum and colon, become rapidly colonized with high numbers of *Campylobacter spp.* quite early on in the life of a chicken (Rosenquist et al., 2006). These bacteria are easily transferred to the skin and muscle of a carcass during processing through leakage or rupture of the intestinal tract (Berrang et al., 2001). The microenvironment of the skin fosters growth of *Campylobacter*, especially the species *C. jejuni* which can survive in crevices, become entrapped inside feather follicles with water, and trapped under the surface water layer, even after rinsing and storage at 4°C (Chantarapanont, et al., 2003). At higher temperatures, *Campylobacter spp.* has been shown to survive and grow within the controlled atmosphere of packaging at room temperature (Scherer et al., 2006). Studies of the impact of environmental temperature reveal that during months with higher temperatures, higher number of isolates have been

obtained from poultry, the highest being during June in the northern hemisphere (Jorgensen et al., 2011). The issue of seasonality will be addressed below.

Methods of rearing chicken

Regarding flock susceptibility to infection, some have suggested that less-intensively reared chickens may have higher exposure to environmental sources of *Campylobacter* species. A Danish study indicated that chicken flocks raised according to an organic and/or free-range system of production exhibit higher rates of *Campylobacter* prevalence than flocks raised in intensive production systems (Heuer et al., 2001). Studies also indicate a difference in *Campylobacter* species according to rearing system. Studies with intensively reared birds generally show 80 to 90% of isolates being *C. jejuni* and the remainder being *C. coli* (Jorgensen et al., 2002). But a British study focusing on interactions between campylobacter species, bacteriophages, and other potential anti-*Campylobacter* agents, compared intensively-reared, organic and free-range poultry flocks and found an alternation of species as the chicks matured. Their study revealed that *C. coli* was the predominant *Campylobacter* species isolated from both organic and free-range chickens, 92% in organic birds and around 43% in free-range birds. Interestingly, results indicated that both types of chickens had been colonized by *C. jejuni* first, and that organic and free-range birds exhibited different species colonization at slaughter (El Shibiny, 2005). The difference may lie in slaughter age: intensively reared birds are slaughtered at 36 to 42 days old, free-range birds are slaughtered at around 56 days old, and organically produced birds are slaughtered at around 73 days old (Colles, 2011). It could be that the intensively reared birds are slaughtered before a shift in *Campylobacter* species, from *C.jejuni* to *C.coli*. These changes in *Campylobacter* type

appear to occur during the life of a broiler chicken and may be influenced by bacteriophages and bacteriocins. The implication is that resident populations of campylobacters may be succeeded by environmental campylobacters through competitive advantage or through synergistic action of antimicrobial agents (El Shibiny, 2005).

Industrial cross-Contamination

The significance of these issues to human campylobacteriosis is two-fold. First, the variation in species dominance could be implicated in a larger, more important issue, namely that of antimicrobial susceptibility. In a 2005 study of retail chicken in Maryland, Cui et al. (2005) found that while *Campylobacter* contamination was more frequent in organic chickens, the pathogens from organic animal production showed more antimicrobial susceptibility (Cui, 2005). The issue of antimicrobial susceptibility will be addressed further on in this thesis.

Secondly, since poultry is a main source of infection among humans, it is important to understand which industry-specific interventions would be the most effective in reducing exposures and subsequent illnesses, given the species and strains of *Campylobacter* present among broiler flocks. According to the European Food and Safety agency (EFSA), exposure to broiler meat, through preparation or consumption, could account for 20-30% of human campylobacteriosis cases in Europe, while 50-80% may be attributed to the chicken reservoir as a whole (ESFA, 2010). Approximately 80% of raw chicken meat sold in the United Kingdom has been found to be contaminated with *Campylobacter* (Jorgensen, Bailey 2002 in El Shibiny). Similarly, Mullner, et al. (2009) provided evidence that poultry caused an estimated 58-76% of cases in New Zealand,

making chicken the leading cause of human campylobacteriosis in New Zealand (Mullner et al., 2009). These and other findings influenced national policy leading to implementation of poultry industry-specific interventions which resulted in observable declines in human notified cases in 2008 (Sears, 2011). Rosenquist et al. (2003) developed model simulations designed to predict the effect of different mitigation strategies of campylobacteriosis and showed that interventions resulting in a 2 log reduction of the number of *Campylobacter* on the carcasses of chickens could reduce the incidence of campylobacteriosis associated with consumption of chicken meals by 30 times (Rosenquist et al., 2003).

Another industrial intervention may involve the use of fly screens. For example, a Danish study focusing on the use of fly screens as biosecurity measures in chicken houses suggested that such screens could reduce prevalence of human campylobacteriosis through a reduction of *Campylobacter spp* infection among broilers. They found that the prevalence of *Campylobacter* spp.–positive flocks was significantly reduced, from 41.4% during 2003–2005, before fly screens, to 10.3% in 2006–2009, with fly screens (Bahrndorff, 2013).

Newell et al. (2001) point to cross-contamination through crate-contamination, which commonly occurs during poultry processing after processing and suggested disinfection of crates as a standard industrial procedure (Newell Shreve, 2001).

Dasti et al. (2010) remind us that in addition to exposure pathways, host factors might also play a role in the pathogenesis of campylobacteriosis in humans, noting that

campylobacters exploit different adaptive strategies in order to establish themselves in avian reservoirs or during the course of human infection. (Dasti, 2010)

Nonetheless, though the exposure pathway is difficult to trace, most past and current point to human exposure to *Campylobacter,* through exposure to raw or undercooked chicken. Since chicken consumption among various human populations is high (Silva, 2011) this remains a significant source of human campylobacteriosis. (Friedman, Niemann, et al., 2000) This issue of exposure to *Campylobacter* through contaminated poultry is further addressed in a subsequent section.

Mammals

In addition to avian species, mammals are also commonly colonized by *C. jejuni* and *C. coli,* most notably ruminants (Van Damme, 2004) associated with farming (Brown, 2004) and domestic pets (Acke 2011).

Livestock, notably ruminants

Among mammals, *C. jejuni* has been isolated from cattle and sheep, animals most often associated with farming. In a UK study examining frequency and distribution of *Campylobacter spp.* across a rural area shared by dairy farm and recreational users, Brown et al. (2004) sampled livestock and wildlife feces, environmental water and soil. They found *C. jejuni* to be the most prevalent species present in the area delimited by the study, and cattle feces to be the greatest source of *C.jejuni* (36%) followed by water (15 %). *C. coli* isolates, of which 17% were found in water and 21% in sheep, were only rarely found in the other samples; *C. lari* was found in all sample times but in moderate numbers (7% in birds, 5% in water) and *C. hyointestinalis* was only found in cattle (7%)

and birds (1%). Their spatial analysis indicated that the greatest risk of *Campylobacter* exposure comes through exposure to cattle feces whose distribution is limited to the farming area, as opposed to being widely distributed throughout the environment (Brown, 2004). Stanley et al. (2003) provide an excellent review article on cattle and sheep as reservoirs of *Campylobacter spp.* pointing out that fecal excretion rates among young cattle are very high, and that both sheep and cattle shed *Campylobacter* intermittently throughout their lives. They cite and summarize numerous studies indicating that animal husbandry, notably of ruminants, plays a key role in the global contamination cycle of *Campylobacter* (Stanley et al., 2003). Another convenient summary table is provided by Humphrey (2007) who took data from publications from 21 countries and compiled a list of "Mean % positive samples" of *Campylobacter* isolated in food animals. His list provides a good overview of *Campylobacter* incidence among dairy cows (30.0%), beef cattle (62.1%), sheep (31.1%), pigs (61.0%) and chicken flocks (58.7%), among others. (Humphrey, 2007).

Extensive studies focused on other farm animals such as pigs will not be addressed here. For further information see: Boes, 2005; Wright, 2005 and Fosse, 2009. The very interesting issue of *Campylobacter* transmission between animal groups will also not be addressed, but in a technical paper prepared for the New Zealand Ministry of Agriculture and Forestry, Marshall and French (2010) provide a very detailed view on modeling of *Campylobacter* carriage and transmission between animal groups (Marshall et al., 2010).

Domestic animals

In addition to avian and mammal species, domestic animals have been shown to carry *Campylobacter*. In Canada, Chaban et al. (2010) conducted an extensive study determining quantifiable levels of many *Campylobacter* species shed by dogs, both healthy and diarrheic, and found that domestic dogs carry a wide range of *Campylobacter*, especially if they have diarrhea. The researchers felt that these findings were relevant to ecological and public health concerns regarding campylobacteriosis.

However, in a cluster analysis of *C. jejuni* isolates from domestic pets in Ireland, Acke et al. (2011) compared the genetic similarity of isolates from pets to isolates in retail food and to clinical cases in humans. They found few clusters containing isolates from dogs and human cases, thus providing little evidence to support the notion that domestic animals represent a real health risk to humans. This contradicts a number of other studies which find increased risk of campylobacteriosis due to presence of a pet. These will be addressed later in the section on Commonly Reported Risk Factors for campylobacteriosis.

Water

The full range of *Campylobacter* species is also found in surface waters but not all are naturally occurring. Natural populations of *C. lari*, have been measured in sea water (Obiri-Danso, 2001). Urease-positive thermophilic campylobacters (UPTC) have also been measured in sea water, but are believed to originate from birds (Obiri-Danso, 2001) as is often the case for coastal waters, as well as estuaries, rivers and lakes. One Finnish study analyzed seven lakes and 15 rivers for five consecutive seasons and found 17.3%

were positive for *Campylobacter* spp., of which 45.8% contained *C. jejuni*, 25.0% contained *C. lari*, 4.2% contained *C.coli*, and 25.0% was comprised of unidentified *Campylobacter* isolates. The researchers attributed higher numbers of campylobacter-positive samples in summer to bird fecal contamination, rather than naturally occurring populations of *Campylobacter spp*. (Hörman, 2004). This is consistent with the fact that the majority of Campylobacteraceae are not able to multiply outside of animal hosts and survive poorly in the environment due to light and temperature sensitivity (Levin, 2007).

Interestingly, these very characteristics have previously led researchers to assume that water systems could be easily purged of contamination. However, as Carter et al. (2009) point out, this turns out not to be the case. High levels of *Campylobacter* species associated with foodborne illness, such as *C. jejuni* and *C. coli,* are continually measured in water systems worldwide (Carter et al., 2009). Since most species of *Campylobacter* tend not to exist naturally in the environment, but derive from direct fecal deposition, pasture runoff, and sewage outflow, (Obiri-Danso, 1999; Savill, 2001; Eyles, 2006; Fong, 2007; Sopwith, 2008; Carter, 2009; Schang, 2012) the potential for human infection remains high. Jones et al. (2001) provide an excellent review of research regarding *Campylobacter* in water, sewage and the environment (Jones et al., 2001).

There is evidence that if introduced to a subsurface aquifer through livestock-contaminated water run-off, *Campylobacter* can persist and be subsequently re-transmitted to animal flocks; in other words, run-off from a dairy farm may be responsible for introducing *Campylobacter* into broiler flocks (Pearson, 1993; Elliott, 2011). Similarly, Nygard (2004) suggests that a water-animal-water cycle may be involved with ruminants such as cattle citing a British study revealing cattle as a source

of contamination for ground water (Stanley, 1998). In a Finnish study, cattle exposed to *Campylobacter* from a nearby lake were more campylobacter-positive in summer or in autumn after the grazing period than after the winter, when the animals were inside and their drinking water source was municipal chlorinated tap water. They were then exposed to the same lake water the following spring, suggesting a water–animal–water cycle as a model for maintaining water contamination (Hänninen, 1998). This is corroborated by Michaud et al. in Canada, (2004) who, after finding stronger associations of campylobacteriosis to ingestion of private well water than ingestion of chicken, suggest that these results are consistent with the hypothesis that the waterborne route of infection may be the common underlying pathway linking infection in humans, poultry, other domestic animals, and wild birds. (Michaud, 2004). Pérez-Boto et al. in Spain (2010) found well water to be epidemiologically linked as the source of *C. coli* infection in poultry breeders (Pérez-Boto, 2010). The implication is that environmental pathways may be more significant than independent risk factors in acquiring campylobacteriosis (see later discussion on strain ST-45 and the link to rurality).

Noteworthy research in this area involves possible bioremediation of *Campylobacter*-infected surface waters through planktonic organisms such as *Daphnia* which could be biomanipulated into enhanced grazing, thus reducing the densities of *C. jejuni* in drinking water reservoirs and recreational water bodies. (Schallenberg, 2005).

