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Geographic and Temporal Epidemiology of Campylobacteriosis

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I am submitting herewith a dissertation written by Jennifer Weisent entitled "Geographic and Temporal Epidemiology of Campylobacteriosis." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.

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Geographic and Temporal Epidemiology of Campylobacteriosis

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Dedication

This dissertation is dedicated to my parents, Marguerite and Francis Weisent. Throughout my life they have offered me the freedom and encouragement to be adventurous, think independently, explore ideas and pursue lofty goals without hesitation or fear. I am eternally grateful for all of their support and love.

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My sincerest gratitude and loyalty go out to my dear circle of closest friends, especially Dr. Kristi Oldham, Siri Karta Barry, and Joanna Rodger—amazing, pioneering women, who challenge me relentlessly and support me unconditionally.

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Abstract

Campylobacteriosis is a leading cause of gastroenteritis in the United States. The focus of this research was to (i) analyze and predict spatial and temporal patterns and associations for campylobacteriosis risk and (ii) compare the utility of advanced modeling methods. Laboratory-confirmed *Campylobacter* case data, obtained from the Foodborne Diseases Active Surveillance Network were used in all investigations.

We compared the accuracy of forecasting techniques for campylobacteriosis risk in Minnesota, Oregon and Georgia and found that time series regression, decomposition, and Box-Jenkins Autoregressive Integrated Moving Averages reliably predict monthly risk of infection for campylobacteriosis. Decomposition provided the fastest, most accurate, user-friendly method.

Secondly, forecasting models were used to predict monthly climatic effects on the risk of campylobacteriosis in Georgia. The objectives were to (i) assess temporal patterns of campylobacteriosis risk (ii) compare univariate forecasting models with those that incorporate precipitation and temperature and (iii) investigate alternatives to random walk series and non random occurrences that could be outliers. We found significant regional associations between campylobacteriosis risk and climatic factors and control charting identified high risk time periods.

Our spatial study in Tennessee compared standardized risk estimates and investigated high risk spatial clustering of campylobacteriosis at three geographic scales. Spatial scan methods identified overlapping clusters ($p<0.05$) at census tract,

zip code and county sub-division levels. A greater number of smaller, finer resolution clusters were identified at the census tract level.

Objectives of the second study were to (i) identify socioeconomic determinants of the geographic disparities of campylobacteriosis risk (ii) investigate if regression coefficients demonstrate spatial variability and (iii) compare the performance of four modeling approaches: negative binomial, spatial lag, global and local Poisson geographically weighted regression. Local models had the best fit and identified associations between socioeconomic factors and geographic disparities in campylobacteriosis risk. Significant variables included race, unemployment rate, education attainment, urbanicity, and divorce rate.

Recent technological advancements have opened a virtually limitless 'toolbox' of analytical methods and offer novel means of identifying temporal spikes, spatial clusters and geographic disparities in campylobacteriosis risk that expand and hone our ability to create cost efficient, needs-based prevention and control measures.

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List of Abbreviations Used

AB: Antibiotic

AFLP: Amplified fragment length polymorphism

AIC: Akaike's Information Criterion

CDT: Cytolethal Distending Toxin

FQ: Fluorquinolones

FoodNet: Foodborne Diseases Active Surveillance Network

GBS: Guillain Barre-syndrome

GIS: Geographic Information Systems

GWR: Geographically weighted regression

IBD: Inflammatory Bowel Disease

LOS: lipooligosaccharide

MFS: Miller-Fisher's syndrome

MLST: Multi locus sequence typing

PCR: Polymerase chain reaction

PFGE: Pulsed field gel electrophoresis

RA: Reactive Arthritis

RAPD: Randomly amplified polymorphic DNA

SES: Socioeconomic status

UK: United Kingdom

U.S.United States

VBNC: Viable but nonculturable state

INTRODUCTION

Campylobacter sp. bacteria are a leading cause of human gastroenteritis in developed nations, infecting an estimated one percent of the U.S. population annually, surpassing pathogens such as *Salmonella* sp. and *E. coli* (1-3). As a result of the pathogen's ubiquitous nature, the establishment of causative associations between human infection and contaminated food or water, animal contact and other environmental sources is a formidable task (4-6).

Campylobacteriosis risk (defined as the probability that an individual will develop campylobacteriosis within a given time period (7)) is affected by a complex set of temporal and spatial factors (geographic regions, climate patterns, human behaviors, and food and water sources) (8-10). The seasonality of campylobacteriosis has been well documented worldwide, and variation in disease risk may be due to the effects of temperature and rainfall on the survival and reproduction of campylobacters in either the environment or on foods sources (11-13). Studies have yet to be performed to determine the importance of climate factors on disease risk or predict the future risk of disease in different geographic regions in the United States. Comparing how temperature and precipitation patterns vary among regions may help identify the sources of transmission, improve local accuracy in forecasting, and alert epidemiologists and public health officials to potential epidemics (14-17).

In addition, outliers and interventions in surveillance data may indicate outbreaks, geographic variation in reporting, and policy or prevention procedures that affect disease risk over time. While it is difficult to predict temporal change, statistical control

charting and past interventions can be incorporated into models to understand and improve process performance (18). Univariate models and those that incorporate exogenous climate variables could be combined with alternative statistical approaches to provide public health officials with an early indication of irregularity in disease incidence and result in more efficient and cost-effective control strategies (14, 19-23).

In the United States, Foodborne Diseases Active Surveillance Network (FoodNet) identifies marked geographic variations in the incidence of *Campylobacter* infection (24). Although the overall risk of disease in the U.S. population is 13 cases per 100,000, some areas in Tennessee have risks as high as 200 cases per 100,000 (25). Therefore, there is interest in identifying the determinants of the geographic disparities seen in disease risk so as to guide disease control efforts. Furthermore, the use of Geographic Information Systems (GIS) and spatial statistics to identify high risk regions serves to support and expand current knowledge on the mechanisms of transmission and geographic risk factors for this disease.

Understanding the impact of spatial scale on the assessment of health outcomes provide meaningful inferences from available geographic data (26, 27). Thus far, there is no consensus on what geographic scale is most appropriate, precise or useful for surveillance data (28-30). A number of studies exist that utilize cluster detection and mapping techniques to identify high risk regions for infectious diseases (31-33), yet few compare the differences in spatial patterns of disease at more than one level. From a public health standpoint, the utility of surveillance data is oftentimes limited by the level to which it can be aggregated and analyzed with reliable results. To address these

problems, we explore the direct impact of geographic scale on results of spatial cluster investigation using aggregated campylobacteriosis data in Tennessee.

Understanding health disparities among racial, ethnic and across socioeconomic subgroups is a priority of public health personnel and policy makers (34, 35). In developed countries, the relationships between socioeconomic status (SES) and campylobacteriosis risk are complex and have been shown to vary geographically, often between rural and urban areas (36-38). The geographic differences in associations between socioeconomic factors and campylobacteriosis risk are a worldwide phenomenon, hence regression models need to account for the fact that regression coefficients, used to assess associations between risk factors and disease, might vary in space. Local modeling approaches enable investigators to more accurately estimate the true relationships between determinants and disease risk since they estimate regression coefficients for each location in the study area (39-41).

Geographically weighted regression (GWR) modeling techniques compute local regression coefficients thereby allowing the estimates of the associations between outcome and explanatory variables to vary spatially (42, 43). This flexible modeling strategy is necessary to improve our understanding of the determinants of geographic disparities of campylobacteriosis risk. For example, local variations were detected in the occurrence of diseases such as obesity and breast cancer when modeled against socioeconomic factors (44-46). In these and other studies, global modeling approaches, that estimate one regression coefficient for each variable in the model, hide local variations in associations (40, 41). Local GWR model estimates allow health

professionals to better assess how the effect of the explanatory variable changes by geographic location. Armed with this knowledge, health planners can better identify the most important disease determinants for different regions and therefore better plan health programs, provision of services and resource allocation to meet the unique needs of different communities.

The potential for emerging infectious disease patterns to change in response to anthropogenic climate and land use changes warrants the continual improvement and updating of current forecasting systems. Technological advances in forecasting software and program capability beget systematic review of methods and their applicability in the realm of public health. Systematic analyses of multiple modelling techniques aims to create an optimal model to be used by public health officials with a state specific, accurate and user-friendly method for predicting disease risk. Risk forecasting could provide public health officials with an early indication of irregularity in disease incidence and act as an epidemic alert system (14, 19-22). Rather than apply a one-size-fits-all approach to control and prevention strategies, methods that identify regional variations in disease risks enable public health officials to provide more cost-effective needs-based population health planning.

The focus of this research was two-fold: To analyze and predict spatial and temporal patterns and associations for campylobacteriosis risk and to assess the utility of advanced temporal and spatial modeling methods and software systems.

This dissertation is comprised of six chapters, including a comprehensive literature review at the beginning, four self-contained studies that address unique spatial

and temporal modeling objectives, and finally, a summary of the discussions, recommendations and conclusions of the research.

CHAPTER 1

Literature Review

1.0 Introduction

The Campylobacteriaceae family are Gram-negative, spiral shaped bacteria that are typically microaerophilic and motile (1-3). The family members are primarily gastrointestinal commensal organisms and include *Campylobacter*, *Arcobacter* and *Bacteroides* (47, 48). The genera together (*Campylobacter, Arcobacter* and *Helicobacter*) comprise over forty species, most of which have been isolated from humans (5). Thermophilic campylobacters, in particular *C. jejuni, C. coli* and *C. lari,* are responsible for the majority of disease in humans (47, 49), with *C. jejuni* comprising 80-90% of infections (1). While *Campylobacter spp*. have been known causative agents of diarrhea in domestic farm animals since the early 1900's, in the last 30 years *Campylobacter* infection has emerged as the most common cause of bacterial gastroenteritis in humans (1, 2). Despite being responsible for over 2 million cases annually in the United States, death due to *Campylobacter* infection is uncommon. Deaths occur, worldwide, in approximately 5 per 10,000 people (50) and in the United States, up to124 die annually (51). Victims are often infants, elderly or other immune compromised, individuals (2).

2.0 History

The organism now known as *Campylobacter* was first found in infants with diarrhea that died in the 1880's (47, 49). Theodore Escherich described the bacterium microscopically from neonatal stool samples in 1886, but was unable to obtain a successful culture (52). Subsequently, the research community lost interest as it was

not considered to be a pathogen of significance. *Campylobacter* was definitively identified in 1913 by McFadyean and Stockman during research into epizootic abortions among sheep in the United Kingdom (UK) (1, 49).

Initial description and naming of *Vibrio fetus* as a major cause of abortion in cattle and sheep was confirmed by Smith in follow-up morphological work performed in 1918 (52) (49, 53). In 1957, Elizabeth King identified two distinct subgroups by visual, serological and clinical characteristics (1, 48). In 1963 the organism was differentiated from the genus *Vibrio*, and given the name *Campylobacter*, derived from the Greek word for 'curved rod' (49).

The first report of human *Campylobacter* enteritis was documented in 1938 and attributed to consumption of contaminated milk (53). It was not until 1972 that the organism was isolated from human blood and feces by Dekyser and Butzler (47). In 1977, Martin Skirrow described a culture and incubation procedure that has since been adopted as a standard laboratory method for fecal isolation. This technique paved the way for further research and classification (5, 49). Most recently, four main species of *Campylobacter* (*jejuni, coli, lari, fetus*) which share less than 35% genetic similarity have been characterized using DNA hybridization (54). To date, thirteen additional species have been identified, the majority of which have been isolated from humans (5, 55, 56).

3.0 *Campylobacter* species/strains

For this review, "*Campylobacter"* refers to either *C. jejuni* or *C. coli* as these two species account for the vast majority (90-99%) of *Campylobacter* strains isolated from humans (57-59). The term may also be used to designate campylobacter isolates when

identification was not undertaken at the species level. The 2007 quarterly report from the European Centre for Disease Prevention and Control identified *C. jejuni* as the predominant species (92.9% of isolates) recorded for 15 European countries under surveillance (60). *Campylobacter coli*, the second most frequently isolated species, accounts for between 7 and 18.6% of human cases (61). A study of poultry house isolates in France detected 77.7% contaminated with *C. jejuni*, 10.7% *C. coli* and 11.6% had both (62). The same study sampled supermarket chicken parts and found *C. jejuni* in 74% and *C. coli* in 26% of positive samples.

Isolation procedures developed to enhance the growth of *C. jejuni* and *C.coli* may be supportive of other species of pathogenic importance (57). In a study of South African pediatric patients, 16.2% of stool isolates had multiple *Campylobacter* isolates. Such pathogenic strains include *C. concisus*, *C. upsaliensis, C. jejuni* subsp. *doylei* and *H. fennelliae* (57). C*ampylobacter lari, C.hyointestinalis,* and *C.fetus* subsp *fetus*, have also been associated with diarrhea in humans (63). A study of feedlot cattle shedding patterns in Alberta, Canada detected *C. lanienae* (55.5%), *C. hyointestinals* (7.7%) and *C. fetus* (1.7%) in fecal samples over five collection periods during a four month period (64). Dogs and cats are considered to be primary reservoirs for *C. helveticus* and *C.upsaliensis* (65, 66). The pathogenic potential of some species in humans remains unknown.

In Poland *C. coli* were detected in 30.1% of positive human samples and 56.9% of poultry meat samples. The authors concluded that a regional component may be of importance in species prevalence (67). Geographic differences among strains from

developed versus developing nations have also been reported (63). In South America, approximately 25% of human cases are due to *C. coli* isolates whereas industrialized nations typically see no more than 5% of this species (68). Similarly, *C.coli* was found in 41% of human isolates from Hong Kong and 39% from the Central African Republic (63). While prevalence of strains may vary geographically, they are found homogenously among hosts. In a study of *C. jejuni* (control) and *C.coli* (case) no significant differences were found in age, gender or geographic locale between the two species (58). In general, *C. jejuni* patients are more likely to be hospitalized, and *C. coli* patients are often linked to consumption of river, stream or spring water, suggesting differences in disease source, transmission and outcome. The reasons for such differences are a major topic of scientific investigation in the field of health geographics.

4.0 Clinical Signs

Onset of human campylobacteriosis is characterized by fever and acute inflammatory enteritis (48). The mean incubation period of three days (range 1-7 days) is longer than most gastrointestinal infections (54, 69, 70). Infection may be accompanied by influenza-like symptoms, abdominal pain and cramping, followed by profuse watery diarrhea (69). In a study of 3489 confirmed cases of campylobacteriosis in 2003, 96% reported diarrhea, 28% bloody diarrhea, 81% fever and 86% abdominal cramps. A second case-control study including 7,360 responses demonstrated very similar results: Diarrhea 95%, abdominal pain 85%, fever 78% and bloody diarrhea 27% (71). In a cohort study of diarrheal illness, *Campylobacter* positive cases were more likely to show signs of fever [OR 3.19 (95% CI 2.36, 4.31)], muscle aches [OR 3.13

(95% CI 2.32, 4.22)] and abdominal pain [OR 3.40 (95% CI 2.32, 5.12)] than patients with *Campylobacter* negative laboratory results (72). Cases due to *C. coli* tend to be milder than those due to *C. jejuni,* although they are not clinically distinguishable without speciation (63, 69).

In children the disease can manifest as abdominal pain without diarrhea (30%), and although patients infrequently vomit (15%) nausea is a common symptom (69). Children are also more likely to pass frank blood in the stool (69). Although most infections are confined to the intestines, systemic infection may accompany intestinal disease, especially in immune compromised, very old and very young patients (48, 54). Immune deficient patients can become chronic carriers of the bacteria and suffer recurrent bouts of enteritis and bacteremia, although the importance of bacteremia associated with enteritis is not clearly understood (54, 69).

Intestinal complications can also occur, such as colitis, toxic megacolon and intestinal hemorrhage (73). Hepatitis, nephritis, pancreatitis and myocarditis are other reported extra-intestinal sequelae (73). The wide array of potential clinical manifestations are postulated to be a result of the organism's unique genetic diversity, a high priority research topic in molecular and clinical epidemiology (74).

5.0 Pathogenesis and Virulence Factors

The details of *Campylobacter* pathogenesis and virulence factors are not clearly understood (48, 63, 74, 75). From an epidemiological standpoint, there are no major differences in virulence among *C .jejuni* strains, and many species (*C. concisus*, *C. upsaliensis, C. jejuni* subsp. *doylei* and *H. fennelliae)* are known to cause similar clinical

signs (76) (57). *Campylobacter jejuni* is implicated in over 90% of human cases in developed nations, therefore, comprehensive knowledge of pathophysiology is limited to this species (54). Pathology of the disease is described for human hosts, as domestic and non-domestic animal species, including poultry, often exhibit asymptomatic intestinal colonization (77).

Following ingestion by the host, *Campylobacter* colonizes both the small intestine and its target organ, the colon (78). Gastric acid in the stomach and bile salts may eliminate much of the consumed dose; however, *C. jejuni's* ability to bypass these natural defense mechanisms enhances overall virulence (54, 79, 80).

Campylobacter jejuni and *C. coli* cause inflammation and may cause tissue damage by translocating across the intestinal barrier (81). During infection, the bacteria is found primarily inside or closely associated with intestinal crypt cells (82). *Campylobacter jejuni* is capable of surviving intracellularly, which helps it to evade the host immune response (75, 82). The pathogen essentially 'hides out', by residing in a membrane bound compartment within the intestinal epithelial cells (75, 81). While inside the cell, the bacteria undergo physiological changes that enable them to avoid delivery to lysosomes and remain viable for a minimum of 24 hours (81).

Results from intestinal biopsies and leukocyte counts in stool samples of *Campylobacter* enteritis cases have helped to classify the disease as inflammatory in nature (48). Phagocytic mononuclear and polymorphonuclear cells have also been detected within the intestinal epithelium (81). Physical adherence of bacteria to the epithelial mucosa initiates the host inflammatory response and results in the

development of diarrhea (53, 83). Tissue injury secondary to this inflammatory response occurs in the jejunum, ileum and colon where marked epithelial gland destruction, crypt abscess formation and infiltration of the lamina propria occur (1).

Recent evidence suggests that the bacteria have also developed efficient strategies for entry into the subcellular epithelial space, leading to infection levels between 10-15 bacteria per cell within 2-3 hours (83). Other postulated virulence factors alter gut motility in the host and enable survival in blood phagocytes (54, 77). The clinical manifestation of diarrhea occurs primarily as a consequence of cell damage resulting from pathogen infiltration and reduction in intestinal absorptive capacity (48) (81). Extensive damage to host intestines can result from the combination of inflammation, epithelial hemorrhage and the release of bacterial toxins (54, 79, 81).

5.1 The role of flagellum in pathogenesis

The flagellum is a necessary component of *Campylobacter* pathogenesis, infectivity and virulence (54, 77, 81). The rapid, erratic motions produced by the major and minor flagellin (flaA and flaB) are vital to overcoming peristalsis and intestinal colonization (48, 78) . *Campylobacter jejuni* motility allows for effective translocation, adherence and invasion in viscous conditions such as those found in the thick mucus linings of the intestinal tract (78). Recent molecular typing studies seek to understand the mechanism of *fla*A, as flagellar characteristics that support successful colonization in the chicken gut may be linked to increased virulence in humans (84). Evidence suggests that other virulence factors undergo co-regulation with flagella and that flagella may contain adhesins for attachment to epithelium (54, 85). The flagellum also secretes

antigens required for invasion of the host epithelium (86). These proteins contribute to virulence via Type III secretory apparati that project into and compromise the host cell (53, 86). Recent microarray studies and genetic characterization of the strain NCTC11168 (the first sequenced strain) variant confirm the association between antigenic proteins and virulence (78).

5.2 The role of bacterial enterotoxins

Several studies report that pathogenic strains of *Campylobacter* produce Cytolethal Distending Toxin (CDT), a three subunit holotoxin that allows binding and penetration of host cells (80, 86). CDT toxins have been found in gastrointestinal pathogens such as *E. coli* and *Shigella* and are a known cause of cell distention and death (87). However, the enterotoxin genes have not been identified in *C. jejuni* and the specific role toxins may play in *Campylobacter* pathogenesis remains unclear (48, 86, 87). Cytolethal Distending Toxin may cause cell cycle arrest, have apoptotic effects, and induce Interleukin-8 (IL-8), a pro-inflammatory cytokine thereby increasing host-pathogen epithelial contact time and immunosuppression (75, 80, 85). The inflammatory response and IL-8 release are considered crucial to induce diarrhea in the host (53, 75). Additional hypotheses suggest that other cholera-like enterotoxins are also involved in the pathogenesis of *C. jejuni* (1, 87). However, enterotoxins have not been found in fecal samples of infected individuals, nor have antibodies to toxins been identified (1, 48, 77).

5.3 The role of cell surface properties

Campylobacter jejuni's success as a gut pathogen and commensal is linked to its ability to modify cell surface properties (84). The lipooligosaccharide (LOS) and capsular structure of *C. jejuni* are highly variable and associated with effective evasion of the host immune system (85, 86). Both structures are implicated in epithelial cell adherence, invasion and virulence (83, 86). Reduced virulence and adherence, in vitro and in vivo, have been found in capsule deficient strains (53, 85). Lipooligosaccharide may also play a role in extraintestinal manifestations of the disease such as Guillaine Barre Syndrome (GBS). For example, LOS can be mimicked on the molecular level by peripheral nerve gangliosides resulting in autoreactive antibodies and subsequently, a damaging inflammatory response (53).

5.4 Other important virulence factors

Secretion and signal transduction mechanisms, O-linked and N-linked glycosylation systems, flagellar proteins, adherence appendage identification, and chemotaxis are areas of *Campylobacter* pathogenesis and virulence that require further investigation (53, 86). Chemotaxis toward mucus and amino acids has been observed, yet protein response, attractants and signal transduction mechanisms are not well understood (86). A series of adhesins (CdF, JlpA, CapA, CBF1) have also been described, each making an important contribution to binding, invasion and inflammation (86). The protein CiaB is secreted by *C. jejuni* as a prerequisite to invasion of cultured epithelial cells (86). This secretion mechanism has been linked to an export system on the flagellum which has yet to be fully characterized. Other studies have tied O-linked

glycosylation with assembly and motility of flagella and N-linked glycosylation with *Campylobacter* virulence factors (53).

Despite the many recent advances in the understanding of *C. jejuni* pathophysiology, it is believed that the majority of virulence factors have yet to be identified (74, 85). In addition, the relative importance of *Campylobacter*-host cell interactions in the role of the infectious process remains unclear (2, 81, 83). Major barriers to understanding *C. jejuni* pathogenesis are attributed to (i) the lack of non-primate models that mimic intestinal pathology, (ii) an inability to fully substantiate hypotheses on the molecular level and (iii) the complex and variable genetic makeup of the organism (79, 85, 86). Advances in microarray and other genetic tools are expected to make great contributions to our knowledge in the near future (84, 86).

6.0 *Campylobacter* biology and the environment

6.1 Biology and survival

Campylobacters oxidate amino acids or tricarboxylic acids as sources of energy and grow in microaerophilic environments (containing 5-10% oxygen) (48, 88). Stringent nutritional requirements and a minimum of adaptive responses limit their ability to grow in the environment, especially when compared to other bacterial pathogens (74). Campylobacters are only capable of reproducing at temperatures found in warm-blooded animals (48, 88), and upon excretion from human, animal and bird hosts, must contend with a host of stress factors. When expelled into aquatic and terrestrial environments they are immediately exposed to variable levels of oxygen, temperature changes, nutrient sources and ultraviolet radiation (89-91). Experimental evidence has

shown that temperature and atmospheric conditions have a significant influence over the expression of genes (92).

Recent molecular studies have identified genes thought to confer protective heat and acid tolerance traits via plasmids (89). Unlike *Salmonella* and *E. coli* which possess numerous genetically expressed defense mechanisms (osmoprotectants, and regulators of oxidative stress and heat-shock) campylobacters have a more limited range of cellular survival adaptations (89, 92, 93). *Escherichia coli* remains culturable for much longer periods of time under the same experimental conditions (>200 days versus <85 days for *C. jejuni*). Thus, although campylobacters remain viable in the environment, detection is a challenge (93).

Campylobacters require high temperatures (32-45 ^{0}C) for replication in the host (88, 94). Paradoxically, the organism survives more readily at low environmental temperatures (4^{o}C versus 22^{o}C or 30^{o}C) and in cold aquatic environments (89, 90). They are also sensitive to ultraviolet-B radiation at experimental levels comparable with sunny conditions (91, 95). Obiri-Danso et al. demonstrated rapid decline in survival rates under light and temperature ranges reflective of summer conditions in the UK (95). Positive environmental samples were detected more frequently on rainy versus sunny days by Louis et al.(96), supporting the fact that campylobacters are sensitive to desiccation (89, 96).