Seasonality

Incidence of *Campylobacter* infection exhibits seasonal variations, tending to rise in the late summer in temperate climates. These fluctuations are important to take into

account when evaluating data regarding fluctuations in rates of human cases of campylobacteriosis. In a surface-water sampling study conducted over five consecutive seasons in Finland, *Campylobacter* was the most frequently isolated enteropathogen (17.3% of the samples collected). The highest number of samples positive for *Campylobacter* was collected in May 2001, 43.3% compared to other sampling times ($P < 0.05$). (Hörman, 2004) As summarized by Humphrey et al. (2007) for the United Kingdom, campylobacteriosis cases peaked in May in Northwest England, (Sopwith et al., 2003), and in late June/early July in Scotland, with this increase evident in more rural or semi-rural areas than urban ones (Miller et al., 2004; Humphrey, 2007). Nichols (2005) found an annual increase in *Campylobacter* infection in England and Wales starting in May and peaking in early June (Nichols, 2005). Sears et al. (2011) found the largest declines in campylobacteriosis notification in New Zealand to take place in the winter months (Sears, 2011). Green et al. (2006) found clear peaks in *Campylobacter* infection in Canada during the summer and fall months, with the lowest number of cases occurring in the winter and spring (Green, 2006).

A number of studies point to temperature variation as being a possible cause of variations in *Campylobacter* prevalence because different species thrive at different temperatures. In a Danish population-based study, Nielsen et al. (2012) found that incidence of *C. jejuni* and *C. coli* infection varied seasonally, with incidence peaking in late summer months, whereas *C. concisus* exhibited an almost constant monthly prevalence (Nielsen, 2012). Similar results were found in Finland by Hörman et al. (2004) who detected *Campylobacter spp* less frequently in lakes and rivers during winter than in spring, summer, or autumn (Hörman, et al.). Kovats et al. (2005) applied a

regression analysis to data linking temperature and cases of campylobacteriosis across Europe, Canada, Australia and New Zealand, finding a distinct peak of infection in the spring for many areas. (Kovats, 2005).

Interestingly, the various species of *Campylobacter* thrive differently depending on water temperature and amount of solar radiation, two factors which have an impact on the survival and recovery of the different *Campylobacter* species (Hörman, 2004). Most studies point to lower temperatures being more optimal to *Campylobacter* growth than higher; for example *C. jejuni* and *C. coli* survive in cold water (at temperatures below 10°C) much longer than they survive in water at temperatures higher than 18°C (Hörman, 2004) though *Campylobacter lari* and urease-positive thermophilic campylobacters (UPTC) seem to survive longer in sea waters (Obiri-Danso, 2000). This would imply that *Campylobacter spp.* should be more frequently isolated in winter months, when water temperatures and solar radiation levels are lower, than in spring and summer. The findings of Obiri-Danso et al. (2000) did corroborate this, finding low numbers of *C. jejuni* and *C. coli* in Danish coastal waters in the summer, especially in the afternoon, suggesting combined effects of higher temperatures and higher levels of U.V. radiation. How then can the numerous studies finding higher numbers of *C. jejuni* and *C. coli* in the late summer be explained? Obiri-Dano et al. (1999b) suggest that the somewhat counter-intuitively high frequency of *Campylobacter spp.* measured in coastal waters may result from the continuous nature of inputs from sewage treatment facilities and agricultural run-off as well as complex interactions between migrating avian species such as mallard ducks and movement of incoming water over sediment surfaces (Obiri-Danso, 1999b).

Campylobacter seasonality driven by air temperature has been studied extensively. Louis et al. (2005) found increased campylobacter rates to be correlated with air temperature with the most striking seasonal effect observed for children under 5 years old (Louis, 2005). Similar studies supporting a seasonal peak in spring and summer are summarized by Sopwith et al. (2008): a European study (Nylen et al., 2002) showed that the timing of the seasonal peak varied, occurring earlier in Wales (weeks 23–27) than in Scotland (weeks 24–27) and the Nordic countries (weeks 29–35), and a New Zealand study indicated a marked difference in the seasonality between the North and South Islands (Hearnden et al., 2003). Some researchers refer to climate-induced temperature changes (Baker 2007) and the effect this may have on populations of flies which transmit *Campylobacter spp.* Nichols (2005) hypothesizes that worldwide seasonal changes in the incidence of campylobacteriosis are due to changes in fly populations and their contact with human and animal feces. Other studies have observed a connection to practices of animal husbandry for both poultry and ruminants. (Louis et al., 2005) Hanninen et al. (1998) measured higher numbers campylobacters shed by dairy cows in the summer, when the cattle were drinking lake water, than in the winter, when they were provided with chlorinated municipal water, providing an opportunity for heightened recontamination of surface water in summer months.

Antimicrobial Susceptibility

Seasonality can be linked to variations in antimicrobial susceptibility in various *Campylobacter* species. In the Netherlands, van Hees et al. (2006) found an inverse relationship between the annual incidence rates of infection from July through September

and the rates of floroquinolone and macrolide resistance among *Campylobacter* isolates (van Hees, 2006). In the U.S., Gupta et al. (2004) compiled data from a variety of broad studies such as the sentinel county study and from the National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria, finding that overall *Campylobacter* resistance to fluoroquinolones has risen over the past decade and the prevalence of ciprofloxacin-resistant *Campylobacter* increased significantly from 13% to 19% from 1997-2001. This is an issue of concern, since severe *Campylobacter* infections in adults are commonly treated with ciprofloxacin. The rise of fluoroquinolone resistance may result in ineffective treatment when fluoroquinolones are used. The authors refer to numerous studies which suggest that the use of fluoroquinolones in food-producing animals might be linked to fluoroquinolone-resistant *Campylobacter* species (Gupta, 2004). As of September, 2005, the Food and Drug Administration (FDA) of the United States suspended all fluoroquinolone use in poultry production, with the goal of eliminating on-farm selection of fluoroquinolone-resistant strains of *Campylobacter.* Price et al. (2007) point out the existence of stable reservoirs of fluoroquinolone-resistant strains in poultry production facilities and suggest that these strains may persist after reduction of fluoroquinolone use (Bull, 2006). Their own research examining the prevalence of fluoroquinolone-resistant strains in two conventional poultry producers in Pennysylvania and Maryland over a two-year period, before and after the ban, suggests that despite reductions of fluoroquinolone, fluoroquinolone-resistant strains of *Campylobacter* may persist even after on-farm use of fluoroquinolones has ceased. (Price, 2007)

Antimicrobial resistance may also be linked to the method of animal production. In Maryland, Cui et al. (2005) analyzed conventionally and organically raised retail chicken found in Maryland stores and found 76% of organic (n=198) and 74% of conventional (n=61) to be contaminated with *Campylobacter spp.* Of these, conventional chickens tended to be more contaminated with *C. jejuni* (62%) than *C. coli* (40%) Antimicrobial susceptibility tests revealed that all campylobacters were susceptible to chloramphenicol, that resistance to tetracycline was most common (78%), followed by resistance to erythromycin (46%). More isolates from the conventional chickens (20%) were resistant to ciprofloxacin than those from the organic chickens (5%), but rates of resistance to erythromycin and tetracycline were higher in organic chicken isolates (49% and 81%, respectively) than conventional chicken isolates (36% and 69%, respectively). The researchers highlighted the need for more abundant baseline data on microbial susceptibility according to varying animal production systems (Cui et al., 2005).

Guévremont et al. (2006) evaluated antimicrobial resistance among isolates from poultry, swine and humans in Canada and observed that among isolates from broiler chickens, rates of resistance to streptomycin and to tetracycline were both 50% as compared to 56% tetracycline-resistant human isolates. Among *C. jejuni* isolates in broilers, 39% were resistant to tetracycline, compared to 67% in humans. Among *C. coli* isolates from pigs, rates of resistance were 59% for clindamycin, 61% for erythromycin, 67% for streptomycin, and 68% for tetracycline. The public health concern is that antimicrobial resistance in animal reservoirs of *Campylobacter* could have a negative impact on treatment of campylobacteriosis infection in both animals and humans. (Guévremont, 2006)

In their review of *Campylobacter* as a foodborne pathogen, Silva et al. (2011) point? out that since there are no international criteria for susceptibility testing of *Campylobacter spp.* and there is a lack of standardization among European countries in how to monitor antimicrobial resistance, a debate exists with regard to how to interpret data reported by numerous studies on this topic and to evaluate their concomitant discrepancies. However, there seems to be a general consensus that antibiotic resistance is evolving quickly and is having a dramatic effect in the environment of food animal production. This is especially true as multiple drug resistance becomes an increasingly cogent issue in public health (Silva, 2011).

CHAPTER 3: RISK FACTORS FOR CAMPYLOBACTERIOSIS IN HUMANS

Commonly Reported Risk Factors for Campylobacteriosis

Having established the ubiquity of *Campylobacter* in the environment and animals, it is now essential to describe some common exposure pathways that have an impact on human health. I will focus mainly on exposure through chicken and contaminated water, although other common risk factors will be mentioned such as exposure to contaminated milk, other contaminated foods, pets, and foreign travel.

Chicken and Poultry

As one of the most commonly reported foodborne pathogens in the world, many studies attempt to elucidate the risk factors involved in *Campylobacter* infection. In general, it is agreed that exposure to contaminated chicken through some form of consumption (raw or undercooked, fresh, or at a restaurant) is related to *Campylobacter* incidence. Since raw chicken can have very high levels of *Campylobacter* contamination, measured by Jørgensen et al. (2002) to be to be greater than 10^7 cells per carcass, cross-contamination can be extensive both in commercial and household food preparation. Rosenquist et al. (2003) point out the many links between chicken consumption and campylobacteriosis in countries where *Campylobacter* incidence has declined as a result of changes in chicken production or consumption for other reasons (Rosenquist, 2003). For example, in 1999 when a dixoin crisis in Belgium caused a dramatic drop in retail chicken consumption – chicken and eggs were abruptly withdrawn from the market due to dioxin-contaminated feed components – the incidence of *Campylobacter* infections

dropped by 40% (Vellinga, 2002).

Raw or undercooked chicken

One of the most commonly reported risk factors for sporadic *Campylobacter* is exposure to raw or undercooked poultry (Friedman 2004). New Zealand has higher rates of campylobacteriosis than most of the developed world, with an annual notification rate of 16,000 cases per 100,000 population in 2006 (Sakkaf et al., 2010). This results from both high rates of poultry contamination and poultry consumption (Carter et al., 2009). Due to these high annual rates, much research focuses on campylobacter occurrence in New Zealand and several studies implicate several poultry-associated risk factors such as consumption of undercooked chicken (French et al., 2008; Sakkaf et al., 2010; Müllner, 2010; Sears et al., 2011). In Europe, a Danish case-control study examining risk factors for sporadic cases of *Campylobacter* infection identified that, in Denmark, consumption of undercooked poultry conferred the highest risk of campylobacteriosis (OR 4.52; 95%CI= 1.33–15.32) (Nieman, 2003). A prospective case control study in Australia was designed to identify risk factors for *Campylobacter* infection in persons >5 years of age. Simulating distributions of infection, the researchers modeled the uncertainty associated with each estimated case number, deriving what they referred to as a "credible value" or CrI. They found that the foodborne risk factor with the highest attributable risk was cooked chicken, with an estimated median of 21.2% (95% CrI 0.0%–36.9%), followed by undercooked chicken, with an estimated median of 8.1% (95% CrI 5.2%–11.1%). Although the adjusted odds ratio (AOR) for cooked chicken was lower than that for undercooked chicken, the higher population-attributable risk (PAR) was explained by a

higher proportion of exposed case-patients (74.3% reported eating cooked chicken) (Stafford 2008).

Fresh vs frozen chicken

In Sweden, Studahl and Andersson (2000) found that after drinking contaminated milk, eating chicken was the second highest risk factor for campylobacter infection (OR 2.29, 95% CI 1.29–4.23). They found that consumption of fresh chicken, as opposed to frozen, conferred higher risk, with cases (23.5 %) having eaten fresh chicken more often than controls (9.4%) (Studahl and Andersson, 2000). A Danish case-control study found that consuming frozen chicken conferred a slightly protective factor (OR 0.97, 95% CI 0.61–1.55) though in that study, exposure to undercooked poultry of all types, fresh or frozen (OR 3.50; 95% CI=1.15–10.63) was the only exposure significantly associated with campylobacter infection (Niemann, 2003).