Evidence shows that *Campylobacter* survival declines in response to warmer environments, in part due to competition with other bacteria (88, 96). Experimentally, the pathogen's ability to adhere and invade host cells diminished as ambient or aquatic

temperatures rose (91). *Campylobacter jejuni* are no longer culturable after 12 hours, at 37^{o}C, whereas in colder conditions (4^{o}C) they remained culturable for up to 5 days (95, 97). Thomas et al. found the greatest change in rate of decay between 15-25^{o} C, with a rapid decline in culturability at temperatures exceeding 15^{o} C (90).

Despite these limitations, campylobacters are commonly present and transmissible in both aquatic environments and animal excreta (98-100). In Wales, human campylobacteriosis rates were correlated with annual increases in temperature and the overall seasonal pattern was associated with environmental factors more so than food consumption (96). Rollins et al. hypothesize that survivability in colder conditions may improve the organism's likelihood of being recycled into animal hosts in the spring (97), thereby playing a key role in maintaining a viable reservoir over winter. The combination of *Campylobacter's* complex response to environmental stressors and unique host interactions continue to perplex the research community.

6.3 Free-living organisms as potential reservoirs

Free-living protozoal organisms and arthropods may provide campylobacters with substantial reservoirs and vehicles for infection. One potential reservoir includes the Darkling beetle (*Alphitobius diaperinus)* and larvae, within which detection of live campylobacters has been recorded (101). The exact mechanism by which arthropod dissemination of the pathogen occurs is poorly understood. *Campylobacter jejuni* also shows improved survivability when cultured with amoebic organisms, as opposed to when cultured alone (102, 103). In laboratory experiments, campylobacters survived within the amoeba (*Acanthamoeba polyphaga*) at temperatures that normally diminish

survival time in the environment (102). Following co-cultivation, campylobacters were alive and motile within amoebic vacuoles, and survived rupture of the host cell. A similar study showed that *C. jejuni* remained viable longer in co-cultures with protozoa and had a significant increase in ability to resist disinfection (103). Further studies to understand and quantify the symbiotic relationships between protozoal species and arthropods in *Campylobacter* survival and disease transmission are needed.

6.4 Viable but Nonculturable State

The viable but nonculturable state (VBNC) is a physiological change of state, described as a biological transition induced by suboptimal environmental conditions. The state may be of transitory dormancy or ultimate decline for the organism, but this has yet to be determined (89, 102). Campylobacters in the VBNC have been found in biofilms, thin adherent groupings of microorganisms that create protective aquatic microenvironments during extreme conditions (89). They have been shown to be less capable of responding adaptively to temperature and acidity in biofilms (104). The effect of biofilms and the VBNC state is of unknown significance to the survivability and pathogenesis of *Campylobacter*.

Studies indicate that conditions of stress such as elevated temperature, atmospheric oxygen and nutrient deficiency have a greater effect on culturability than survivability, with nutrient deficiency being the most important stress factor overall (91). Metabolic activity in the VBNC state has been recorded for up to 30 days and under favorable conditions, a return to culturable form has been reported (89, 97, 105). Moen et al. showed that *C. jejuni* expresses genetic traits that activate mechanisms for

survival under non growth conditions (92). Cook and Bolster observed that viability of *C. jejuni* was present under laboratory conditions more than 50 days after loss of culturability (93). *Campylobacter* cells tend to lose their recoverability prior to undergoing actual membrane disruption (92). Thus, is it difficult to quantify the outcome of environmental stress and the role of the VBNC state in the transmission of disease. In essence, campylobacters that transition into this form are capable of 'slipping under the radar.'

Scientists and public health regulators often quantify human exposure to bacteria as a function of the pathogen load in the environment. For example, water safety standards rely on the absence of bacteria to indicate safety (106). The inability to detect the VBNC form has been implicated in inconsistent findings between the presence of *Campylobacter* and more easily detectable fecal indicators in water and sewage (93). Difficulties in detecting VBNC campylobacters hinder our understanding of the disease ecology and may be an important factor in identifying associations between the risk of disease and environmental sources (107).

6.5 Environmental conditions and survivability

Campylobacter survivability declines as temperature and light increase, causing oxidative stress (108). In contrast, the risk of campylobacteriosis in human populations increases during warmer months with longer daylight hours. One explanation for the dichotomy is that *C. jejuni's* ability to survive in water improves as turbidity, oxygen and nutrient levels vary seasonally (91-93, 99). Under conditions of high oxygen tension campylobacters have lower survivability, as determined by the expression of 'stress

genes' (92). Warmer waters tend to maintain lower aquatic oxygen levels, thereby enhancing survival.

A combination of land use factors, farming strategies and summer weather patterns may serve to elevate bacterial loads, and offset the detrimental effect of temperature. A study in the U.S. coastal region of Georgia that encompassed a mixed-use watershed with known agricultural run-off showed that increased concentration of campylobacters in aquatic systems during warmer months was associated with increased precipitation and turbidity (99). The hypothesis was that elevated pathogen levels contributed directly to high prevalence of human campylobacteriosis in the county of study (17 per 100,000 persons versus 6.8/100,000 for the remainder of the state). While this seems logical, the environmental load of bacteria was highest during the summer months when both ambient and aquatic temperatures were at their annual peak. This complex relationship between ecological factors and bacterial adaptation make it difficult to identify individual environmental contributions to disease burden.

7.0 Transmission

7.1 Common routes

Campylobacter primarily undergoes fecal-oral transmission. The infective dose has been documented between 500-10,000 organisms (2, 47, 54, 62, 69). The likelihood of colonization increases with dose and the estimated number of organisms necessary for diarrheal illness can range from 53-20,000,000 (109). Direct person-to-person transmission is rare. However, a recent case-control study identified 'exposure to household members with diarrheal disease' as a risk factor [OR 3.5 (95% CI: 1.6-

8.0)] for campylobacteriosis (110) . In untreated patients with normal immune function the organism can be excreted for up to 3 months (range 2 weeks to 3 months) (54, 111). Although it is hypothesized that campylobacters have a low level of infectivity after passage through humans (76), further investigation is required to determine if direct transmission is more common than suspected.

Conlan et al. assert that the key to understanding transmission of *Campylobacter* lies in unraveling the complex process of colonization in poultry (70). While recent molecular evidence suggests that vertical transmission occurs in broiler flocks (112), horizontal transmission from on-farm sources is the most likely mechanism of colonization (70). Current research aims to find ways to decrease transmission and colonization in poultry and other farm animals and thereby decrease the level of contamination in human food sources, drinking water and the environment.

7.2 Flies and rodents as vectors

The ubiquitous nature of campylobacters, coupled with the low infective dose in humans, contribute to the theory that flies and rodents are important mechanical vectors in the transmission cycle (113-115). Flies have also been implicated as part of the chain of transmission and infection associated with warmer months (113, 116). Guerin et al. modeled temperature as a function of fly activity and found an increased prevalence in poultry flocks during conditions when flies are active and abundant in Iceland (117). It is theorized that poultry may become colonized after ingestion of either *Campylobacter*-positive flies, their excrement or regurgitated gut contents (117). In another study, *C. coli* were isolated from flies during environmental sampling on pig farms (61). In 2004, a

New Zealand study of urban and farm sources, found a 9% prevalence of *C. jejuni* in flies (114). In a Danish case-control study, broiler houses without screening had a *Campylobacte*r positive flock prevalence of 52.4% while those with screens had a prevalence of 15.4% (118). This suggests that the physical barrier to flies may prevent colonization of poultry. The importance of flies and rodents in the disease cycle has yet to be substantiated and is under current investigation.

8.0 Immunity

8.1 Acquired Immunity

The human immune response to campylobacters, an inflammatory gastroenteritis, is paramount to understanding its complex epidemiology (48). Currently, a number of *Campylobacter* specific antigens have been identified, with flagellin (a flagellar protein subunit) being immunodominant (79). Infection does not guarantee clinical disease, and a better understanding of how host factors affect variation in individual susceptibility and clinical signs would improve our ability to identify important risk factors (79).

The humoral and cell-mediated immune systems have been implicated in host immune response (79, 81, 119). Infection confers short term immunity of unknown duration by stimulating antibodies within five to seven days of infection (69, 79). Peak antibody response occurs within 2-4 weeks, declines over several months and can be detected in serum and mucosal secretions (56). Higher antibody responses have been associated with milder clinical signs (120), less excretion of the organism, and an immune host may render *Campylobacter* nonpathogenic (63). Although asymptomatic

excretion has been commonly documented in immune individuals the mechanism remains unknown (54, 121).

Evidence for partial immunity is seen in the disproportionate number of *Campylobacter* cases among travelers versus indigenous population from developing nations (63). Exposure to indigenous strains can protect an individual within a given region. Partial immunity is thought to be the primary reason why children in developing nations excrete the organism for a shorter duration than those in developed countries (63, 79). Children in developing countries are exposed to many *Campylobacter* species at an early age, whereas in developed nations exposure may be strain specific (122). This difference is mainly attributable to lifestyle and behavioral factors. In all children, for the first six months of life, immunoglobulin (IgA, IgM and IgG) levels remain low despite exposure. This is due to both maternal antibodies and poor serologic response by young children and contributes to the elevated risk in this age group (123). However, breast feeding may be protective and has been shown to decrease incidence of symptomatic infection in children (56, 123). As age increases beyond six months, all baseline levels of IgA and IgM (lesser extent IgG) increase in response to infection (63, 79). These exposure and immune status differences help to explain why young children in developed regions have a higher incidence of clinical disease when compared to those in underdeveloped regions.

The risk of acquiring the disease is likely to be affected by the number of immune individuals in the population (herd immunity) (119). Acquired immunity to specific strains is one hypothesis for why *Campylobacter* rates in some regions have been declining

(124). As the frequency of infection increases, there is a resulting decrease in disease due to increased prevalence of acquired immunity in the population.

Immune response differences have been identified between the general population and workers on farms and in abattoirs (120). For example, workers with direct exposure to the organism demonstrate a higher level of conferred immunity, despite having a higher overall risk of disease (125). One food-borne outbreak study found a lower risk of infection (RR 0.76, 95% CI 0.39-1.52) in animal workers versus non-animal workers (124). In a study of surveillance data in Scotland, Miller et al. found that older age groups had lower incidence of disease and were less likely to be infected by common serotypes (126). This supports the hypothesis that exposure over time leads to increased immunity. Currently, efforts are being made to document how animal exposure confers immunity and identify differences in attack rates between human subgroups (Ie. farmers and animal workers versus others) (124). The goals are to characterize antibody titers of high exposure groups to help clarify how the immune system response correlates to the risk of disease.

8.2 Immune compromise

People with genetic or acquired defects in humoral or cell-mediated immunity are at higher risk for developing campylobacteriosis (79). More severe clinical manifestations of the disease are associated with immune deficiency disorders such as AIDS, hypogammaglobulinemia and agammaglobulinemia (73, 79). In AIDS patients, incidence rates of infection are up to 40 times that of immune competent individuals (69, 79). Individuals with chronic intestinal illness (Inflammatory Bowel Disease (IBD), celiac

disease), rheumatism and asthma are also at higher risk for *Campylobacter* infection (127). It is important to note that individuals who suffer from these conditions have increased susceptibility to a host of other pathogens and it often remains unclear which disease process occurred first.

Higher infection rates have also been associated with immune compromise resulting from increased use of antibiotics and from use of antacids (128). Antacids decrease the ability of the stomach, a first line of defense, to kill bacteria. Many drugs alter normal host defense mechanisms, and *Campylobacter* infections associated with prior antibiotic use may be a result of selective advantage (129). In essence, drug resistant *Campylobacter* outcompete normal flora, leading to colonization and clinical disease. The immunological effect of drugs such as antacids and antibiotics on disease acquisition is currently a hot topic of research.

8.3 Vaccination in humans and animals

Due to the strain specific nature of immunity and complexity of antigens, an effective human vaccine has not yet been developed (79, 121, 130). However, promising vaccine formulations are currently being tested, specifically through military avenues such as the Navy Medical Research Institute in Bethesda, Maryland (121). Vaccine development in humans is supported by clinical trials that demonstrate acquired immunity and resistance to colonization following initial infection (130). In contrast, safety issues such as incomplete knowledge of *Campylobacter* pathogenesis and the complication of Guillaine Barre-syndrome continue to hamper vaccine efforts (130).