Eating in restaurants

In their U.S. based case-control study across FoodNet sites, Friedman et al. (2004) found that eating poultry at a restaurant was associated with a high risk of campylobacteriosis, especially for chicken (matched odds ratio or mOR, 2.4; 95% CI, 1.9-2.9) but also for turkey (mOR, 2.1; 95% CI, 1.57-4.0) and other meat (mOR, 2.1; 95% CI, 1.7-2.5) (Friedman, 2004). Michaud et al. found very similar results in Québec, Canada: the three top risk factors for campylobacteriosis were consumption of raw, rare, or undercooked poultry (OR 5.00, 95% CI, 1.79–13.98, p = 0.002); raw milk or raw milk products (OR 3.67, 95% CI 1.95–6.90, p = 0.0001); and turkey or chicken eaten in a

restaurant, a fast food establishment or a buffet (OR 1.96, 95% CI 1.24–3.11, p = 0.004). These factors accounted for 8%, 18%, and 20% of cases, respectively (Michaud et al., 2004). In a comprehensive risk assessment study conducted in three counties of Washington state, Denno et al. (2009) provide interesting data on the risk factors associated with eating in a variety of restaurant settings, such as eating with high frequency at table-service restaurants (OR: 4.2; 95% CI= 1.6–11.3), eating at fast-food restaurants (OR: 1.7; 95% CI= 1.0–2.8), at self-serve buffets, and mobile food stands (OR: 4.4; 95% CI= 1.7–11.5) (Denno et al., 2009).

Poultry husbandry

Another exposure pathway to contaminated chicken comes through contact with chickens themselves, often through animal husbandry. Interestingly, in a Michigan-based prospective case-control study, Potter et al. (2003) found that the association between consumption of undercooked poultry and illness was not statistically significant but that exposure to poultry through husbandry was significantly associated with *C.jejuni* infection (OR=6.884; 95% CI=1.438, 32.954) (Potter et al., 2003). This is corroborated by Studahl et al. (2000), whose small case-control study found that working on a farm brought with it significantly higher risk of contracting campylobacteriosis and direct contact with hens or chickens on a poultry farm constituted a greater risk of contracting campylobacteriosis than just visiting the farm (OR: 11.83; 95% CI= 3.41, 62.03). This high odds ratio should be contextualized by the study's relatively small sample size (cases: n= 101; controls: n-198) (Studahl et al., 2000). As Studhal et al. (2000) point out, however, farm inhabitants are at higher risk of campylobacteriosis, but since they have

contact with a variety of animals (cows, pigs, chicken and wild animals), drink well water and sometimes ingest unpasteurized milk, it is difficult to characterize a single risk factor (Studahl et al., 2000).

Contaminated Water

As previously mentioned, *Campylobacter* can be found in a variety of water sources and therefore, the ingestion of water is another common exposure pathway for campylobacteriosis in humans.

Overview of Contamination Sources

As mentioned in an earlier section, *Campylobacter* has been isolated in surface waters; hence, exposure to *Campylobacter* through surface water is a commonly reported risk factor. The isolation of most *Campylobacter* species in surface waters is due to contamination of human or animal origin, deriving from sewage outflow, direct fecal deposition, and pasture runoff (Jones, 2001). In a two-year Spanish study, Rodriguez et al. (2010) collected surface water and wastewater samples and found that 82% of the samples (mean of 1.3 MPN 100 ml(-1)) contained *Campylobacter*. The highest counts were in poultry wastewater and urban sewage, with a predominance of *C .jejuni*, while *C. coli* predominated in pig slurry. The two species were also found to co-exist in water samples (Rodriguez, 2010).

Groundwater

Groundwater is normally considered to be microbiologically clean, but it can become contaminated. Close et al. (2010) demonstrated that under experimental

conditions *Campylobacter* can be transported through contaminated soil to groundwater (Close et al., 2010). Many major outbreaks of campylobacteriosis have involved groundwater contamination. In South Bass Island on Lake Erie in Ohio, groundwater contamination originated from wastewater-treatment facilities and septic tank overflows due to precipitation events (Fong, 2007). In southwestern France, a massive community outbreak of campylobacteriosis was part of a multi-pathogen drinking water contamination event due to agricultural run-off and malfunctions in the water treatment facilities for the village of Gourdon (Gallay, 2006). In both cases, water contamination derived from human and animal sources of *Campylobacter.*

Recreational Ingestion of Untreated Surface Water

It is thus not entirely surprising that exposure to contaminated water is a risk factor for human campylobacteriosis. Past studies have shown that ingestion of untreated water from lakes, rivers and streams can be a risk factor for campylobacteriosis. Sopwith et al. (2008) examined a single prevalent human strain of *C. jejuni*, ST-45, one strongly associated with the early summer seasonal peak of campylobacteriosis incidence in northwestern England and found a striking concordance between periods of ST-45 isolation in water and reported incidence in humans, suggesting a relationship between the presence of this strain in the environment and human infection (Sopwith, 2008). In a case-control study conducted in the early 1990s by Adak et al. (1995) in the U.K., the univariate analysis revealed that human ingestion of untreated water while participating in recreational activities was one of the four top factors associated with an increase in risk for campylobacteriosis ($p= 0.013$) This study's multivariable analysis showed an odds ratio of 4.16 (OR 4.16;95% CI= 1.45,1.9) (Adak, 1995) for exposure to campylobacter

through recreational water use. More specifically, a recent case-control study in Finland focusing on independent risk factors for domestically acquired sporadic *Campylobacter* infections, showed that swimming in natural sources of water was a novel risk factor (Adjusted OR 2.80; 95% CI= 1.23–6.39, p= 0.0145) (Schönberg-Norio, 2004). This is plausible since the infectious dose for *Campylobacter* is fairly low. (Humphrey, 2007) Conversely, a Norwegian case-control study found that swimming in the sea, a lake, or a pool was protective rather than a risk factor (OR 0.7, 95% CI= 0.5, 1.0, p=0.03) (Kapperud, 2003) but this does not appear to be widely corroborated. In fact, Denno et al. (2009) found that aquatic recreation was the most important factor associated with *Campylobacter* infection (OR 2.7; 95%CI=1.5– 4.8) (Denno, 2009).

Untreated Drinking Water

However, the study conducted by Kapperud et al. (2003) pointed to a much more significant risk factor for campylobacteriosis, namely drinking untreated water. Twenty-nine of the 101 cases and 41 of the 149 controls who had intentionally drunk non-disinfected water in this study (as opposed to incidental ingestion during recreational activities such as swimming) had done so directly from a surface water source during outdoor activities such as hiking or camping, and cases had consumed such water significantly more times than their matched controls. Both multivariate models, which included and excluded protective factors, showed that drinking non-disinfected water was a leading risk factor for campylobacteriosis (OR 1.9, 95% CI= 1.1, 3.3, p=0.03 and OR 2.5, 95% CI= 1.2, 5.4, p=0.02, respectively) (Kapperud, 2003). Similarly, in a Swedish prospective case-control study, which examined domestically acquired *C. jejuni* and *C.*

coli infections among children younger than six years old, Carrique-Mas (2005) reported an adjusted OR of 6.0 (95% CI= 1.3, 27, p=0.02) among those who had drunk water from a lake or river (Carrique-Mas, 2005). Humourously, Jones points out that Scandinavians suffer from a divine right to drink from streams which appear to be clean (Gunnarsson, pers. comm. in Jones, 2001).

Private well water

Carrique-Mas found that living in a household with a private well was slightly associated with the risk of Campylobacter infection, though not statistically significant (OR 2.6; 95% CI=0.9-7.4, p=0.08). Other studies corroborate this finding. Schönberg-Norio et al. (2004) found that drinking dug-well water was an independent risk factor for *Campylobacter* infection (OR 3.19, 95% CI= 1.58, 6.45, p=0.0017). They also found that drinking water from a large water plant protected against sporadic *Campylobacter* infection, since large water plants use surface water as their source and use multistage purification and disinfection procedures before distributing drinking water to consumers (Schönberg-Norio, 2004). Michaud (2004) reported similar findings in Québec, Canada: drinking tap water from a deep well at home was the only risk factor identified (53% of cases compared to 23% of controls; OR 3.83, p = 0.06 by univariate analysis and OR 3.96, p = 0.06 after adjusting for age group and sex (Michaud, 2004). Similarly, an ecological study conducted among municipalities in Sweden found a slightly protective effect of having a public water supply instead of a private supply (IRR 0.93; 95%CI= 0.90–0.95 (Nygard, 2004). A population-based surveillance case-control study among infants 0-6 months of age, using U.S. FoodNet data, found that drinking well water (OR

4.4; CI, 1.4-14) brought with it an increased risk of *Campylobacter* infection among infants (Fullerton, 2007).

Despite this accumulation of evidence, Schallenberg et al. (2005) warn us that clear and direct links between these potential sources and campylobacters in drinking or recreational waters are difficult to establish, owing partly to the complex behavior of campylobacters once in the aquatic environment (Schallenberg, 2005). Along similar lines, upon examining data on waterborne disease and water collated by the Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre, the results of which indicated that outbreaks of campylobacteriosis derived from drinking water are confined to private water supplies, Jones et al. (2001) made a point of calling the association 'probable' at best, since *Campylobacter* is quite difficult to isolate in the actual water source. Nonetheless, this association is plausible since private water supplies are predominantly found in small rural systems which are more likely to be contaminated with animal waste (Wyn-Jones, 2000).

Private water sources and poultry flocks

Along parallel lines, in a study focusing on molecular identification of a common source of *C. coli* infection on poultry breeder farms, well-water was epidemiologically linked as the source of *C. coli* infection (Perez-Boto, 2010). A similar study was conducted among broiler flocks in Iceland and researchers found that farms using municipal water sources had approximately one-third to half the risk of campylobacteriosis than farms using "non-official" (non-municipal) untreated sources and approximately one-third the risk of treated municipal water. Their data showed that using a non-municipal UV-treated water supply did not pose a significantly different risk

from using non-municipal untreated water ($p < 0.05$) (Guérin, et al, 2007).

Contaminated Foods

A large body of research addresses the problem of exposure to *Campylobacter* through contaminated foods other than poultry, but only some of these issues will be mentioned here briefly.

Contaminated Milk

Exposure to contaminated milk has been implicated in a number of outbreak investigations such as two milk-associated outbreaks of *Campylobacter* enteritis in the Netherlands involving schoolchildren visiting dairy farms (Heuvelink et al., 2009). Hunt et al. (2009) document a similar outbreak due to exposure to unpasteurized milk at a community fair in Kansas, 2007 (Hunt, 2009). But exposure to unpasteurized milk in general is considered to be a risk factor for *Campylobacter* infection. In Denmark, Niemann et al. (2003) found similar results finding that unpasteurized milk was associated with an increased risk of infection (OR 1.89, 95% CI 0.89–6.16). In Finland, Schönberg-Norio et al. (2004) found that drinking pasteurized milk conferred a protective effect (adjusted OR 0.44; 95% CI= 0.22–0.85) (Schönberg-Norio, 2004). In the U.S., Friedman et al. (2004) found that unpasteurized milk was associated with illness regardless of where it was prepared.

Other Contaminated Foods

Contaminated shellfish also have been implicated in sporadic and outbreak cases

of campylobacteriosis. Friedman et al. (2004) found that raw seafood was a food item associated with illness regardless of where it was prepared. Similarly, Niemann et al. (2003) found that frequent consumption of pork chops was associated with illness (OR 1.71, 95% CI 1.01–3.27) and that eating beef, organs from pigs, ham and game was more common among controls than cases in this case-control study focusing on risk factors for campylobacteriosis in Denmark.

In terms of foods conferring protection from campylobacter, produce such as grapes were found to be associated with increased risk in Denmark (OR 1.47, 95% CI 0.94–2.13) but eating raw carrots, cabbage and unpeeled apples/pears conferred protection (OR 0.67; 95% CI=0.44–0.99, 0.50 0.27–0.90 and 0.48; 95% CI= 0.31–0.73, respectively) (Niemann, 2003). Schönberg-Norio found that eating black and red currants and blueberries (adjusted OR 0.17; 95% CI=0.07–0.41 and 0.43; 95% CI= 0.21–0.89, respectively) reduced the risk of campylobacter infection (Schönberg-Norio, 2004).

Common risk factors in developing countries include wide exposure to contaminated foods through local markets (Bodhidatta, 2013) but this will not be addressed here.

Food Preparation Practices

Inadequate precaution to safety measures in food preparation, especially of poultry, is a widely reported risk factor for *Campylobacter* infection and has been extensively documented. In general, insufficient temperatures and cooking times as well as cross-contamination lead to *Campylobacter* exposure and thus increased risk of

infection. In a multicenter, prospective case-control study conducted by Stafford et al. (2008) in Australia, the consumption of undercooked chicken was associated with a high risk of *Campylobacter* infection in individuals over the age of 5 years (adjusted OR: 4.7; 95% CI=2.6– 8.4) (Stafford, 1008). In their tri-county risk assessment study, Denno et al. (2009) reported that suboptimal kitchen hygiene after preparation of raw meat or chicken (OR, 7.1; 95%CI= 2.1–24.1) conferred a high risk for *Campylobacter* infection in Washington state. Friedman et al. (2004) reported that preventing cross-contamination through washing the cutting board after use with raw chicken (mOR, 0.5; 95% CI, 0.3–0.7) and washing hands after handling raw chicken (mOR, 0.5; 95% CI, 0.4–0.6), were practices associated with a reduced risk of campylobacteriosis (Friedman, 2004).

Regarding food prepared on the barbecue or grill. Niemann et al. (2003) found that meat prepared at a barbecue, which included pork, veal and beef was a risk factor (OR 1.93;95% CI= 1.13–2.94). Along similar lines, Carrique-Mas et al. (2005) found that consumption of grilled meat conferred a higher risk of infection (adjusted OR: 5.15; 95% CI=1.7-18.1, $p<0.01$) though this was no longer significant in a second model eliminating protective factors from the multivariate analysis (adjusted OR: 2.1; 95% CI=0.9-4.7, $p=0.07$) (Carrique-Mas, 2005).

In a very revealing study conducted in the Netherlands by Nynke et al. (2007), the researchers reported that even when consumers were educated about safe food preparation practices, especially regarding poultry, they often mistakenly relied on visual confirmation of thorough cooking or "doneness" rather than actually measuring temperature, leading to *C. jejuni* exposure through undercooked poultry (Nynke, 2007). Studahl et al. (2000) lament the fact that although industrial food safety programs have

been instituted in Sweden--reducing the proportion of campylobacter infected chickens to a level of 10-15%--and widespread public health campaigns have educated the public regarding safe food preparation practices, the number of indigenous campylobacter infections has actually increased (Studahl, 2000). Redmond et al. (2002) provide an extremely comprehensive review of food safety studies in Europe, North America, Australia, and New Zealand. They point out that although the importance of adequate consumer food-handling practices is widely acknowledged, the consumer remains the least studied link in the food chain (Redmond, 2002).

Other factors

Pets

It was mentioned earlier that in Ireland, Acke et al. (2011) compared the genetic similarity of isolates from pets to isolates in retail food and to clinical cases in humans and found few clusters containing isolates from dogs and human cases, thus providing little evidence to support the notion that domestic animals represent a real health risk to humans. This contradicts a number of other studies which have identified an increased risk of campylobacteriosis due to the presence of a pet. In their U.S. population-based case-control study, Friedman et al. (2004) found that the AOR was 3.4 (95% CI=1.8–6.5) for individuals who had a puppy. Fullerton et al. (2007) found the risk to be even higher for infants exposed to a pet with diarrhea in the home (OR 7.6; 95% CI=2.1-28) (Fullerton, 2007). Carrique-Mas et al. (2005) found having a dog in the household to be a significant contributor to risk of *Campylobacter* infection (adjusted OR 3.8; 95% CI=1.5-9.7).

Travel Abroad

Traveling abroad has also been associated with both outbreaks and sporadic (non-outbreak) cases of campylobacteriosis (Kassenborg, 2004; Ravel, 2011). Nielsen et al. (2012) found that in Denmark, travel abroad in the last month was found to be associated with an increased risk for infection. A total of 52 (18.4%) of 282 cases had been abroad in the month prior to onset of disease compared with 30 (9.4%) of 319 controls (OR 2.51; 95% CI= 1.49– 4.24) (Nielsen, 2012). Nichols et al. (2012) report that travel from the UK to both EU and non-EU countries was associated with almost a fifth of all *Campylobacter* infections (Nichols, 2012). In the U.S., Denno et al. (2008) found domestic travel to be associated with *Campylobacter* infection (OR: 2.5; 95%CI= 1.4–4.6) (Denno, 2009). Examining FoodNet data, Fullerton et al. (2007) found that *Campylobacter* infection was associated with travel outside the United States at all ages (OR 19.3; CI, 4.5-82.1).

Malnutrition

A newly opening area of research concerns potential predisposition to enteric infection arising from malnutrition, especially in children of the developing world. (Brown, 2003; Gupta, 2011). Brown et al. (2003) found that malnutrition can predispose a child to *Campylobacter* infection (Brown, 2003). Upon examining the gut microbiome of a malnourished child, Gupta et al. (2011) drew similar conclusions (Gupta, 2011). Similarly, Fernandez et al. (2008) found that malnourished children in Chile were more frequent carriers of *Campylobacter spp.* (31.4%) than well-nourished children (9.9%).

Lee et al. (2013) delved into the iterative relationship between growth stunting and *Campylobacter* infections among children in Peru and found that *Campylobacter* infections were associated with reduced weight gain over a three-month period (65.5 g (95% CI: −128.0, −3.0)(p = 0.040) and 43.9 g (95% CI:−87.6, −1.0)(p = 0.049) less weight gain, respectively). Although symptomatic *Campylobacter* infections were only marginally associated with reduced linear growth over a nine month period (−0.059 cm per episode, 95% CI: −0.118, 0.001)(p = 0.054), severe episodes of campylobacteriosis were associated with reduced linear growth (−0.169 cm/episode, 95% CI −0.310, −0.028)(p = 0.019) (Lee, 2013).

Environmental Risk Factors for Campylobacteriosis

Rurality

Many studies regarding incidence rates of *Campylobacter* infection point to rurality as a key risk factor. An extensive study on degrees of rurality in Germany found significant associations with higher campylobacteriosis rates among children under five living in inner rural areas (Incidence Rate Ratio: 2.9, 95% CI= 1.9–4.4), for children aged 5–14 years, living in inner rural areas (IRR: 2.1, 95% CI= 1.3–3.1) and in inner intermediate areas (IRR: 1.8, 95% CI= 1.2–2.7) (Fitzenberger et al., 2010). Bessel et al. (2010) reported similar findings in Scotland, showing that, overall, low rurality (or high urbanicity) had a significant protective effect, especially among those under the age of 15 (RR: 0.745, 95% CI= 0.700, 0.792). Sears et al. (2011) examined the decline in notification rates of campylobacteriosis in New Zealand after the implementation of

poultry-specific interventions. They found that the decline in infection notifications was the smallest among rural populations when the average annual rate for 2002-2006 was compared to that of 2008 (RR: 0.66, 95% CI= 0.62-0.70). (Sears 2011) Similarly, in their geospatial analysis of *Campylobacter* infection in rural Canada, Green et al., (2006) found that in almost every age and gender category in rural Manitoba, *Campylobacter* incidence rates were higher among rural children, especially in the 0-4 year age group (rural males: 97.5 cases/100,000 vs. urban males: 13.2 cases/100,000; rural females 72.8 cases/100,000 vs. urban females: 10.5 cases/100,000) (Green, 2006). Green et al. (2006), point out that this pattern of higher incidence among the youngest population groups in rural areas is not unlike the *Campylobacter* infection patterns found in the developing world.

In terms of genotyping, it could be that certain strains of *Campylobacter* are more highly associated with rural environments. Sopwith et al. (2008) examined a particular strain of *C. jejuni* in northwest England, the multilocus sequence type (ST)–45, and found that among other factors, persons infected with ST-45 were more likely to live in rural areas and to be <5 years of age than case-patients infected by other sequence types of *C. jejuni*. This could have significant environmental implications since ST-45 has been reported to be well-adapted to surviving outside of animal hosts and thus more available to infect humans through transmission routes other than food, such as water, outdoor activities, and pets (Sheppard, 2009). Results pointing to a greater association of human ST-45 with residence in more rural areas support this hypothesis. If ST-45 is indeed a key driver of transmission between livestock, environmental, and human settings, as suggested, this would imply that the ST-45 strain of *Campylobacter* should be a key

target for intervention in reducing *Campylobacter* prevalence (Sopwith, 2008).

Farming, Farm Animals and Animal Husbandry

An important component of rurality is exposure to farming and farm animals and, as noted above, numerous studies point to an association between contact with farm animals and incidence of *Campylobacter* infection. This is plausible because an extensive body of research exists documenting that most, if not all, livestock and poultry carry *Campylobacter* and that the numbers of bacteria can be very large, especially in poultry (Jones, 2001). Quantitative data for intestinal carriage of campylobacters is available for most farm animals and birds, such as beef and dairy cattle (Stanley et al. 1998c), sheep (Stanley et al. 1998d), pigs (Weijtens et al. 1997), chickens (Wallace et al. 1997) and turkeys (Wallace et al. 1998). This is neatly summarized by Jones, et al. (2001) who point out that *Campylobacter* present in the intestines of farm animals and poultry readily enter the wider environment through discharge of slaughterhouse effluent, and fecal distribution during grazing (Jones, 2001). Therefore it is plausible that farm visits, especially by children, can result in campylobacteriosis. For example, a Norwegian investigation into a nationwide *E.coli* outbreak in 2009, revealed that among nursery school children who had recently visited farms, the same strain of *C. jejuni* was isolated in the feces from infected children and from the lambs on the farms, implicating animal feces as the source (Møller-Stray, 2012). In a study that examined cases of severe gastroenteritis among children living in rural Québec, Canada, Levallois et al. (2004) found a link between campylobacteriosis and intensive livestock activities. (Levallois, 2004)

In the United States, univariate analyses conducted in rural Michigan by Potter et al. (2003) found that contact with any food-producing animal significantly increased the odds of campylobacteriosis (OR: 4.722, 95% CI= 1.737-12.833), and that contact with adult domestic poultry was highly significant (OR: 3.216, 95% CI= 0.811, 12.763). The care and raising of cattle and swine were also associated with increased risk of illness, though to a lesser degree (cattle OR: 3.058, 95% CI: 0.907, 10.307 and swine OR: 7.358, 95% CI: 0.845, 64.079). Multivariate analyses in the same study found that exposure to animal husbandry had a greater association than farm exposures, with poultry husbandry having the strongest association with enteritis due to *Campylobacter jejuni* (OR: 6.884, 95% CI= 1.438, 32.954) (Potter, 2003). The study conducted by Friedman et al. (2004) focused mainly on consumption of poultry and on improper food preparation practices, but did find that exposures associated with campylobacter infection included having contact with farm animals (mOR: 2.2, 95% CI= 1.5-3.2), contact with a live chicken (m OR: 2.4, 95% CI= 1.4-4.2), visiting a farm where there were animals (mOR: 2.0, 95% CI= 1.4-2.9), and having contact with animal stool (mOR: 1.6, 95% CI= 1.2-2.0), all p values < 0.01 (Friedman, 2004).

Animal Density

Related to both rurality and farming is animal density, a key component which has also been found to be associated with incidence of campylobacter infection. Nygard et al. (2004) examined environmental risk factors for campylobacteriosis in Sweden and their multivariable Poisson regression analysis identified ruminant density as one of the top three independent risk factors for infection (Incidence rate ratio or IRR: 1.08, 95%

CI=1.05–1.11). After excluding the three largest cities from the model, which accounted for 1377 cases of infection, the IRR for ruminant density increased slightly to IRR: 1.12 1.09–1.15 (Nygard, 2004). Green et al. (date) employed geospatial analyses to compare rates of *Campylobacter* infection in rural Canadian provinces compared to those in urban centers. Using data from the 2001 Canadian Census of Agriculture, these researchers overlaid thematic maps of composite animal density (CADI) onto thematic maps of *Campylobacter* incidence and found that the areas of highest animal density corresponded to observed areas of high infection incidence. Despite near certainty that rates of infection were underestimated due to underreporting, the incidence rate ratio of campylobacteriosis for overall animal density was 1.68 (95%CI: 1.39-2.02) in the highest group when compared to the reference category. Rates varied for specific animals: for chicken density, the highest grouping had an incidence rate ratio of 2.11 (95% CI: 1.73-2.58), for pig density 1.99 (95%CI: 1.61-2.46), for cow density 1.54 (95%CI: 1.30-1.83). When fully adjusted, the model used in this study indicates that animal density can be used as a significant predictor of *Campylobacter* incidence (Green 2006).