Campylobacter rarely causes clinical disease in domestic animals, yet vaccination of animal species has been considered as an indirect means of human disease prevention by decreasing the bacterial reservoirs (131). The theory for reducing shedding in nonclinical animals is based on the assumption that vaccines may confer a nonspecific boost to the immune system that results in overall reduction in pathogen load (131). Poultry are the largest known sources of infection and are the primary animal species in which vaccination might have a positive impact on public health. Some researchers hypothesize that vaccination would be the most effective strategy for prevention and control of human disease caused by poultry (56). Promising strategies, such as CmeC subunit vaccines for subcutaneous injection in chickens are currently under investigation (132). However, as is the case with humans, the genetic variability and instability of campylobacters hinder the formulation of an effective and economical vaccine in poultry (133). To date, cost effective vaccines are not available and have not yet been universally approved or implemented in animals.

9.0 Diagnosis, isolation and identification

Campylobacters can be identified in fresh stool samples, particularly during the acute stage of illness. Gram stain analysis, the most common method, is sensitive (66-94%) and specific (>95%) (134). Other identification methods include phase contrast and dark field microscopy. Following primary isolation, colonies should test oxidase positive and be visually identified by their grey coloration and irregular, flat appearance (134). Commercial culture systems are also available to isolate *Campylobacter* species from blood samples, but this detection method is much less commonly performed.

Selective culture media and passive filtration methods have been developed over the past thirty years as a means of transporting and isolating *Campylobacter* (135). Clinical samples are grown and isolated using selective antibiotics whereas environmental samples often require pre-enrichment procedures to increase bacterial numbers (48). Thermophilic species are usually incubated at 42^{o} C, and isolation of a broader range of species can be achieved at 37^{o} C (135). Ideal atmospheric conditions include 5% oxygen, 10% carbon dioxide and 85% nitrogen (134). Ninety seven percent of fecal samples from acute infections can produce positive isolates within 48 hours of incubation on appropriate culture medium, under microaerophilic conditions, thereby providing a turn-around time for identification of 2-4 days (135, 136).

Preparation of campylobacters prior to molecular typing is an important component of successful identification and typically requires culturing in enriched broth or agar. Commonly used Preston broth enrichment procedures eliminate the need for plating to identify the bacteria, and result in rapid detection (3-4 days). A study by Denis et al. found no significant difference in PCR identification methods for *Campylobacter* species extracted after 24 hours in Preston broth when compared with colony collection after 72 hours on agar plates (62). Other selective media are routinely used for isolation of clinically important campylobacters. Most are commercially available, charcoal based, blood-free or blood-containing, and include antibiotics to inhibit unwanted intestinal flora (134).

10.0 Treatment and Prognosis

Human campylobacteriosis is typically self limiting; the vast majority of individuals clear the infection without chemotherapy (48, 79). Oral replacement of fluids and electrolytes are the primary treatment and antibiotic therapy in the early stages of infection has been shown to shorten the duration of illness (69). Patients do not typically seek medical attention until they have had clinical signs for 4-6 days, at which point the illness is often improving (69). Up to 20% of patients experience mild relapse several days after remission of signs and immune compromised patients may be permanently unable to clear the organism (48). The drug of choice for treatment includes a 5-7 day course of erythromycin (111). Emerging resistance to macrolides has prompted the use of fluoroquinolones such as Ciprofloxacin, tetracyclines and chloramphenicol as alternative antibiotics (73). Antimicrobial treatment, in general, is only indicated in severe, recurrent or systemic cases or in immunosuppressed individuals (79, 111).

11.0 Complications

11.1 Guillain Barre-syndrome (GBS)

Guillain Barre-syndrome (GBS) is characterized by an acute peripheral neuropathy, histologically similar to autoimmune neuritis (137). While two thirds of GBS patients have had some form of infection within the previous six weeks, approximately 25% of patients were infected with *Campylobacter jejuni*, the most frequently identified pathogen (137-139). *Campylobacter* related GBS is labeled as a disease of true "molecular mimicry" with host immune response implicated as the underlying cause (48,

79, 139). *Campylobacter* patients typically have the axonal form of GBS which has been linked to antibody production resulting from cross reactivity to ganglioside structures within the lipo-oligopolysacharide of the bacterial cell wall (137, 139, 140). The antibody response ultimately leads to nerve damage in the host. Genetic strain differences and host factors are thought to contribute to the auto immune response, yet exact mechanisms that elicit complications following *Campylobacter* enteritis remain unknown (140).

Guillain Barre-syndrome cases are usually sporadic. The disease is 1.5 times more likely in males, and has an overall estimated incidence between 0.6-4.0 cases per 100,000 annually (137, 139). Mortality rates in industrialized countries range between 2 and 3% (79) with 11 to 76 annual deaths estimated in the United States (141). Worldwide, between 4-15% of GBS patients die and 20% suffer lifelong disabilities (139). *Campylobacter* patients are likely to have a more severe form of the disease and a delayed recovery than GBS acquired for other reasons (137).

The likelihood of developing GBS after infection with *Campylobacter* was recently explored using a cohort of over 29,000 laboratory confirmed cases of campylobacteriosis from Swedish surveillance data (138). The study found GBS at a rate of 30.4 per 100,000 (95% CI: 13.9; 57.8), one hundred times higher than the expected rate in the population as a whole. The study noted that no cases were found in children, whereas people over the age of 59 had the highest rates (248 cases per 100,000 persons). Other studies of patients with campylobacteriosis in developed nations cite less than 1 case in 1,000 and less than 2 in 10,000 will develop GBS (139,

140, 142). The total annual estimated cost for patients with GBS resulting from campylobacteriosis is between 57.5 and 420.5 million USD (141).

11.2 Miller-Fisher's Syndrome (MFS)

Miller-Fisher's syndrome (MFS), also associated with *C. jejuni* infection, is a form of GBS that includes facial and lower cranial nerve involvement and manifests from cross reactivity between epitopes on axons or Schwann cells and *C. jejuni* lipo-oligosacharides (137). In approximately 20% of MFS cases *Campylobacter* infections are diagnosed three weeks prior to the onset of disease (140). The discriminatory reason for *Campylobacter* infection to manifest as either GBS or MFS is unknown.

11.3 Other important potential sequelae

Other secondary manifestations of *Campylobacter* infection include Reactive Arthritis (RA) and Reiter's syndrome (a disease which causes inflammation of the urethra or conjunctiva) (79). Reactive arthritis is characterized by acute and/or chronic, sterile joint inflammation and musculoskeletal symptoms (143). It is hypothesized that host HLA-B27 interacts with persistent campylobacters, resulting in clinical disease four weeks following intestinal infection (79, 143). Reactive arthritis is rare in children, affects males and females equally and is most prevalent in young adults (143). Duration of symptoms is approximately 6 months yet, over half of affected patients report symptoms for greater than one year and long term prognosis remains a mystery (143).

Incidence of RA is between 1-5% of confirmed cases of campylobacteriosis, with an annual risk of 4.3 per 100,000 persons in developed countries (143). A Swedish study found the risk of RA within one year of *Campylobacter* infection to be higher (RR

6.3, 95% CI 3.5-10.4) than the baseline risk in the general population (142). A case-control study involving 457 cases and 687 controls found that in women, use of gastric proton pump inhibitors was independently associated with reactive arthritis, which occurred in 5% of cases and 2% of controls (127). None of the participants in the study reported Guillain-Barre, Miller-Fisher or Reiter's syndrome. Whether or not host susceptibility is a factor in chronic sequelae remains unknown and chronic disease or relapse occurs in 5% of *Campylobacter* reactive arthritis patients (127).

Campylobacteriosis has also been associated with Crohn's disease, inflammatory bowel disease and hemolytic-uremic syndrome (79). A Swedish registry-based study identified an elevated risk in *Campylobacter* patients (RR 2.8, 95% CI 2.0-3.8) for ulcerative colitis within one year of infection (142). The same study demonstrated a 0.02% risk for bacteremia in all campylobacteriosis cases (142). A potential link between *Campylobacter* enteritis and the development of myocarditis and pericardits has been proposed in multiple case reports but has yet to be substantiated (144). Infrequent instances of renal and urinary complications, peritonitis and splenic rupture have also been reported (73). Although campylobacters are rarely isolated outside the gastrointestinal tract, blood or oral cavity, the bacteria have been detected in brain, vertebral and intraorbital abscesses (145). The origin and mechanism of these infections are unknown.

12.0 Antimicrobial resistance

Resistance to macrolides and fluorquinolones (FQ) , the antimicrobial treatments of choice for patients with campylobacteriosis, has been on the rise in both humans and

animals since the 1990's (146). The rise has sparked global research interest in the epidemiology, and potential health consequences of this problem (111, 147). Patients with FQ or erythromycin resistant strains of *Campylobacter* have higher hospitalization rates, longer duration of illness and more severe diarrhea (111, 147). Overall, treatment of human campylobacteriosis with commonly used antibiotics has become less effective due to the high prevalence of resistant strains (148).

In many countries, a clear connection has been identified between resistant human strains and the use of antibiotics in animal production (149). The licensed use of FQ in veterinary medicine and the poultry industry is implicated as a major contributor to increased resistance patterns (49, 111). Prudent use of antibiotics within the human and animal medical and production industries is needed in order to curtail the selective pressure that leads to the development of resistant campylobacters. Investigation into the transmission mechanisms for antibiotic resistant genes is also warranted to further our understanding of the temporal and spatial resistance dynamics (150).

12.1 Mechanisms of Resistance

The mechanisms by which campylobacters acquire antibiotic (AB) resistance stem from selective pressures that cause the bacteria to evolve and mutate in response to environmental insults. Such pressures initiate genetic mutations, while plasmids, bacteriophages and transposons allow for intercellular mobility of activated resistance genes (49). Fluoroquinolone resistance mechanisms are mediated by spontaneous point mutations, while target modification and active efflux mechanisms are activated in macrolides (148). Tetracycline resistance is conferred by conformational changes that

cause cell binding sites to release the tetracycline molecule (148). Multidrug efflux pumps play an important role in the physiology of antibiotic resistance and current investigations may help to elucidate control strategies at the cellular level (151).

12.2 Prevalence in humans

In Europe in the late 1980's, human *Campylobacter* infections were first noted to be resistant to FQ. Between 1989 and 2009, England and Wales had an increase in *Campylobacter* isolates that were resistant to ampicillin, ciprofloxicin, tetracycline and erythromycin(128). Studies in the U.S. showed a rise in FQ resistance from 13% to 19% between 1997 and 2001 while erythromycin resistance stayed stable at 2% (146). A French study showed resistance to nalidixic acid (26.3%) and reported that resistance to tetracycline, ampicillin and erythromycin was prevalent but occurred at lower levels in 2004 (152). In Austria, approximately 40% of human isolates were resistant to ciprofloxacin, paralleling a rise in resistance in poultry from 38.4% to 57.7% between 2004 and 2007 (153). Despite the high prevalence of *Campylobacter* infection in Australia and New Zealand, little information exists with regard to antibiotic sensitivity in these countries.

12.3 Prevalence in animals

Antibiotic use for growth promotion of food animals, in conjunction with the increase of intensive farming systems has lead to the development of highly resistant bacterial strains, worldwide (152). In the United States, it is estimated that 1-2% of broiler chickens slaughtered annually have received fluoroquinolones (146). A temporal study of beef cattle administered chlortetracycline and oxytetracycline in feed in Alberta

Canada showed significant increases in prevalence of antimicrobial resistance in *C. coli*, *C. fetus* and *C. jejuni* to tetracycline and doxycycline over the six month study period (150). In New Zealand, erythromycin resistant, offal, or organ meats in pigs showed campylobacters were associated with the preventive use of Tylosin (147). In Denmark, a Tylosin ban resulted in decreased macrolide resistance in *C. coli* from pigs (66.5% to 20% between 1998 and 2005) (154). A 2007 study showed that the economic effects on the poultry and pig industries were minimal with the discontinuation of growth promoters in European countries (154).

A study by Englen et al. characterized resistance patterns in dairy cattle that purported to represent 85.5% of the U.S. dairy population, (96 operations in 21 states) (155). The results showed that 94 out of 96 farms had at least one positive fecal sample that included an antibiotic resistant strain of *Campylobacter spp*. In Australia, 31% of *C. jejuni* and 22% of *C. coli* isolates from poultry were resistant to erythromycin. The difference in resistance rates were associated with antibiotic practices between farms (147). A UK study showed that *Campylobacter* isolates from all raw red meats exhibited resistance to antibiotics, including quinolones (156). The same study found multidrug resistant *C. jejuni* and *C. coli* in samples of beef (16.2%, 0%), lamb (6.3%, 19.2%), and pork (22.2%, 21.9%), respectively. In 1999, 10% of retail chicken meat in the U.S. was found to be contaminated with FQ resistant campylobacters (146).

Research is currently needed to quantify the effect of withdrawing antimicrobial drugs from the food animal production system (148). Campylobacters are zoonotic, and while most human infections are self-limiting, treatment of the disease in young, old and

immune suppressed patients may be compromised with the acquisition of AB resistant strains from animals and animal food products. The increasing prevalence of antibiotic resistance strains of campylobacters may warrant international control programs to limit the use of antibiotics in animal husbandry systems, specifically as growth promoters in the poultry industry (111).

13.0 Genetic Characteristics and Molecular Identification Methods

The genome sequence for *C. jejuni* has now been completely characterized, an advancement that may pave the way to novel prevention and control strategies in the future (121). However, the *Campylobacter* genome is characteristically unstable and undergoes frequent recombination and rearrangement (59, 157). Four major hurdles hamper the identification of *Campylobacter* sources and routes of transmission. These include the (i) lack of a clear cut discriminatory method, (ii) lack of a universal molecular typing standard, (iii) plasticity of the genome, and (iv) sporadic disease distribution. According to a Finnish research group, “Discriminatory typing methods for use in the study of molecular epidemiology and population genetics of *Campylobacter* isolates are crucial to better understand the epidemiology and ecology of the organism.”(158)

The sporadic spatial and temporal distribution of this disease present a challenge for molecualr investigation of transmission routes (159). Communication and collaboration among researchers, along with standardization of molecular methods on an international level, would be of great benefit in understanding the epidemiology of campylobacteriosis (48, 136).

13.1 Phenotypic vs. genotypic typing methods

Campylobacters can be identified, with varying reliability, by a variety of techniques based on genetic and biochemical properties of the organism (48). There are two major groups of typing methods used to distinguish campylobacters–phenotypic and genotypic. Phenotypic methods are generally less discriminatory and include serotyping, biotyping, phage typing, protein electrophoresis and fatty acid profiling (59, 159). Biotyping is the least discriminatory method overall (59). Although less discriminatory, these techniques are useful due to *Campylobacter's* capacity for recombination and weak clonal structure. Serotyping (Heat stable Penner scheme and heat labile Lior scheme) is the most commonly used phenotypic method. Penner serotyping is useful in coarse comparisons for large numbers of isolates where the source distribution is of primary interest (59, 157). These techniques require technical expertise, are time consuming and encounter a high number of untypeable strains, thus limiting their widespread use (160).

Genetic content of the organism can be examined directly for changes via genotypic methods such as pulsed field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), all of which can cover the entire genome. Pulsed field gel electrophoresis is the most discriminatory whereas riboprinting has medium discriminatory power but is definitive in its ability to make direct comparisons of profiles (59, 161). To identify specific loci, multi locus sequence typing (MLST) techniques are currently being tested (159). Genotyping methods have the major advantage of being available worldwide (160).

13.2 Combined method strategies

A variety of polymerase chain reaction (PCR) detection combinations can identify thermophilic campylobacters at the species level (48). Amplified fragment length polymorphism is based on PCR and electrophoresis and is rapid, highly discriminatory and universally applicable (162). Pulsed field gel electrophoresis and riboprinting combined enable accurate determination of polymorphisms in the entire genome (59). Another method of verifying similarity between isolates is through the combined use of Penner serotyping and PFGE (157, 163-165). Furthermore, Penner and *fla*-RFLP were found to be useful in identifying waterborne outbreak strains as isolates could first be grouped into large clusters that could then be genetically matched using PFGE (157). A disadvantage with *fla* techniques is that they lack technical standardization between laboratories, despite high reliability within laboratories (160).

13.3 Problems and solutions

The genetic plasticity of *Campyobacter* species continues to hinder efforts to accurately link strains with disease (70). High antigenic diversity, including over fifty antigenic serotypes have been identified in *C. jejuni* and *C. coli* isolates (77). *Campylobacter jejuni* has a high rate of recombination and subsequently, a highly variable phylogenetic tree. As a result, identifiable gene frequencies among common source isolates differ significantly (136, 166). In Switzerland, authors were unable to identify a dominant clone among different sources within the country (167). In the UK, human disease strains characterized and confirmed by Penner serotyping, Preston phage typing and biotyping were found to be phenotypically diverse (136). In addition,

information on long term patterns have not yet been accrued for PFGE (168). The utility of epidemiological and molecular data as a means of confirming sources of infection have not been incontrovertibly successful (169), and while modern typing methods have the potential to fill in these gaps, much work in this area is still needed (166).

Recently, standardized protocols have been put into use by PulseNet, a U.S. surveillance system for foodborne pathogens (134). The hope is that improved availability of rapid, standardized techniques will unify the genotyping process. Standardization and harmonization of typing methods has also been initiated among European laboratories (CAMPYNET) for the purpose of creating comparable typing results over time (59). This is important because a singular genotyping method cannot reliably and consistently distinguish between two *Campylobacter* (67, 167). Proper matching and strain characterizations of molecular epidemiological data would provide a more comprehensive picture of the disease dynamics (58).

14.0 Epidemiology of Campylobacteriosis

14.1.0 Incidence and prevalence

14.1.1 United States and developed nations

The general category of enteric diseases are estimated to cause 1.7-2.5 million deaths worldwide per year with 400 million cases attributable to *Campyobacter* (121). Campylobacteriosis is a leading cause of bacterial gastroenteritis in the United States and many other developed countries (4, 47, 49, 50, 99, 170). The epidemiology of this organism is complex and remains poorly defined, in part because it is ubiquitous in the environment and distributed throughout the food chain (4, 59, 94, 170). Differences in

surveillance systems, reported incidence rates and primary risk factors for campylobacteriosis vary between countries (79). Difficulties in assessing the disease burden may also be a function of underreporting and surveillance methods implemented in individual countries (170).

The annual estimate of *Campylobacter* cases in the U.S. is 2.4 million (approximately 1% of the population) (171), and despite active surveillance systems such as the Foodborne Disease Active Surveillance Network (FoodNet) implemented in 1996, the majority of *Campylobacter* infections go undiagnosed (1, 171). In the U.S., reported incidence of infection was 23.5/100,000 population in 1996, and decreased by 27% from 1996 to 2001(51). The risk of infection fell further to 12.7 (5,712 cases total) in 2006 (172) and stabilized at approximately 13/100,000 in 2007 (173, 174). In 1997, the highest annual incidence of *C. jejuni* infection to date was reported at 69 per 100,000 in the state of Hawaii (129). The exact reason for the overall decline in cases observed since 1996 remains unclear, however, underreporting is thought to be a factor. The decline is also hypothesized to be related to improved disinfection and slaughter procedures within the poultry industry (3).

In 1999, 55,000 cases were reported in the UK but the true case counts were estimated at over 400,000 (4, 58) accounting for 27% of all food-borne disease in that region (47). Surveillance data show that infectious gastrointestinal diseases affect one in five people annually in England and Wales (169, 175). Approximately 90% of reported *Campylobacter* cases are due to *C. jejuni* (176), and only 1.7 of every 1,000 cases are confirmed by laboratory diagnosis (169). Over 23 years of surveillance,

(ending in 2011) showed an overall increase in campylobacteriosis in these two countries, with a prevalence in 2009 of 105 per 100,000 (128).

Campylobacter is the most frequent cause of bacterial gastroenteritis in Denmark as well, with a fourfold rise in incidence between 1991 and 2001(11, 177). It is estimated that 1% of the population in Western Europe is infected annually (47, 178) with risks for European countries ranging from 60-90/100,000 (89). In 2005, 6,200 laboratory-confirmed cases of *Campylobacter* were reported in the Netherlands, an incidence rate of 1.5-3.7 per 1,000 person years (127, 179).

In New Zealand, *Campylobacter* is the most common notifiable disease (49) with 334 cases per 100,000 reported in 2002 (98). The incidence increased to approximately 400/100,000 in 2006 and comprised 73% of the country's annual economic cost of infectious intestinal disease (180). Experts from the World Health Organization agree that the increased incidence cannot be explained by changes in reporting or medical and laboratory practices (170). In 2006 a concurrent rise in incidence of *Campylobacter* infection and hospitalization rates was seen in New Zealand, suggesting a 'real' as opposed to surveillance related increase (36). For example, the number of hospitalized cases between 1996 and 2003 tripled for campylobacteriosis whereas increases were not reported in other enteric diseases such as *Salmonella* (36).

In Canada, there were 30.2 cases per 100,000 people in 2004 (50). A recent study using statistical models in the province of Ontario estimated that for each reported case of gastroenteritis there are several hundred cases that go unreported (181). A second study estimated that there were 23-49 cases of campylobacteriosis for every

reported, laboratory-confirmed case annually (182). In Japan, a steady rise in campylobacteriosis risk throughout the 1980's and 1990's is attributed to the implementation of improved surveillance (183) and in Australia, incidence of campylobacteriosis is calculated to be12 times that of the U.S.(184). These studies highlight the effect of underreporting on the estimated incidence of human campylobacteriosis.

14.1.2 Developing nations

The lack of public health infrastructure and national surveillance greatly hinders epidemiologic information in underdeveloped nations. Studies suggest that the epidemiology of campylobacteriosis differs between developed and developing nations (63, 185, 186). *Campylobacter* is hyperendemic in developing countries, in part due to poor sanitation, inadequately treated drinking water and close human-animal contact (53, 170). In nations such as Zimbabwe, Egypt, China, Nigeria, and the Central African Republics, exposure to fowl and other free-living animals around the home has been linked to disease (187). Contaminated meat products, human and animal fecal contamination of the environment and drinking water sources are other reported risk factors for infection (187).

Consumption of undercooked chicken and contaminated milk are important risk factors in industrialized and underdeveloped nations, (63). However, the disease patterns are thought to differ due to ethnicity, climate and populations dynamics combined with host and strain factors (63). Isolation rates for adults with diarrhea in developing nations ranged from 5-20% (90 per 100,000 people) (123, 187). Over 50%

of infected adults with *Campylobacter* enteritis report bloody diarrhea (1) whereas children under 5 years old exhibit milder clinical signs and are infected much more frequently (40-60,000 per 100,000) (53, 123).

In Latin America and Africa, *Campylobacter* is the most frequently isolated pathogen from stool samples in children under two years of age with diarrhea (63, 187, 188). Isolation rates in children under five years old range from 7.7-18% in Africa, to 10.5-13.8% in Latin America and 10-17.6% in Southeast Asia and China (187). In some developing countries clinical disease is commonly reported in two year olds and rarely seen in adults (69, 189, 190). In addition, *Campylobacter* is frequently recovered from stool samples of children in developing nations without clinical signs of disease (4-40% isolation rates found in various studies) (63, 77, 187).

The disparity between prevalance and clinical manifestation in children versus adults is due to immune status, animal exposure, poor sanitation and hygiene (123). In children from developing countries, serum antibodies to *Campylobacter* develop early in life and become protective (63, 69, 123). Transmission from natural sources (animal and water reservoirs) is implicated in early exposure, and repeated exposure is thought to confer a high level of immunity (68, 122).

Patients in developing nations are frequently coinfected with other enteric pathogens such as *Giardia lamblia* or rotavirus (187). Also, complications such as Guillaine-Barre syndrome (GBS) are rarely reported, despite high incidence rates (63). Linking bacterial gastroenteritis with complications requires adequate health care and follow-up and may go unreported in developing countries. The precise reasons for

disparities in reported *Campylobacter* incidence and prevalence between developed and developing nations require further research and documentation.

14.2 Burden of Disease

Despite the increase in incidence in *Campylobacter* infections over the past twenty years, inadequacy of surveillance systems for human, animal and foodborne sources, and variability in results of epidemiological studies hinder accurate estimation of the burden of disease (170, 179). Burden of disease is a summary measure of health effects, such as morbidity, mortality or calculable economic losses, that offer insight into the impact of disease on a population (191). Disease burden estimates help public health and policy makers compare health effects between different diseases, make risk management decisions and create guidelines for prevention.