Similar results were found by Potter et al. (2002) in an ecological study focusing solely on animal density, more specifically poultry. Researchers compared incidence rate ratios of *C. jejuni* enteritis in high- and low-poultry-density counties of Michigan between 1992 and 1999. Using poultry density per county as a proxy variable for occupational exposure to *C. jejuni* , they found that the risk for *C. jejuni* enteritis was 1.31 (95%CI=1.21,1.42) times higher in high-density counties than in low-density counties: the incidence rate (IR) for high-density counties was 11.99/100,000 person-years (95%CI=11.07,12.95) while for low-density counties, the IR was 8.6/100,000

person-years (95%CI=8.38,8.82) (Potter, 2002). The researchers did point out that differences may be due to differences in rates of care-seeking or illness reporting which would lead to biases in data results, as would variations in the number of laboratories present. Using Bureau of Census Current Population Survey data for 1997-1999, they did find that there was no difference in the mean proportion of uninsured between high- and low-density counties (*t* test; $p > 0.05$).

Nonetheless, unlike Green (2006) who found strong statistical significance between animal density and campylobacteriosis, Potter et al. (2002) found that the impact of their data results were limited by crude estimates of poultry density by county, rather than per person. This is especially true for Michigan counties which have high poultry density, but are, in fact, highly populated and considered urban. Better data availability on poultry density would lead to more precise measures of exposure, especially insofar as occupational exposure is concerned (Potter, 2002). In Denmark, Nygard et al. (2004) found similar limitations when examining the association of environmental factors and campylobacter incidence in municipalities of Sweden, 1998–2000. In terms of animal density, they found an IRR of 1.03 (95%CI=1.01,1.05) for poultry and found an IRR of 1.08 (95% CI= 1.05, 1.11) for ruminant density. When the three largest cities were excluded from the model, this IRR increased to IRR 1.12 (95% CI =1.09,1.15), suggesting a strong association between living in an area with high ruminant density and increased incidence of campylobacteriosis. However, when rurality was included in the multivariable analysis with the two other variables of ruminant density and water-pipe length, living in a rural area did not show any significant independent effect. The researchers posited that in a rural area, there is a high risk for contamination of

drinking-water sources due to seepage from cattle manure used as fertilizer to ground-water well. But they pointed out an increased risk of campylobacteriosis in high-animal density areas could originate from higher rates of direct transmission from cattle and small ruminants. Since measuring indirect transmission from animals through water is difficult to do, they suggest further research combining data from individual based studies with the data on environmental factors (Nygard, 2004).

Sociodemographic Risk Factors for Campylobacteriosis

Gender and Age

In terms of sociodemographic factors, the two most consistent independent variables associated with campylobacteriosis are gender and age. Multiple studies point toward higher incidence of campylobacteriosis in males, especially those under five years of age and among those under five years of age living in rural areas. Using FoodNet data on sporadic *Campylobacter* infection incidence in the United States between 1996 and 1999, Samuel et al. (2004) found that male subjects had the highest incidence of campylobacteriosis each year across all age groups (24.4/100,000 males vs. 19.9/100,000 females). They also found that the distribution across age categories exhibited a bimodal pattern, with a peak at <1 yr. (56.2 cases/100,000) and 1-4 yrs. (41.2 cases/100,000), and another peak in the 20-29 yr category. (30.3 cases/100,000) (Samuel, 2004). In a case-control study in 1998-1999 using FoodNet data, Friedman et al. (2004) found a similar bimodal pattern with a peak at 4 yrs. and another peak in the 22–52 yr. category. Being female was a protective factor (AOR: 0.5, 95% CI: 0.4-0.5) (Friedman, 2004). These

findings are corroborated worldwide. In Canada, Green et al. (2006) found the highest rate of campylobacter infection to be among 0–4 yr. olds and 20–39 yr olds, with slightly higher rates occurring in males (Green, 2006). In Germany, the study conducted by Fitzenberger et al. (2010) corroborates this pattern: campylobacteriosis incidence being highest among those under five years of age (61 cases/100,000), and those aged 15–44 years (56 cases/100,000) (Fitzenberger, 2010). Gillespie et al. (2008) also found that in England and Wales, overall Campylobacter incidence in males slightly higher than in females (RR: 1.06, 95% CI 1.03–1.10), and a bimodal age distribution pattern, but with the first decline occurring at 2 yrs. of age and with incidence in females exceeding that for males in the 20-36 yr. age category (RR: 1.21, 95% CI: 1.14-1.29) (Gillespie, 2008). In Sweden, Carrique-Mas et al. (2005) also found a greater proportion of cases among males (57.1%) and a preponderance of cases among those younger than 2 yrs (Carrique-Mas, 2005). In New Zealand, a country in which rates of campylobacteriosis have been steadily rising for the past two decades (Baker, 2007), Baker et al. (2007) examined data from the national notifiable disease surveillance system and found that for the period 2001–2003, campylobacteriosis showed highest average annual notification rates in children aged 1–4 years (578.1/100, 000), and adults aged 20–29 years (470.0/100, 000). Average annual notification rates were higher in males than females (362.1/100,000 and 295.9/100,000 respectively) (Baker, 2007).

In terms of species distribution, a population-based study conducted in Denmark by Nielsen et al. (2012) found that species varied by age group, with *C. concisus* being isolated more frequently among small children (<1 year) and the elderly (≥65 years) instead of *C. jejuni or C. coli* (Nielsen, 2012). In Israel, Weinberger et al. (2013) found a

sharp increase in campylobacteriosis. Although rates tripled within just 12 years across all age groups, for both *C. jejuni* and *C. coli,* the highest infection rates were seen among children <2 years of age. Within this age group, the observed rates were significantly higher than those reported in Western countries (European Centre for Disease Prevention and Control, 2011; CDC FoodNet Annual Report, 2011) but were comparable to that reported for New Zealand (Nelson, 2008).

Interestingly, this same study found a hormetic curve in *Campylobacter* incidence according to age, with the lowest and highest age groups having the highest rates: 363.39 and 348.80 cases/100,000 population were measured for 1st and 2nd years of life respectively, the lowest incidence rate measured in the fifth decade of life (12.82 cases/100,000 population) and a slight increase toward the eighth decade of life (26.44 cases/100,000 population) (Weinberger, 2013). Researchers suggest that this difference in incidence resembles rates for developing countries and may be indicative of repeated exposure to *Campylobacter* spp. in early childhood which results in acquisition of protective immunity at older ages.

Ethnicity

Unlike many other foodborne pathogens, *Campylobacter* tends to be associated with white individuals and infection incidence is reported to be higher among Caucasians than among other racial and ethnic groups. In their overview of the incidence of common foodborne pathogens, by age, sex, race and ethnicity, the CDC found the highest rates for campylobacteriosis among whites (12.19 per 100,000 population), then Indians/Native Alaskans (9.92 per 100,000 population), then Asians/Pacific Islanders (9.80 per 100,000

population), those self-identifying as multiple race (3.97 per 100,000 population), and finally African-Americans (3.64 per 100,000 population) (CDC FoodNet Annual Report, 2011). In their study of incidence trends across all ten U.S. FoodNet sites, Samuel et al. (2004) report that although overall average incidence rates between 1996 and 1999 were highest among Asians and Hispanics (33.5 cases/100,000 and 31.6 cases/100,000 respectively) and lowest among African-Americans (13.0 cases/100,000), the only consistent pattern by race/ethnicity was that incidence among African-Americans was lower at each site, though hospitalization rates for this group was higher. (Samuel, 2004) Similarly, in their descriptive study of *Campylobacter* patients in England and Wales between 1989 and 2011, Nichols et al. (2012) found a higher prevalence of *Campylobacter* in communities where more than 95% of the population was 'white British' than in communities in which fewer than 50% were white, positing poorer access to healthcare, greater susceptibility or increased exposure. In New Zealand, known for very high rates of campylobacteriosis, Baker et al. (2007) also found higher rates of campylobacteriosis in New Zealand among individuals of European ethnicity. They noted that ethnic differences typical for Maori and Pacific people, who generally experience higher rates of infectious diseases in New Zealand, were less marked for campylobacteriosis hospitalizations compared with notifications. Baker et al. (2007) also suggested that these differences were related to poorer access to primary care and diagnostic services resulting in lower rates of notified disease (Baker, 2007).

Socioeconomic Status and Level of Education

In terms of socioeconomic factors, many research studies report that incidence of

Campylobacter infection tends to be associated with white middle class individuals who are educated. Friedman et al. (2004) report that overall, more patients in their case-control study were white (mOR: 1.5, 95%CI: 1.1-1.9) and earned above the median income category of $30,000-$59,999 (mOR: 1.8, 95%CI: 1.4-2.3) than did controls. (Friedman, 2004). Bessell et al. (2010) report that in Scotland campylobacteriosis occurs more frequently among the least deprived (Bessell, 2010). Similarly, the fully adjusted regression model applied by Green et al. (2006) indicates that high socioeconomic status confers increased risk of *Campylobacter* incidence (Green, 2006). In England and Wales between 1989 and 2011, Nichols et al. (2012) found an inverse relationship between *Campylobacter* prevalence and the Oxford Index of Multiple Deprivation. This corroborates the findings of Bessel et al. (2010) in Scotland Greater indicating lower case incidences associated with deprivation according to the Carstairs Deprivation index, mean relative risk (RR) being 0.965 (95% CI=0.959, 0.971) (Bessel, 2010). It has been suggested that the eating habits of individuals with increased income involve more high risk foods such as undercooked "pink" pork or sushi. (Tan, 2008)

Additionally, it has been reported that unsafe food preparation practices may actually rise as total annual household income increases. Although this might appear counter-intuitive, this association has been documented by Nesbitt et al. (2009) in a Canadian study on high-risk food consumption and food safety practices (Nesbitt, 2009). If incidence of campylobacter is associated with higher levels of education, this does not necessarily imply those knowledgeable about safe food preparation practices apply these practices systematically in the home.

Overall Research Gaps in Risk Factors for Campylobacteriosis

Of the many issues raised in this Background section, the confluence of sociodemographic characteristics combined with environmental risk factors was of key interest. How are community-level environmental factors, such as rurality and exposure to contaminated water, intertwined with socioeconomic risk factors for the individuals included in our FoodNet data catchment area? By conducting an ecological study using small geographic units such as the zip code, I was able to examine the degree to which rurality might be a factor, more specifically, whether or not the presence of animal operations might impact the risk of campylobacteriosis. Although comprehensive data on animal operations is fairly difficult to obtain, my study did reveal interesting findings.

In the future, it would be of interest to obtain more accurate and complete data on animal operations for the state of Maryland in order to refine the association between animal density and risk of *Campylobacter* infection. Instead of relying on a binary variable (presence or absence) for animal operations, it would be more useful to include a continuous variable such as high-, medium-, or low- density of animal operations by zip code, assuming that gaining access to this kind of data is feasible. Pork and poultry operations figure heavily in the Maryland economy and precise data on such operations is not always easily accessible. Using a continuous variable would provide a more accurate view of the association between animal operations and risk of campylobacteriosis, further elucidating the exposure pathways involved in infection in humans. Constructing a study in this way would be similar to the research led by Potter, et al, in their Michigan studies (Potter et al, 2002, 2003) and Green et al, in Manitoba, Canada (Green et al, 2006).

It would also be interesting to obtain data on what types of water are available in each zip code. For example, individuals residing in rural zip codes may tend to have

private wells and thus have a greater likelihood of risk of campylobacteriosis through ingestion of contaminated water. This would continue the process of identifying, and potentially predicting “hot-spot” communities that bear high burdens of campylobacteriosis.

The next chapter is comprised of the manuscript pertaining to the research conducted for this thesis. A discussion of the public health implications of the findings follows.

CHAPTER 4: MANUSCRIPT

Rurality, Presence of Broiler Operations, and Community Socioeconomic Factors Influence the Risk of Campylobacteriosis in Maryland

Abstract:

Objectives: Environmental and socioeconomic factors can play an important role in the risk of *Campylobacter* infections. Here, we evaluate for the first time in the U.S., the combined impact of community-level environmental and socioeconomic factors on the risk of campylobacteriosis.

Methods: *Campylobacter* case data (2002-2010, n=3,694) were obtained from the Maryland Foodborne Diseases Active Surveillance Network. Community-level socioeconomic and environmental data were obtained from the 2000 U.S. Census and the 2007 U.S. Census of Agriculture. Data were linked by zip code. Incidence rate ratios were derived by Poisson regressions. A subset of zip code-level characteristics was mapped.

Results: In zip codes that were 100% rural, incidence rates of campylobacteriosis were 6 times (IRR=6.18; 95%CI=3.19-11.97) that of urban zip codes. In zip codes with broiler chicken operations, incidence rates were 1.45 times that of zip codes without broilers (IRR=1.45, 95%CI=1.34-1.58). Higher rates were also observed for zip codes that were predominantly white and had high median incomes.

Conclusions: Our findings show that the risk of campylobacteriosis could be significantly influenced by the community and environment where one lives.

Introduction

Campylobacter is a leading cause of bacterial gastroenteritis in much of the developed and developing world[1;2]. In addition to the diarrhea and vomiting associated with gastroenteritis, infection with *Campylobacter* can lead to more serious sequelae such as Guillain-Barré syndrome, a demyelinating autoimmune disorder that can sometimes lead to death[3]. Scallan et al. (2011)[4] estimated that *Campylobacter* causes approximately 845,000 domestically acquired illnesses in the United States each year, along with 8,463 hospitalizations and 76 deaths. While the majority of these illnesses are estimated to be foodborne [4], attributing specific infections to specific sources has been challenging.

Commonly reported risk factors for *Campylobacter* outbreaks include exposure to undercooked poultry[5], unpasteurized milk,[6;7] and contaminated water[8]. Eating in restaurants[9], not observing proper food preparation practices[10] and traveling abroad[9;11], have also been associated with both outbreaks and sporadic (non-outbreak) cases of campylobacteriosis. Additional risk factors for sporadic infections include contact with pets[5;12], contact with farm animals and livestock[13;14], and contact with animal feces[15]. Significant associations between living in rural areas and risk of campylobacteriosis also have been identified in Europe and Canada[16-18]. Moreover, a specific feature of rural environments, animal density, has been identified as a significant predictor of *Campylobacter* incidence in Canada and New Zealand[16;17].

Several sociodemographic risk factors for campylobacteriosis have also been identified, the two most consistent being gender (males) and age (less than 5 yr)[8;16-19]. Previous studies have also evaluated socioeconomic factors associated with the incidence of *Campylobacter* infection and the findings suggest that these infections may occur more frequently among individuals characterized by higher socioeconomic status[16;20]. Moreover, Samuel et al.(2004)[21] reported that the incidence of campylobacteriosis among African-Americans was lower than that among other ethnic groups across multiple sites in the United States, although hospitalization rates for this group were higher. These findings, however, could be influenced by differentials in illness reporting among varying races and ethnic groups.

Nonetheless, these previous reports have largely resulted from population-based case-control studies focused on individual-level data. To our knowledge, no U.S. study has examined the combined effect of community-level environmental and socioeconomic risk factors on the risk of campylobacteriosis. Such an analysis can be useful in 1) identifying (and possibly predicting) "hot-spot" communities that bear high burdens of this illness; and 2) addressing significant research gaps concerning potential health disparities in the risk of infectious diseases[22]. The purpose of this study was to link Maryland Foodborne Diseases Active Surveillance (FoodNet) data to U.S. Census data and USDA Census of Agriculture data at the zip code level to evaluate associations between community-level environmental and socioeconomic risk factors and the incidence of *Campylobacter* infections in Maryland.

Materials and Methods

Data Sources

Campylobacter case data were obtained from the Maryland FoodNet. The Maryland FoodNet program is one of ten FoodNet sites funded by the Centers for Disease Control and Prevention (CDC) that conducts active surveillance on culture-confirmed cases of *Campylobacter*, as well as nine other pathogens[23]. This study focused on culture-confirmed cases of *Campylobacter* infection occurring in Maryland between 2002 and 2010. A case was defined as an individual whose biological specimen (stool, blood, or other) was culture-confirmed for the presence of *Campylobacter,* regardless of symptoms or date of onset. For each *Campylobacter* case, clinical data (i.e. date of onset and outcome) and demographic data (i.e. age, gender and race) were also obtained.

Socioeconomic data were obtained from the 2000 Census of Population and Housing, Summary File 1 (SF1) and Summary File 3 (SF3), by 5-digit zip code tabulation area (ZCTA)[24]. ZCTAs are statistical geographical units developed by the U.S. Census. They were produced to ameliorate the challenges in defining areas represented by individual zip codes since census data is not collected by zip code. ZCTA data, therefore, serve as a proxy to zip code level data and have been used in this capacity in other studies[25-27]. The following eight socioeconomic variables were obtained at the ZCTA level and analyzed as potential predictor variables, based on recommendations by Krieger et al.(1997)[28]: 1) median household income in 1999 (US$) (SF3); 2) per capita

income in 1999 (US$) (SF3); 3) percent owner-occupied housing units 1999 (SF1); 4) percent of households with public assistance income for 1999 (SF3); 5) percent of individuals living below the poverty level in 1999 (SF3); 6) percent of the population ≥ 25 years of age without a high school diploma (SF3) ;7) percent of the population composed of whites (who consider themselves white only), African Americans and Hispanics (SF1); and 8) percent of individuals living in rural areas, on a scale of 0% to 100% (SF3). The Census 2000 defines "rural" as "all territory, population, and housing units located outside of [urbanized areas] (UAs) and [urban clusters] (UCs)"[29]. UAs and UCs comprise densely populated territory which consists of "core census block groups or blocks that have a population density of at least 1,000 people per square mile; and surrounding census blocks that have an overall density of at least 500 people per square mile"[29].

To delve deeper into specific environmental factors present in rural areas that may impact the risk of campylobacteriosis, data were obtained from the 2007 U.S. Census of Agriculture, National Agricultural Statistics Service[30]. Specifically, data on the number of animal operations with sales by zip code were obtained for the following: broiler chickens, turkey, aquaculture, sheep/goats, hogs, dairy, and beef cattle.

Data Linking, Statistical Analysis, and Mapping

Data from all sources were linked by zip code and 5-digit ZCTA. Campylobacteriosis rates were then calculated by zip code (using zip code population estimates from the 2000 Census); by year for the state of Maryland (using Maryland

intercensal population estimates); by season for the state of Maryland (based on the National Weather Service's and National Aeronautics and Space Administration's dates of equinoxes and solstices); and by age group, sex, and degree of rurality.

Categorical variables were created for dependent U.S. Census and U.S. Census of Agriculture variables based on the individual distributions for each variable. Univariate Poisson regressions were then performed to investigate the individual effect of each variable on rates of campylobacteriosis (by zip code).

Pearson's correlation was then performed between all dependent socioeconomic (U.S. Census) and environmental (U.S. Census of Agriculture) variables to avoid using highly correlated variables in the multivariate regression model. Because a large proportion of zip codes had a campylobacteriosis rate of zero, a multivariate, zero-inflated Poisson regression model was used. In the zero-inflated model, the rurality variable was used as the predictor of the excess zeroes, and a Vuong test was used to confirm that the zero-inflated Poisson regression model was a better fit compared to the standard Poisson regression model ($Z=15.18$, $p<0.0001$). The final multivariate model included rurality, and was adjusted using the following variables: presence or absence of broiler chicken operations; presence or absence of turkey operations; presence or absence of dairy operations; presence or absence of aquaculture operations; median household income in 1999; percent owner-occupied housing units 1999; percent of the population ≥ 25 years of age without a high school diploma; percent of the population composed of

African Americans; and percent of the population composed of Hispanics. All statistical analyses were performed using Stata 10.1 I.C (StataCorp LP, College Station, TX).

Once statistical analyses were performed, campylobacteriosis rates and a subset of the dependent variables that significantly influenced the risk of campylobacteriosis were mapped by zip code to provide a visual representation of our findings. All mapping was performed using ArcGIS 10.1 (ESRI, Redlands, CA).

Results

Campylobacter Cases

Between 2002 and 2010, 3,694 cases of culture-confirmed *Campylobacter* infections were reported to the FoodNet active surveillance system in Maryland. A total of 3,687 cases (99.8%) had valid zip codes and were included in this study (Table 1). The majority of cases were among adults 20-59 years old (59.3%) with males representing a slight majority (53.6%). Whites comprised 57.4% of the cases. Only 0.2% of the total infections resulted in death.

Incidence and Seasonality of Campylobacteriosis

The annual incidence of campylobacteriosis for Maryland ranged from 5.23 per 100,000 population in 2004 to 8.71 per 100,000 population in 2010 (Figure 1, Panel A). As expected, higher rates occurred in summer and most springs, while lower rates occurred in fall and winter (Figure 1, Panel A).

Impact of Community-level Environmental Factors

Our multivariate, zero-inflated Poisson regression model (that included both environmental and socioeconomic variables) provided evidence that several environmental factors at the zip-code level influenced rates of campylobacteriosis (Table 2). In the final adjusted model, in zip codes that are characterized as 100% rural by the

U.S. Census the incidence rate of *Campylobacter* infection was 6 times that of zip codes that are less than 0.9% rural (IRR=6.18; 95%CI=3.19-11.97) (Table 2). In zip codes that contain broiler chicken operations, the incidence rate of campylobacteriosis was 1.45 times that of zip codes that do not contain broiler operations (IRR=1.45; 95%CI=1.34-1.58). The presence of turkey, dairy and aquaculture operations was slightly protective (Table 2); however, the effect associated with aquaculture was not significant.

Impact of Rurality by Age and Sex

Our descriptive, individual-level *Campylobacter* case data confirmed the effect of rurality on campylobacteriosis rates. In general, males and females residing in rural zip codes had higher rates of *Campylobacter* infection compared to their urban counterparts (Figure 1, Panel B). This trend was consistent across all age categories, except the 10 to ≤19 yrs. age group where the rate for rural females was similar to the rate for urban males. In both rural and urban zip codes, male rates of infection were higher than corresponding female rates, except for rural males and females >60 yrs, where the rate for females was slightly greater than that of males. Furthermore, in both rural and urban zip codes, the highest rates of infection were among children under 5 years old. Rates for rural children in this age category exceeded all other rates: 26.40 per 100,000 population for males and 19.17 per 100,000 population for females.

Impact of Community-level Socioeconomic Factors

Beyond rurality and its associated environmental factors, community socioeconomic factors were also correlated with the risk of campylobacteriosis. For example, our multivariate model showed that the incidence of Campylobacteriosis in zip codes with the highest median incomes (≥ $66,500) was 2 times that of zip codes with the lowest median incomes (<$40,000) (IRR=2.09; 95%CI=1.79-2.44). In addition, zip codes characterized by the very lowest (<6.6%) and the very highest (≥16.1%) percentages of the population ≥ 25 yr. without a high school diploma had higher incidence rates of *Campylobacter* infection (Table 2). Meanwhile, living in zip codes with higher owner occupancy rates seemed to impart a slightly protective effect with regard to the risk of *Campylobacter* infection. Similarly, living in zip codes that were characterized by high percentages of African Americans and Hispanics was protective (Table 2).

Descriptive Spatial Analysis

Maps providing insight into the spatial distribution of *Campylobacter* infection rates in Maryland, as well as the spatial distribution of a subset of the significant environmental predictor variables, are provided in Figure 2. The maps visually emphasize the relationship between *Campylobacter* infection rates (Figure 2, Panel A), rurality (Figure 2, Panel B), and presence of broiler chicken operations (Figure 2, Panel C), particularly on Maryland's Eastern Shore.

Discussion

In this study, we found that higher incidence rates of campylobacteriosis in Maryland occur in zip codes characterized by certain environmental and socioeconomic factors. Our multivariate analysis showed that zip codes that are 100% rural, contain broiler chicken operations, and have the highest median incomes have the highest incidence rates of *Campylobacter* infection. To our knowledge, this is the first U.S. study to evaluate the combined impact of community-level environmental and socioeconomic factors on the risk of campylobacteriosis, an illness that is traditionally viewed as foodborne. Our results provide evidence that, beyond individual-level behavioral factors, a person's risk of campylobacteriosis also may be associated with where she or he lives.

Campylobacteriosis Rates in Maryland

Our descriptive analysis showed that the annual average incidence of campylobacteriosis in Maryland was 7.30 per 100,000 population between 2002 and 2010. This rate was slightly higher than the average annual rate (6.8 per 100,000) reported by Ailes et al.(2008)[31] who analyzed Maryland FoodNet data from an earlier time period, 1996 to 2006. In comparison with other states, rates of campylobacteriosis in Maryland remain the lowest among the ten FoodNet sites, including Colorado (19.6 per 100,000) and California (34.0 per 100,000 population). Similarly, rates of *Campylobacter* infection in Maryland remain well below the national rate of 13.6 per

100,000 population[32]. In terms of seasonality, we observed higher rates of campylobacteriosis in spring and summer (Figure 1, Panel A), and these findings are consistent with previous studies conducted in New Zealand, Canada, and Philadelphia, USA to name a few[16;17;33]. White et al. (2009) observed that the specific factors associated with summer that increased disease risk were warm and humid weather.