In the United States, approximately 76 million cases of food-borne illness occur annually (192). Seventeen percent of all foodborne disease hospitalizations in the U.S. are due to *Campylobacter* (157) and worldwide hospitalization rates are the third highest, exceeding rotavirus and *Salmonella* infections (111). When underreporting factors were applied to U.S. data, annual incidence ranged from 300-470/100,000 people and between 7.6 and 18 people are thought to be infected for every reported case (191). Up to 8 billion dollars are spent in the U.S. annually due to campylobacteriosis when productivity and extraintestinal complications such as GBS are factored into analyses (141).

Mortality rates for *Campylobacter* at 30 days post infection are estimated, worldwide, at 4 in 1000 persons (170, 193). In Denmark between 1991 and 1999,

campylobacteriosis patients had a 1.9 times excess mortality (than the unexposed population) two years post infection (193). Deaths were associated with diseases of chronicity and immune compromise such as HIV. Ternhag et al. found standardized mortality ratio for domestically acquired cases in Sweden to be 2.9 (95% CI: 1.9-4.0) within the first month after infection as compared to 0.3 (95% CI: 0.04-0.8) for those infected abroad (194).

In Canada, *Campylobacter* accounts for over 50% of water and foodborne diarrhea cases combined (143). It is also the second highest reported cause of traveler's diarrhea worldwide, second only to enterotoxic *E. coli* (ETEC) (121). An annual estimated cost of 225 million U.S. dollars was attributed to campylobacteriosis in the UK in 1999 (71), the highest cost associated with a single pathogen (111). In a UK study performed in 2003, close to 3,500 cases resulted in the accrual of over 37,000 sick days and 1,400 hospitalized days (10% of cases were admitted to the hospital) (195). A study in the Netherlands equated the health burden of *Campylobacter* with diseases such as meningitis, sepsis, upper respiratory infections, ulcers and Down Syndrome (38).

In Australia, foodborne diseases, affect 5.4 million people annually, and are responsible for 15,000 hospitalizations and 80 deaths (196). Surveillance data, simulation and multiplier techniques used to quantify campylobacteriosis incidence in Australia showed that 90% of cases go unreported, with an annual estimated incidence of 1,184 per 100,000 persons (95% CI: 756-2670) (197).

The burden attributable to species other than *C. jejuni* remains unknown, and the financial and health-related effects of antibiotic resistance in *Campylobacter* species has yet to be fully quantified (170). In developing nations, and many countries around the globe, the disease burden also is unknown, resulting in an enormous economic strain on both government and industry. A priority for decreasing this burden must include a combination of targeted surveillance and improved public education. The World Health Organization is currently developing a Foodborne Disease Burden Epidemiology Reference Group to unite disciplines on a global level, and shed light on the burden of disease for campylobacteriosis and other gastrointestinal illnesses (191).

14.3 Sporadic versus common source infections

The majority of reported campylobacteriosis cases are classified as sporadic (isolated, scattered or occasional pattern of occurrence) and outbreaks arising from a single, definable source are infrequent (4, 50, 51, 159, 161, 169, 180). In 2005, approximately 250 of the almost 14,000 cases of campylobacteriosis in New Zealand were associated with outbreaks (180). In England and Wales *Campylobacter* comprised only 2% of reported outbreaks due to infectious intestinal disease between1992 and 1999 (195) and only 81 outbreaks were reported between 1989 and 1999 (58). Annually, there are rarely more than ten recorded outbreaks of campylobacteriosis in England and Wales (169). Large outbreaks, although very rare, are almost always associated with untreated water or milk (76, 190, 198). This is true in Finland, where 9 of 21 total waterborne outbreaks between 1998 and 2000 were caused by campylobacters (163).

Common source outbreaks arising from either direct contact or shared exposure within households has been shown to occur. In June 1996 and May 1998, 34% of documented cases in Japan were outbreak related (183). In England and Wales, surveillance data showed higher than expected reported cases of concurrent disease in either the home or community in 2003 (195). The study found that 506 (17%) of 3070 patients with *C. jejuni* reported other household members simultaneously had similar signs of illness, and 41 (8%) of these reported a sick individual in the household greater than one week from the confirmed case (195). Ten percent of cases reported knowing an individual outside of the household with similar symptoms. An Australian case-control study also linked exposure to household members with diarrheal disease as a risk factor [OR 3.5 (95% CI: 1.6-8.0)], supporting the hypothesis that point source outbreaks, though difficult to confirm, might be more common than previously suspected (110). Furthermore, some researchers believe that the increasing mobility of foods and populations may mask outbreak detection and lead to an overestimation of sporadic incidence (175).

Molecular studies in the UK and Denmark indicate that indistinguishable strains can be found in poultry, dogs, pigs, sheep, and cattle, supporting the theory that sporadic human infections may arise from multiple sources (159). Coinfection with more than one species has been documented in 5-10% of sporadic cases and up to 50% of outbreak related cases in the UK (124). Difficulty in recognizing outbreaks may result from the complex interactions between disease determinants that hinder our ability to properly identify risk factors (195).

14.3.1 Poultry

Exposure to contaminated poultry might occur sporadically, as point source due to work or group-related activities or individually in a home. The twenty to forty percent of campylobacteriosis cases associated with chicken consumption in the UK are considered sporadic (71). In 2008, 97% of sporadic disease was attributable to cattle and chicken after molecular sequencing and source identification in England (166). A case-control study performed in Hawaii showed that eating chicken prepared 'outside the home' and eating chicken from restaurants were separate risk factors for infection (129). The study showed that chicken prepared at home was inversely associated with disease.

Campylobacteriosis outbreaks due to chicken/poultry are hypothesized to be infrequent, as the pathogen is unable to multiply on food (199). This biological constraint differentiates *Campylobacter* from other food-borne pathogens (*Salmonella, E.coli*) known to cause outbreaks. Furthermore, common source contamination is likely to be masked by modern methods of food distribution (180). The time between exposure, clinical signs and identifying an outbreak is crucial to case follow-up, linking disease to source and confirming the strain (58, 200). As a result, source identification is challenging and *Campylobacter* outbreaks that occur in food establishments that serve or prepare chicken may remain unidentified (195).

14.3.2 Outbreak detection-successes and challenges

Large social gatherings such as parties and buffets have tight temporal and spatial continuity and can be effectively investigated using current epidemiological

methods. In recent years, improved surveillance and investigation standards have enhanced outbreak detection success. During a three week long outbreak in 2006, the combined use of Penner typing and MLST helped epidemiologists identify multiple infection strains and coinfection in three infected individuals among 48 people (201). The outbreak was associated with improperly cooked chicken liver pate and the exposure occurred in December when the restaurant hosted eleven Christmas parties that contained the contaminated dish. In another success story in Sweden, investigators used web-based questionnaires to link ten cases of diarrheal illness (only four stool samples positive for *Campylobacter*) to marinated chicken served at an anniversary buffet hosting 100 people in October 2007 (198). In Scotland, chicken liver pate was identified in November 2005, an outbreak that affected 68 of 165 people attending a dance (202).

In typical restaurant situations, individuals at risk for consumption of a contaminated food item are virtually undetectable–linking the individual to the pathogen equates to finding a 'needle in a haystack'. Point source campylobacteriosis arising from restaurant patronage may remain hidden due to the lengthy incubation period prior to clinical disease, low likelihood of reporting, wide range of disease severity and geographic diversity within the patron population. Mild cases of *Campylobacter* diarrhea emerging 5-7 days after consumption of contaminated restaurant food in healthy adults from diverse neighborhoods are unlikely to be traced. The long interval between illness and follow-up may also introduce significant recall bias and decrease the likelihood of

correct source identification (200). Shortening the reporting time by improving surveillance and laboratory confirmation may improve efforts in early identification.

14.3.3 Molecular evidence for sporadic and outbreak cases

Common source infections may occur more frequently than current research and statistics show, and molecular typing is the best and most current tool for strain detection. McTavish et al. identified 32 of 112 serotyped isolates of *Campylobacter* that had spatial patterns consistent with a common source of contamination in New Zealand (180). Molecular typing can help identify *Campylobacter* strains in temporal and spatial clusters suggestive of outbreaks (180). Investigating cases that are spread out over time and space is costly and logistically difficult. In well-defined outbreak situations, microbiological information is readily obtained and verified; the geographic and chronologic limitations generally make confirmation by typing feasible (159). In the case of *Campylobacter* infection, identifying common source contamination is more difficult, especially when prolonged common source infections occur over a wide geographic region (136).

The diversity of *Campylobacter* subtypes among human cases and reservoir sources is daunting (136, 161). Host specificity between *Campylobacter* strains and sources is poorly understood (124) , and molecular typing performed during confirmed outbreaks often identifies multiple vehicles as sources of infection (159). During waterborne outbreaks, the timeline between sampling of water sources and matching them to human cases may be too long to reliably verify the source of infection (58, 163). Over time, the strains responsible for disease may be eliminated by the host or disperse

in the environment and be replaced by other environmental strains, thereby masking the original source and hindering our understanding of the disease epidemiology.

Part of the difficulty in confirming sources of *Campylobacter* infection may be due to the public health sector's reliance on survey information. Retrospective questionnaires targeting laboratory-confirmed cases are a primary method for linking risk factors with temporal and geographical information and molecular typing. Recall bias is a frequent confounding factor that is worsened by the lengthy incubation period. Studies have also found that genotypes may vary in different geographic regions, making source identification problematic (158). The reason for this variation remains unclear. Despite statistically significant identification of temporal and spatial clustering, retrospective source verification efforts encounter the challenge of obtaining reliable typing information over long time periods and distances (195).

14.4 An overview of campylobacteriosis risk factors

Defining risk factors is a crucial step in planning and implementing disease prevention strategies. Definitive risk factors remain unexplained for the vast majority of *Campylobacter* cases. Risk factors can be broken down into three basic categories: pathogen specific, host specific, and environmental risk factors. Pathogen specific factors are not well understood and therefore play an unclear role in *Campylobacter* epidemiology. The most important host specific risk factors include immune compromised individuals, such as the very young, the elderly and those with preexisting illnesses. Individuals with immune related diseases such as HIV, chronic intestinal diseases, asthma and rheumatism are at higher risk for disease than the general

population. It remains unknown if these diseases occur more frequently prior to or as a consequence of *Campylobacter* infection (127). In recent years, exposure to medications such as antibiotics and antacids have been implicated as risk factors (127, 129). These exposures weaken the host defense system prior to infection by either altering normal gut flora or diminishing the natural acid barrier in the stomach.

Environment-related risk factors are, by far, the most extensively researched for campylobacteriosis. The most commonly reported risk factors for infection, across studies in the United States and abroad, include consumption or contact with contaminated poultry, raw milk and inadequately treated drinking water and contact with farm animals and pets (51, 106, 125, 170, 177, 190). Poultry consumption causes an estimated 10-40% of cases of infection (171). Other important factors include foreign travel and consumption of undercooked poultry and red meat (161), eating at restaurants and poor kitchen hygiene (199) and consumption of raw fish and shellfish (190). An early study of sporadic, laboratory-confirmed cases in Colorado identified consumption of untreated water [(OR 3.33 (95% CI 1.03,12.29)], raw milk [(OR 3.30 (95% CI 1.04,10.45)], undercooked chicken [OR 2.77 (95% CI 1.01,12.7)], and cat ownership [(OR 3.05 (95% CI 1.33,7.0)] as primary risk factors (203). In a UK cohort study of sporadic cases by Evans et al. in 2003 significant risk factors for infection included eating chicken [OR 1.73 (95% CI 1.09,2.73)], salad vegetables [OR 1.6 (95% CI 1.03,2.50)], bottled water [OR 1.39 (95% CI 0.98,1.96)] and contact with cows or calves [OR 5.44 (95% CI 1.05,28.10)](72). Direct contact as a mode of *Campylobacter* transmission is thought to be rare, however, contact with a person who had diarrhea

prior to onset of disease was an independent risk factor (OR, 3.32 [95% CI 1.72-6.4]) in a recent study in France (199). The overall contribution of environmental contamination and nonfood routes of exposure remains unclear (176).

To understand campylobacteriosis epidemiology and pinpoint the sources of disease, several questions must be addressed. First, the correct combination of relevant socioeconomic and environmental contributors to disease must be identified. Secondly, the seasonal component of risk, often linked to human activities, behaviors and contact with environmental sources and reservoirs must be investigated. Third, geographic differences in the relationship between natural and anthropogenic reservoirs of disease must be clearly defined, and require further investigation.