Impact of Rurality and Broiler Chicken Operations

One of our major findings is that the degree of rurality of a zip code strongly influences the risk of campylobacteriosis. Our multivariate model showed that incidence rates of campylobacteriosis in zip codes that are 100% rural are 6 times that of zip codes that are <0.9% rural, after adjusting for other factors (IRR 6.18; 95 CI:3.19-11.97). Our case-based descriptive data supported these findings, showing that rates of *Campylobacter* infection were particularly high among rural children under the age of five (Figure 1, Panel B). Fitzenberger et al.(2010)[18] and Bessel et al. (2010)[34] also reported significantly higher campylobacteriosis rates among children under five living in inner rural areas in Germany and rural areas of Scotland, respectively. Similarly, in their geospatial analysis of *Campylobacter* infection in rural Canada, Green et al.(2006)[16] found that in almost every age and gender category for rural Manitoba, *Campylobacter* incidence rates were higher for rural populations.

These high campylobacteriosis rates among rural populations, and especially among children, could be attributed to higher exposures to both animals and animal waste[14]. We hypothesize that increased exposure to contaminated groundwater and

surface water in rural environments may also impact the risk of campylobacteriosis and deserves further study. Close et al.(2010)[35] demonstrated that, under experimental conditions, *Campylobacter* can be transported through contaminated soil to groundwater. Moreover, Carrique-Mas et al.(2005)[8] found that living in a household with a private well was a risk factor for campylobacteriosis (Odds Ratio(OR)=2.6) in Sweden. These authors also reported that drinking water from a lake or river (ORs=7.4 and 6.0, respectively) was associated with an increased risk of illness.

One significant feature of rural environments in the United States is the presence of industrial food animal production operations. Our findings indicate that living in a zip code with broiler chicken operations equates to 1.45 times the risk of campylobacteriosis compared to living in a zip code without broiler operations (IRR 1.45; 95%CI:1.34-1.58). Potter et al.(2002)[36] showed similar results after comparing the incidence rates of *Campylobacter jejuni* in high and low poultry density counties in the state of Michigan: the risk of *C. jejuni* gastroenteritis was 1.31 (95% CI:1.21-1.42) times higher in high poultry density counties compared to low poultry density counties[36].

The presence of broiler operations in a zip code may influence risk of human campylobacteriosis because broilers are commonly colonized with *Campylobacter* spp.[37;38]. In a 2004 FDA report, where the prevalence of *Campylobacter* among retail meats from major food producing animals was described, the prevalence of *Campylobacter* on retail chicken breast (derived from broilers) was 60.2%, compared to the prevalence of that on retail ground turkey, ground beef and pork chops which was

1.0%, 0.3% and 0.0%, respectively.[39] Once *Campylobacter* colonizes a broiler flock, the microorganism can then be dispersed into the environment during land application of poultry manure[40], and ultimately transferred into nearby surface waters and groundwater as noted above[35]. Wilkes et al. (2011) recently reported that 25% of surface water samples collected in an agricultural area of Ontario, Canada were positive for *Campylobacter* spp. The authors also provided evidence that meteorological factors occurring during periods of land application of manure may have promoted the increased frequency of *Campylobacter* spp. detected in surface waters[40].

Impact of Socioeconomic Factors

Beyond rurality and the presence of broiler chicken operations, our analysis showed that even after adjusting for these factors, community-level socioeconomic factors also play a role with regard to the risk of campylobacteriosis. For example, our findings indicate that campylobacteriosis incidence rates in zip codes characterized by the highest median incomes (≥$66,500) were two times that of zip codes with the lowest median incomes (IRR=2.09; 95%CI=1.79-2.44). This is consistent with a population-based study by Friedman et al.(2004)[5] that found higher numbers of *Campylobacter* cases among U.S. patients whose income was above the median income bracket of $30,000-$59,000. Simonsen et al.(2008)[20] also found a linear increase of infection corresponding to incremental increases in average gross annual income per adult in Danish households. The authors of this cohort study suggest that higher rates of campylobacteriosis among higher income groups may be attributed to the consumption of fresh, rather than frozen

poultry, a more expensive item available to individuals with higher income levels.[20] Another explanation may be that individuals with higher incomes and higher socioeconomic status (SES) overall may have more access to healthcare compared to lower SES groups, and are therefore, more likely to seek care for a diarrheal illness and ultimately be counted as a case in an active surveillance system such as FoodNet.

Our findings also indicate that the highest campylobacteriosis incidence rates occur in zip-codes having the lowest owner-occupancy rates. This may be attributed to differences in reporting among populations living in different housing situations. In a study examining factors associated with individuals reporting diarrheal illness (overall) in England, Tam et al. (2003) found that individuals living in privately-rented housing units were more likely to seek medical care for diarrheal illness than those living in owned or mortgaged properties[41]. Several other studies have also observed that living in rented housing units is associated with higher rates of general practitioner consultation[42;43].

Interestingly, in our final adjusted model, the association between the percentage of the population ≥25 yr. without a high-school diploma and *Campylobacter* infection rates exhibited a hormetic curve. Zip codes with the very lowest (<6.6%) and the very highest (≥16.1%) percentages of the population ≥25 yr. without a high school diploma were characterized by higher incidence rates. Since we observed that rates of campylobacteriosis are higher in wealthier neighborhoods, it seems plausible to concurrently find high rates of infection

among the most educated as well. However, it is somewhat surprising to find almost equally high incidence rates among zip codes characterized by the highest percentages of individuals ≥25 yrs without high school diplomas (IRR 1.38, 95%CI:1.21-1.57). This finding also may be attributed to differences in reporting frequency. Tam et al. (2003) found that individuals with lower educational levels were more likely to consult a general practitioner than more highly educated individuals. Scallan et al. (2006) observed a similar trend, although the difference was not statistically significant[44]. An individual's educational level can influence one's health beliefs, perceptions of health status, and ultimate need for seeking healthcare[41;45]. As a result, individuals with more education may feel more able to not only judge the severity of an illness but also know how to manage it without feeling the need to seek expert advice[41].

In addition to economic and educational factors, zip codes characterized by higher percentages of African Americans and Hispanics exhibited lower rates of campylobacteriosis. This protective effect has been observed in previous population-based studies; notably, Samuel et al. (2004), whose study of *Campylobacter* incidence across all ten U.S. FoodNet sites, found the lowest incidence rates among African-Americans compared to whites. As alluded to above, these findings may be attributed to differential access to healthcare between differing races and ethnic groups.

Limitations

Our study has several notable limitations. First, we were limited by the fact that the zip code level was the smallest level of analysis that we were able to perform at this time based on the FoodNet data available. A census block or census tract level analysis may have provided finer resolution and captured "community" environmental and socioeconomic impacts with more precision. For example, the concept of rurality is more precisely measured at the census tract and census block levels. Similarly, since we performed the analysis at the zip code level, we obtained U.S. Census data by ZCTAs as noted above. However, zip codes and ZCTAs do not correlate 100% of the time, resulting in some zip codes for which Census data is unavailable. Finally, data on the specific number of animals produced in a given zip code is not currently publicly available in Maryland and could not be included in our analyses. Given the importance of the presence of broiler chicken operations with regard to campylobacteriosis incidence, more transparency on the magnitude of animal operations would allow for more accurate assessments of the impacts of these facilities on rural health.

Conclusions

To our knowledge, this is the first U.S. study of its kind to evaluate community-level environmental and socioeconomic factors simultaneously with regard to their impacts on the risk of campylobacteriosis. We demonstrate that where you live may be as important as who you are, what you eat, and how you behave in terms of your risk of infection with a traditionally foodborne microorganism such as *Campylobacter*.

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Amy R. Sapkota: organized and conceived the entire collaboration; conceived the study design and supervised the study; contributed heavily to the writing; approved final version

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Table 1: Characteristics of reported campylobacteriosis cases in Maryland, 2002-2010.

Variable	***n* (%)**
Age (Years)	
0-4	381 (10.3)
5-9	173 (4.7)
10-19	366 (9.9)
20-59	2,188 (59.3)
60+	579 (15.7)
Gender	
Female	1,705 (46.2)
Male	1,976 (53.6)
Unknown	6 (0.2)
Race	
White	2,118 (57.4)
African-American	363 (9.8)
Other/Unknown	1,206 (32.7)
Outcome[a]	
Alive	3,574 (97.4)
Deceased	7 (0.2)
Unknown	87 (2.4)

[a]Data was missing for 19 cases.

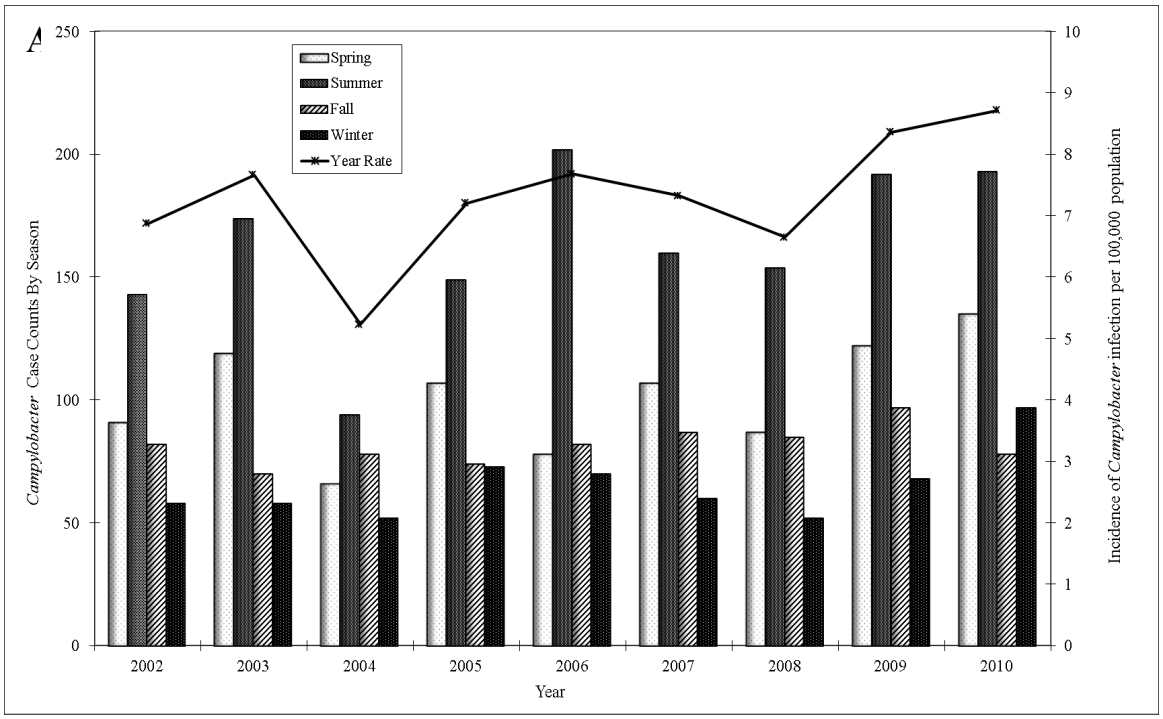
Table 2: Incidence rate ratios (IRRs) and 95% confidence intervals (95% CIs) for campylobacteriosis in association with environmental and socioeconomic factors.

		Multivariate, zero-inflated, Poisson regression[a]	
Zip Code Variable	**Category**	**IRR**	**95%CI**
Rurality	≤ 0.09%	-	-
	> 0.09% to ≤ 39%	0.81	0.35-1.91
	> 39% to < 100%	0.75	0.27-2.09
	100%	6.18	3.19-11.97
Broiler Chicken Operations	Absent	-	-
	Present	1.45	1.34-1.58
Turkey Operations	Absent	-	-
	Present	0.62	0.54-0.70
Aquaculture Operations	Absent	-	-
	Present	0.86	0.75-1.00
Dairy Operations	Absent	-	-
	Present	0.84	0.77-0.90
Median Income	< $40,000	-	-
	≥$40,000 to < $51,250	1.21	1.08-1.35
	≥ $51,250 to < $66,500	1.86	1.64-2.10
	≥ $66,500	2.09	1.79-2.44
Owner Occupancy	< 69%	-	-
	≥ 69% to < 80%	0.91	0.83-1.00
	≥ 80% to < 87%	0.80	0.72-0.88
	≥ 87%	0.80	0.72-0.90
Population ≥ 25 yr. without a high school diploma	< 6.6%	-	-
	≥ 6.6% to < 11%	0.66	0.60-0.73
	≥ 11% to < 16.1%	0.92	0.82-1.03
	≥ 16.1%	1.38	1.21-1.57
African American population	< 2.8%	-	-

	≥ 2.8% to < 9.4%	0.94	0.86–1.02
	≥ 9.4% to<21.2%	0.88	0.80-0.97
	≥ 21.2%	0.52	0.46-0.58
Hispanic population	< 0.8%	-	-
	≥ 0.8% to< 1.4%	0.43	0.39-0.46
	≥ 1.4% to< 2.4%	0.45	0.41-0.50
	≥ 2.4%	0.41	0.37-0.45

[a]The rurality variable was used as the predictor of excess zeroes and the model was fully adjusted, including the rurality variable, as well as all animal operation and socioeconomic variables shown in this table.

Figure 1: (A) Incidence of *Campylobacter* infection per 100,000 population and *Campylobacter* case counts by season for the state of Maryland, 2002-2010. **(B)** Incidence of *Campylobacter* infection per 100,000 population by age, sex, and degree of rurality in the state of Maryland.



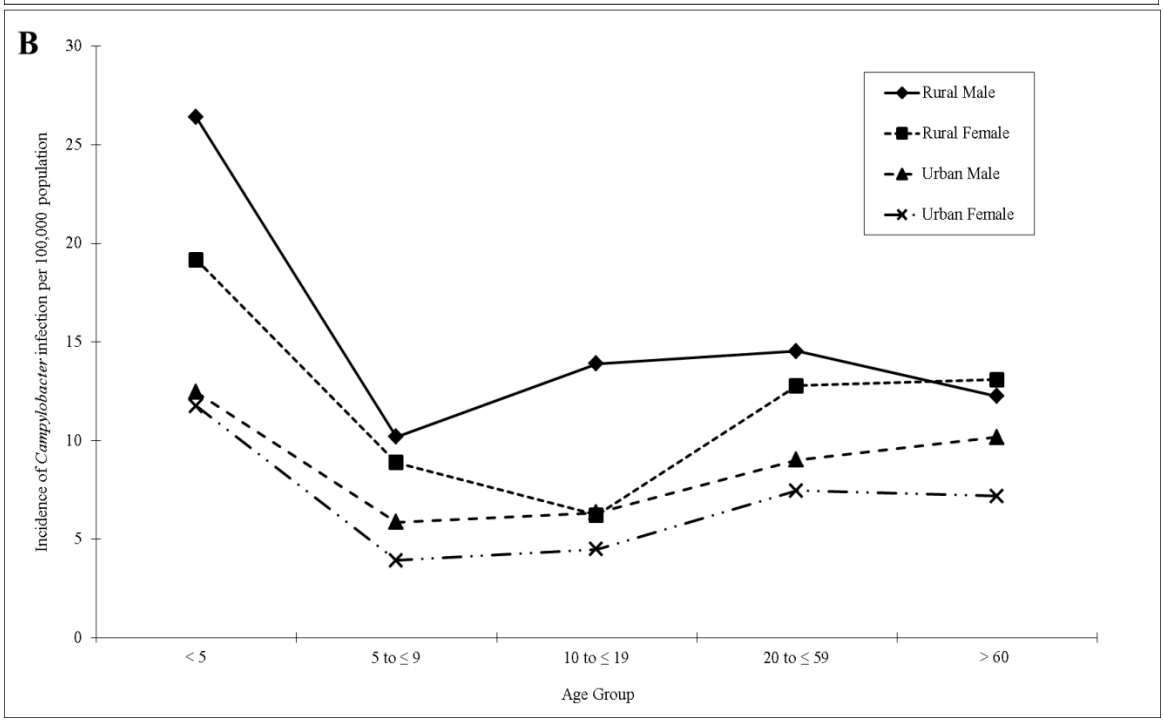
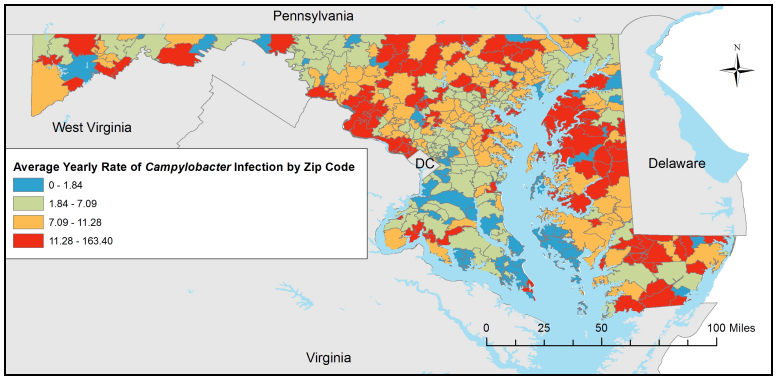


Figure 2: Spatial distribution of campylobacteriosis incidence rates, degree of rurality and presence/absence of broiler chicken operations by zip code in Maryland: **(A)** average annual campylobacteriosis incidence rates by zip code; **(B)** degree of rurality by zip code; and **(C)** presence or absence of broiler chicken operations by zip code.

A



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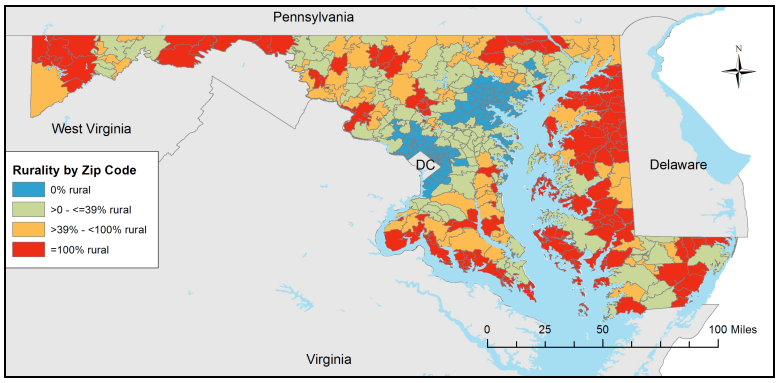
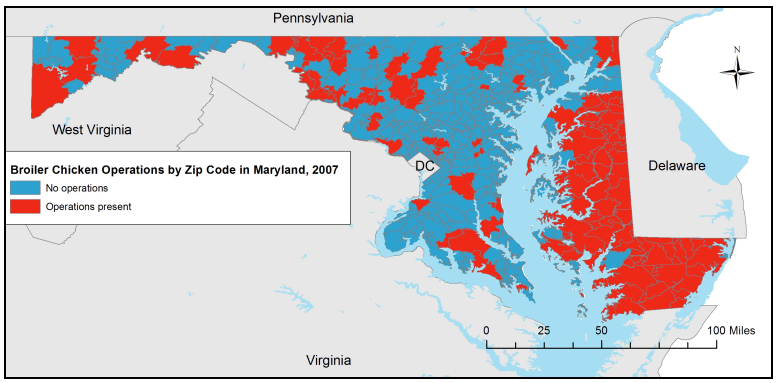


C



CHAPTER 5: DISCUSSION AND OVERALL CONCLUSIONS

As can be inferred from the background information provided at the beginning of this thesis, the issues surrounding risk factors for campylobacteriosis are numerous, and the literature review included in this thesis only begins to scratch the surface of the various environmental and sociodemographic risk factors involved. Until recently, many studies have focused either on sampling and isolating *Campylobacter* in the natural environment, including water sources, birds and mammals, or on identifying potential exposure sources in the human environment within which individuals might be exposed to *Campylobacter spp*, such as drinking water, retail foods, livestock or domestic animals. Sampling studies provide invaluable information as to detectable presence of the bacteria, but a direct link to associated human infection is more difficult to trace. Population-based case-control studies provide essential data on exposure and risk factors, but source attribution within a complex web of risk factors remains a challenge. A community-based ecological study can provide an additional component of information, namely a description of individuals within their own community. Hence, ecological studies constitute an important piece of the overall puzzle.

Ecological studies bring with them their own inherent limitations. Nygard et al. (2004) remind us that data collected for populations, rather than for individuals, do not necessarily reflect associations at the individual level. This can lead data analysis to a potential "ecological fallacy" in which a risk factor derived from aggregate data and subsequently ascribed to individuals does not, in fact, reflect the biological effect at the individual level (Morgenstern, 1998: Nygard, 2004). Nonetheless, since the effect of

environmental variables on those individuals can be difficult to assess, ecological studies are useful in examining the effects of environmental exposures, an approach made more feasible through geographical information systems which can analyze surveillance data according to various geographical units. In their research study, Nygard et al. (2004) found noticeable differences in associations between campylobacter incidence and environmental factors related to water and livestock in Sweden, depending on whether or not data was analyzed by place of infection and/or residence, by county or by municipality. Changing the unit of analysis altered associations between rurality, animal density, water sources and water-pipe length and rates of individual cases of campylobacteriosis: when the unit of analysis excluded major metropolitan areas, increased incidence rate ratios (IRR) were found for ruminant density and water-pipe length and decreased IRRs were found for percentage of the population using the public water supply. The researchers suggest that a multi-level study incorporating data from Individual-based studies with the data on environmental factors would further elucidate the risk factors between indirect transmission from farm animals through water and the environment, three factors which are currently difficult to measure (Nygard, 2004).

The issue of geographic units is a crucial one in ecological studies such as ours. This "Modifiable Areal Unit Problem" or MAUP, a term coined by Openshaw and Taylor in 1979, refers to variations in correlation values when different boundary systems are used, such as administrative boundaries or census enumeration units, leading to the generation of different data sets (Wong, 2004). So-called "zoning effects" become an issue as well as the potential introduction of bias into data analysis. Taking this into account, Arsenault, et al. (2012) attempted to model the incidence of reported cases of

campylobacteriosis in Canada according to environmental variables such as poultry density, ruminant density, slaughterhouse presence, temperature, and precipitation, and demographic variables such as population density and level of education at different levels of geographical organization. They categorized their model geographic scales as administrative (municipalities, census consolidated subdivisions and census divisions), health services (local community service center), natural (watershed) and custom (smallest, agriculture). They found that the direction of the associations was consistent and in agreement with current biological knowledge for all variables, but the strength and statistical significance of the associations varied among scales. They recommended that municipality or census consolidated subdivision scales were preferable in examining spatial distribution of campylobacteriosis in ecological studies, but cautioned that geographical scales should be carefully selected and inferences based on observed data at particular scales should be made judiciously (Arsenault, 2012).

Our research study was conceived along similar lines, examining the association between residence in a rural zip code and campylobacteriosis incidence. We obtained data from the 2000 Census of Population and Housing Summary File (SF1) and Summary File 3 (SF3) by 5-digit zip code tabulation area (ZCTA). (US Census Bureau, 2000) These are statistical geographical units developed by the U.S. census to mitigate the limitations of defining areas by individual zip code, administrative units which do not correspond to the data collection units applied by the U.S. Census Bureau. ZCTA data has been used in other studies and serves as a commonly accepted proxy variable for zip code-level data. (Tanaka, 2007; Drewnowski, 2007; Lurie, 2009) Like Arsenault et al. (2012), we then linked data on 8 socioeconomic variables including residence in a rural

ZCTA and race to sociodemographic information (age, gender) to environmental variables such as animal density, using data from the 2007 U.S. Census of Agriculture, National Agricultural Statistics Service (USDA, NASS, 2007).

Although it only scratches the surface of the many community-level factors which play a role in determining risk of infection from exposure to *Campylobacter,* our study is a first step. In fact, as far as we know, we are the first U.S. study to combine data on environmental factors, with sociodemographic risk factors, at the community level. The results contribute to the growing body of evidence that risk of campylobacteriosis might derive less from individual behaviors or sociodemographic background than from geographic location of an individual's residence (Zappe Pasturel, 2013). In public health discussions of environmental justice, *Campylobacter* is often brought up to be an exception to the common rule: unlike most foodborne pathogens, the whiter, richer and more educated you are, the more you are vulnerable to campylobacteriosis. Our findings, which link geographic residence to risk of infection, shed additional light on the occurrence and exposure to this foodborne pathogen.

To summarize, a leading cause of bacterial gastroenteritis in much of the developed and developing world, the significance of campylobacteriosis cannot be underestimated. Although the majority of these illnesses are still considered to be foodborne, the challenge of attributing specific infections to specific sources remains daunting. It is in this context that community-level data be combined with data on environmental and sociodemographic risk factors in order to obtain a more complete picture of how *Campylobacter* infection affects individuals and communities. This

research study contributes to the widening body of knowledge regarding the underlying causes of campylobacteriosis that may go beyond individual exposures and behaviors.

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