14.4.1 The importance of poultry in human *Campylobacter* infection

Poultry consumption has been associated with up to fifty percent of all cases of campylobacteriosis and therefore will be addressed in detail (76, 190, 193). A case-control study in Australia estimated that annually, over 50,000 cases in those ages five and older could be attributed directly to chicken consumption [OR 4.7 (95% CI:2.6-8.4)] (196). In England and Wales, chicken was implicated in nine of forty-six outbreaks between 1996 and 1999 (169). Of 169 reported outbreaks in Japan between 1993 and 1998, 39 were traced to chicken meat or dishes containing chicken (183). In an ecological study in Michigan, counties with high densities of poultry were associated with higher risk of disease (120). Poultry is thought to be a major source of infection due to the high prevalence of the bacterium in poultry meat (80, 112, 179) and chicken products are considered the most frequently contaminated type of food (179).

14.4.2 Prevalence in poultry flocks and contamination of retail meat

Rapid spread of *Campylobacter* in poultry flocks and retail meat contamination is well documented (124, 159, 179, 190). The average prevalence in broiler flocks from Denmark was estimated by a national monitoring program to be 42% (11). In Sweden, *Campylobacter* was isolated outside broiler houses in 42% of farms sampled, with lower incidence in flocks from broiler houses with rigorous hygiene barriers (204). Workman et al. found 94.2% of sampled chickens and 58.4% of chicken food products were contaminated (205). Chicks that tested negative were less than three weeks old, suggesting that intensive broiler farming leads to environmental contamination and subsequently, rapid intestinal colonization after hatching (94, 205, 206). A study using PCR in France detected *Campylobacter* in 79.2% of 24 poultry houses and 17.5 % of supermarket chicken samples (62). In New Zealand, *C. jejuni* was found in 76.9% of poultry samples tested (84) and poultry meat samples from Poland and other eastern European countries were found to have 34.7% prevalence of *C .jejuni* or *C. coli* (67).

Campylobacter is a commensal organism found in the mucosal crypts of the caeca and small intestine in poultry (56, 70, 207). Arsenault et al. detected 35% *Campylobacter* prevalence in chicken caecal samples and 46% in turkeys (208). Researchers have found the organism in blood as well as in deep tissue of young chicks including the spleen and liver (56). Cross contamination between poultry products at slaughter houses is a widespread occurrence (207) and has been verified by genotyping in two-thirds of *Campylobacter* strains tested (175). Studies using MLST in the Netherlands grouped 70% of human *Campylobacter* strains with a subset lineage

of poultry isolates (179). A Finnish study showed 46% genetic overlap between chicken and human strains, and also found turkey flock colonization to be 3.2 times higher nearby the manure heap and 4.2 times higher in flocks drinking unchlorinated water (209).

Risk factors for flock colonization include poor hygiene and maintenance of facilities, elevated insect burden, inadequate disinfection and inadequate time periods between flock repopulation (206). *Campylobacter* strains persist in the environment between flock rotations (159) and flies have been implicated in the flock contamination as well. In 2004, Hald et al. found that 8.2% of 96 flies captured outside of a broiler house cultured positive for *C. jejuni* and hundreds of flies enter the broiler house through the ventilation system (210). In a follow up study, *Campylobacte*r positive flocks had a 52.4% prevalence of infection without screened housing , which was significantly higher than 15.4% with screens ($P<0.001$) (118). The authors of these studies hypothesize that a proportional reduction in human incidence due to chicken could be expected if the chicken burden at the house level was decreased.

In 1999, dioxin contamination of chicken feed components in Belgium led to a real-life study to document the potential for withdrawing poultry products from the marketplace on the incidence of human (211). Authors modeled the incidence of campylobacteriosis in the Belgian population following a mandatory withdrawal of chicken from the market and found that *Campylobacter* infections attributable to poultry declined by 40%. Mathematical modeling of human campylobacteriosis and prevalence in chickens also showed that infection associated with chicken consumption could be 30

times lower with reduction on the chicken carcass or similar reduction in flock prevalence (109).

14.4.3 Evidence against the importance of poultry as a source of human infection

chickens may be colonized with multiple strains of genetically diverse campylobacters making definitive molecular associations between humans and chickens difficult (56). In Scotland, MLST was used to type and quantify campylobacters in retail chickens and human stool samples from 2001 and 2006 (124). Results showed that despite significant regional declines in human campylobacteriosis, carcass pathogen load had not changed. The research also showed higher genetic diversity in the human samples suggesting (i) a possible, yet controversial, difference in pathogenicity between strains and (ii) that the decline in disease rate was not due to pathogen concentration in the chicken (124).

Other research shows that only 10% of human infections are explained by eating or handling poultry (161, 168). In England and Wales, poultry production was not well correlated with human infection over a 23 year study period (128). Poultry consumption in the U.S. is estimated to be four times that of Iceland, yet annual incidence of campylobacteriosis (per 100,000) is consistently higher in Iceland (112). In a study focusing on young Swedish children with campylobacteriosis, no association was found between infection and consumption of chicken (189). It is also unclear if *C. jejuni* strains associated with poultry are the same as those that cause disease in humans (80). In one three year study, annual variation in the incidence of infection could not be explained by chicken contamination levels (168). In Sweden, despite the declining

prevalence of campylobacters in broilers (10% decline following control measures) human infections have risen steadily since 1991(212). A typing study of human and poultry isolates in Poland found diverse PFGE patterns with only 2 of 75 human isolates matching those taken from meat (67). The role of carcass bacterial count in the incidence of infection has yet to be quantified.

Pope et al. tested poultry and human samples using RFLP in Dunedin, New Zealand and found that the *fla*A virulence type recognized in human infections was found in only 5% of poultry isolates (84). Humans were commonly exposed to less virulent chicken *fla*A types yet human infections resulted primarily from the more virulent *fla*A types, suggesting disease sources other than chicken. In a study of virulence factors in 2007, *C. jejuni* isolates from humans were more resistant to bile salts (P=0.006) and had more pronounced production of CDT (P=0.041) than those from poultry (80). The same study showed that none of the PFGE patterns between poultry and humans were identical, the closest clinical clusters reaching only 80% similarity. Despite the dissimilarities, the authors conclude that all poultry isolates should be capable of human infection due to invasiveness and virulence factors. As yet researchers have been unable to consistently quantify the level of disease that is linked directly to poultry.

14.4.4 *Campylobacter* as a food-borne pathogen

The rise in the incidence of *Campylobacter* in developed nations may be due, in part, to changes in dietary habits, an increase in fast food consumption, and large-scale food processing practices (213). An extensive case-control study found that the total

combined foodborne attributable risk for campylobacteriosis in Australia was 31.4% (95% CI: 10.4-46.8%) (196). Over 65% of the population attributable risk could be explained by eating chicken, salad vegetables and bottled water in a retrospective cohort study of sporadic *Campylobacter* cases in the UK (72). *Campylobacters* have been isolated in high numbers in chicken livers therefore, not surprisingly, eating pate has been associated with increased risk of infection (71, 201). *Campylobacter* has been detected in seafood, including oysters, clams, mussels, scallops and blue crab (214). The bacteria is capable of inhabiting marine environments transitorily and contamination may occur by way of water fowl, sewage effluent and farm runoff (214). Outbreaks have also identified foods such as lettuce, lasagna and barbecued chicken as vehicles linked to cross contamination with raw or undercooked poultry (175, 215). Detection rates on vegetables are typically low (less than 3%) and while rare, outbreaks due to vegetable consumption are usually linked to cross contamination during food preparation (214).

Foodborne campylobacteriosis can be caused by simultaneous ingestion of several strains of the bacteria (169, 175). Between 1996 and 1999, more than one strain of *C. jejuni* were implicated in infection in 9 of 46 outbreaks in England and Wales (169). A number of potential food vehicles, along with several possible routes for cross-contamination, were identified in these investigations. In a study by Nielsen et al. in 2005, 1,285 *Campylobacter* isolates were compared using Penner serotyping, during a one year period. The researchres identified overlap between isolates from food sources (66%), chicken (59%) and cattle (83%) (161). The chicken and turkey samples had a

Campylobacter prevalence of 38.7% and 27.5% respectively and 35% of retail poultry products were contaminated (161). Upon PFGE confirmation of 212 samples, 44 isolates from human infections were identical to food sources and 28 matched cattle isolates. The occurrence of multiple strains in human infection and identical strains between animals and humans supports the idea that a wide variety of sources serve as contaminants.

14.4.4.1 The role of human behavior

Improper food handling and hygiene are estimated to cause 40-60% of all food-borne illness (216). One study identified poor hygiene as an important risk factor (OR, 2.1[95% CI, 1.33-3.37]) for *Campylobacter* in France (199). In a 1988 British survey, less than one third of individuals fully understood the need to handle raw meats separately from other foods (190). Cooking practices such as barbequing meat also account for an increased risk of infection (193). In one outbreak, using a brush to apply sauce to barbequed chicken was implicated as a vehicle for transmission (217). In a Colorado case-control study one third of the cases were considered to be preventable by modification of personal behaviors (203). Observational studies have shown that up to 100% of people do not properly wash their hands and the majority do not properly clean cutlery and cutting boards between use with meat, poultry and ready-to eat foods (216).

In a contrary case-control study (1,316 laboratory-confirmed cases) questionnaire responses showed no association between risk of infection and handling or cooking raw chicken in the home (51). Improvement in cooking practices and hygiene

in the kitchen are thought to be the primary reason for the recent decline in incidence in some regions of the world (124). A majority of risk factors may be preventable through the improvement of personal hygiene (203, 217) and improved education may reduce the reduce the risk of campylobacteriois due to improper food handling and cross contamination (216).

14.4.4.2 Cross contamination and undercooked food

The slaughter and dressing process for meats and offal frequently results in carcass contamination with campylobacters (62, 190) and sets the stage for cross contamination due to improper food handling practices (156, 214-216). Contamination at the slaughterhouse is considered to be almost impossible to avoid due to mechanical evisceration, washing and water chilling procedures (62). In a study by Luber and Bartelt, fresh retail chicken breast fillets had an 87% prevalence of surface *Campylobacter* contamination and 20% prevalence of the pathogen in the deep tissue, indicating that cross contamination and undercooked meat may both play a role in transmission of disease (218). The study also found that poultry with skin had higher pathogen loads than that of skinless fillets. The nature of poultry skin is such that it prevents desiccation of the bacteria, even during typical air chilling procedures at the slaughter plant (214).

A foodborne outbreak investigation and case-control study were undertaken in Connecticut in 1997 nearly one week after clinical signs of campylobacteriosis were reported at a senior center (219). Samples taken from the premises revealed a high likelihood of cross contamination between raw meats and other prepared foods (in this

case the culprits were sweet potatoes, coleslaw and coconut pudding). Four people were hospitalized with laboratory-confirmed *Campylobacter* in the stool and one died. The case is a good example of how cross contamination of unsuspected food sources can result in an outbreak in a group eating scenario.

The most important risk factor in a case-control study in France was undercooked beef (OR 2.26 [95% CI, 1.2-4.24]) (199). A prevalence study of retail meats obtained from 59 stores in Washington D.C. found 91% of the stores carried contaminated chicken (192). The study found 70.7% of chicken, 14% of turkey, 1.7% of pork and 0.5% of beef samples were positive for *Campylobacter spp*. with two of the chicken samples positive for three different species of *Campylobacter* (192). In a similar UK study, contamination with *Campylobacter* was detected in 7.2% of all raw meats sampled and 12.6% in lamb (156). The cause of internal meat contamination remains unclear, and overall, poultry meat becomes more heavily contaminated than red meat during the process of evisceration (205).

14.4.4.3 Restaurants

A FoodNet study in 2004, showed that the largest population attributable risk for campylobacteriosis was due to restaurant consumption of poultry and meats (51). Authors of a case-control study in Quebec, Canada found an odds ratio of 1.96 (95% CI 1.24-3.11) after consuming poultry in a restaurant, fast food or buffet (220). Another case-control study in Australia found that restaurant prepared meats (ham, red meat, roast beef and chicken) and 'greater than two weekly fast food meals' were associated with increased risk of infection (110). The same study found eating at 'burger chains'

was protective [OR 0.6 (95% CI: 0.4-0.9)] and the authors hypothesize that national franchises follow more stringent practices based on national regulations, thereby decreasing the risk of foodborne disease. Logically, the standard of food preparation and cleanliness upheld by individual restaurants directly reflects the potential for *Campylobacter* transmission.

14.4.4.4 Unpasteurized milk

Unpasteurized milk is a recognized risk factor for *Campylobacter* infection and has been associated with outbreaks in the UK and United States (195, 221-223). Two out of forty six outbreaks between 1996 and 1999 implicated unpasteurized milk as the vehicle for *Campylobacter* infection in England and Wales (169). In 1981 three U.S. outbreaks, affecting 50, 100, and nearly 200 people, respectively, were linked to consumption of raw milk (222). In one outbreak, 79% of those who consumed raw milk reported drinking less than one glass of milk (222).

Fecal contamination of cow and goat milk, as well as *Campylobacter* mastitis, has been implicated in several disease outbreaks (88, 190, 222). Human infection has been traced to the excretion of campylobacters into milk as a result of asymptomatic mastitis (88). The bacteria can be isolated from milk and fecal samples of healthy dairy cows (214). While *Campylobacter* is unable to grow in milk, the infective dose is very low (221). One study reported that less than 500 organisms ingested in milk can result in illness (222). *Campylobacter jejuni* can survive for weeks at 4^0C, therefore pasteurization is the only fully protective method of eliminating the organism from milk

(222). The reported health benefits of raw milk and dairy products, and their subsequent increase in popularity, may increase the impact of this risk factor in the future.

14.4.4.5 Organic Foods

Organic meat consumption is a recently documented risk factor for campylobacteriosis (195). While exposure to organic meats remains low in the general population, health concerns stem from higher percentages of *C. jejuni* positive organic vs. conventional poultry flocks (195). The use of livestock manure as fertilizer is of particular concern in organic production systems. Enteric pathogens such as *Campylobacter, Salmonella, Yersinia and E. coli* O157 are known to contaminate ready-to-eat vegetables, and the perception that organic produce does not require cleaning prior to consumption is a major concern (224). Currently, researchers are unable to predict whether organic management of soils will increase or decrease pathogen load (224). Public awareness around pesticide residues, animal welfare issues and environmental degradation has lead to increased demand for organic crops and outdoor/free-range livestock systems (224). The health and safety of these products is likely to emerge as a field of importance in the epidemiology of *Campylobacter*.

14.5 Socioeconomic risk factors for campylobacteriosis

A recent Canadian study found an increased risk of disease in those of higher socioeconomic status and hypothesized that the relationship was due to increased foreign travel, restaurant dining and consumption of raw seafood (225). Gillespie et al. found “white collar” workers (professionals) to have marginally higher risk than “blue collar” workers in England and Wales [RR 1.06 (95% CI: 1.01-1.11)] with the highest incidence of

Campylobacter infection [RR 1.73 (95% CI: 1.64-1.81)] in the working class (housewives, chefs, sales assistants, receptionists, and postal workers) (122). Farm-related and occupational exposures are implicated in the higher incidence of disease among working age groups (20-60 years of age) in rural areas (120).

Social and cultural influences that are associated with disease risk can be difficult to extract and quantify, and interactions among socioeconomic determinants remain poorly understood. One problem resides in the measurement and reporting biases inherent in socioeconomic data, making consistent and reliable use of these factors an important emerging goal for public health officials (226). These combined difficulties result in gaps in the knowledge of how socioeconomic status affects the risk of campylobacteriosis.

14.5.1 Race and ethnicity

A case-control study in England and Wales revealed a significant ethnic hierarchy in the risk of campylobacteriosis, with highest incidence found in the Pakistani population, followed by White, Indian, Bangladeshi, Black and Chinese (122). The same study found no significant difference by age and gender in the Indian and Black communities, yet confirmed that young children in all ethnic groups were at higher risk than older people. In another study, Asian patients were more likely to be infected by *C. coli* than Europeans (71). In New Zealand it was found that across ethnic groups, notification of disease and rates of hospitalizations differed (Maori and Pacific people versus all others). The implication is that people of certain ethnicity, or of lower socioeconomic status have less access to health care, leading to lower disease

notification (36). The importance of race and ethnicity on risk for campylobacteriosis remains unclear.

14.5.2 Age and Gender

Campylobacter incidence has a bimodal distribution with peaks in age groups 0-4 and young adults (179, 190, 227) and age-specific risk factors exist (110, 122). FoodNet calculated incidence of disease for 2004 in infants under one year of age was 28.4/100,000 (the highest risk age group) as compared to the national average of 12/100,000 (228). In Sweden, incidence for 0-4 year olds in 2000 was 44.8/100,000 with the national average of 27.5/100,000 (189). *Campylobacter* was found most frequently in children less than 2 years of age, and adults over the age of 20 in the Navajo and Hopi tribes of the southwestern U.S. (185) Higher incidence and isolation rates of campylobacters in young children have also been reported in studies from Denmark, Quebec, Barbados and Spain (186, 193, 220, 227).

Risk and protective factors for young children may differ from other age groups due to immune status, dietary, behavioral and developmental differences (126, 228). Breast feeding is a protective factor in children under one year of age (63, 228, 229). Odds ratios for breast feeding in two studies were 0.31 (95% CI, 0-1.69) (229) and 0.1 (95% CI,0.1-0.6) (228). Breast feeding confers immunity to the child and the elevated risk for campylobacteriosis seen among children up to five years of age may be due to naïve exposure to the pathogen.

Increased risk in those ages 1-4 and 75-79 in high-density poultry counties of Michigan is thought to be due to direct contact with both poultry and environmental

sources (120). The highest incidence in males age 0-19 years old was reported in rural, heavily agricultural occupations of Manitoba province in Canada (225). Other risk factors for children include drinking well water (OR 4.4, [95% CI, 1.4-14]) and riding in a shopping cart next to meat or poultry (OR 4.0, [95% CI, 1.2-13]) (228), pet contact, day care attendance and exposure to the kitchen environment (229). An Australian study determined that restaurant prepared meat consumption and swimming were risk factors associated with age groups over five years only (110). Incidence in those over the age of 50 is on the rise in England and Wales, and thought to be a result an increase in the aging population, possibly associated with the World War II baby boomer generation (128).

A case-control study that incorporated FoodNet data and included 33% of all identified cases of *Campylobacter* in the United States in 1998-1999, revealed that the incidence of disease in all age groups was higher among males versus females (51, 230). Laboratory-confirmed cases in Colorado 1991-1992 showed higher rates in males (all ages) ($P<.001$) and rates in male infants (under one year) were twice that of females (50.9 versus 23.5 per 100,000) (231). The difference is supported by other studies in developed nations, that found the incidence in males to be higher than females, especially in teenagers (3, 122, 128).

Hormonal changes in humans may affect *Campylobacter* growth and pathogenicity and may be associated with higher incidence in males from birth until age 17 [RR 1.54 (95% CI: 1.43-1.55)] and in females 20-36 years of age [RR 1.21 (95% CI: 1.14-1.29)] (122). Behavioral and exposure issues may also play a role in the incidence

difference between genders. A South Australia survey of children with gastroenteritis found that only 38% of boys washed their hands compared to 45% of girls (965 children surveyed, age 4-6) (232). This may hold true in underdeveloped countries as well. Studies from Nigeria found male children were twice as likely as females to be infected (233). Males may be less likely to seek medical assistance, may engage in different kitchen and food-handling practices than females (51), and may have greater exposure to the outside environment (122). This explanation does not account for elevated rates in male infants and the reason for this gender difference remains unclear.

14.4.6 Rural versus urban

Rural regions and farm environments are more likely to provide direct and indirect exposure opportunities such as farm animals, unpasteurized milk and contaminated surface waters, than urban areas (120, 212, 228, 234). In spatial analyses performed in Manitoba, Canada, the incidence of *Campylobacter* infection was significantly higher in rural and agricultural areas and highest in close proximity to high densities of animals (225). In Michigan, counties with a high-density of poultry farms also had higher risk of infection compared with low-density counties [RR 1.31 (95% CI: 1.21-1.42)] (120). Rural areas with high ruminant, swine and poultry densities were also significantly associated with a higher incidence of campylobacteriosis in Sweden (212). In a molecular comparison of *C. jejuni* subtypes taken from human and environmental samples, infections in rural settings were linked to animal contact (235). A study on Hopi and Navajo reservations showed that farm owners were more likely to develop the disease, (185) and ownership of domestic chickens was a risk factor [OR 12.4 (95% CI:

2.6-59.3)] in Australia (196). These studies suggest that occupational and environmental exposure plays an important role in the risk of disease in rural regions.

Although higher exposure in rural regions is associated with a higher overall incidence of disease, baseline immunity also may differ between regions (79). Notifications and hospitalizations for campylobacteriosis are significantly lower in rural versus urban areas (36, 37). In the Netherlands, incidence in rural regions was half that in urban areas (38) and chronic antigenic stimuli from the rural or farm environment is one hypothesis for lower risk.

In a New Zealand study, adults in urban areas and children in rural areas had higher rates of disease signifying an age-based disparity in the patterns of notification (36). In Manitoba, the incidence for the 0-4 year age group was seven times higher in rural regions than in the City of Winnipeg (225). In Wisconsin, sero-positivity for *C. jejuni* was higher with age and farm residence [OR 2.8, 95% CI 1.9-4.1] (37). Although exposure to campylobacters may be higher for farm vs. non-farm residences, the incidence of clinical disease does not necessarily follow this pattern, supporting the idea that differences in baseline immunity exist between populations living in urban vs. rural regions.

14.4.7 Travel-Associated Diarrhea

Travel-related *Campylobacter* infections are estimated to account for 5-10% of cases in the United States and 10-15% in Great Britain, Denmark, and South Asia (3, 187). Diarrhea and other clinical signs of campylobacteriosis are typically more severe in travelers and more frequently associated with antibiotic resistance (68, 77, 123, 187).

In a FoodNet case-control study, 13% of laboratory-confirmed cases (n=1316) reported travel within the week prior to illness (51). Destinations included Europe (31%), Mexico (21%), Asia (20%), Central and South America (10%) and Canada (6%). Over the last ten years, U.S. troops in Thailand consistently reported campylobacteriosis as the leading cause of diarrhea (63). Worldwide, campylobacteriosis is estimated to occur annually in 27-38% of travelers to developing nations who develop gastroenteritis (63).

The top three sources for European travel-related cases identified by the European Center for Disease Prevention and Control were Spain (24.9%), Turkey (21.1%) and Bulgaria (17.4%)(60). In Sweden, travel-associated campylobacteriosis is estimated to account for two-thirds of all reported infections (189) and in Norway, they account for about half (236). Ekdahl et al. found that there were over 28,000 travel-related cases in Sweden between 1997 and 2003, with the highest risk in those who traveled to India (237). Large regional differences were seen in prevalence of travel-related cases throughout East versus West Africa (502 vs. 76 per 100,000, respectively). In a study of European travel destinations from Sweden, the risk of infection was 100/100,000 in travelers to Portugal and Turkey and 1/100,000 in travelers to Finland (238). Risk factors for tourists are thought to be due to increased exposure to restaurant food and differences in immunity to foreign pathogen strains (238).

It is difficult to pinpoint the sources of travel-associated illness, even when temporally and geographically distinct strains of *Campylobacter* have been identified (159). A molecular study in Denmark showed 31% of travel-related cases were from

food sources, in contrast to 61% in domestically acquired subtypes (161). The same study showed a greater frequency of *C. coli* in travel-related cases. Higher diversity was also seen in travel-related strains, with 42% having a unique molecular subtype as opposed to only 12% in domestic cases. Acquisition of travel-related campylobacteriosis does not always require long distance travel or crossing known political or international boundaries. In a UK study in 2000, 16% of patients who were positive for *Campylobacter* reported travel to rural and coastal regions within the UK (58). More research is needed to better understand the role of immunity and pathogen strain type in travel-associated cases.

14.4.8 Pets

Close contact with pets has been associated with increased likelihood of campylobacteriosis, especially in households with children (195, 203, 239). Dogs are risk factors for *Campylobacter*, *Arcobacter* and *Helicobacter* infections in humans (240), and a 10% co-infection rate with *Helicobacter* and *Campylobacter* has been identified in dogs and cats. Workman et al. found human isolates that were indistinguishable from canine strains, suggesting that dogs are significant reservoirs of disease (205). This is important because in nations such as the U.S. and Australia, almost 65% of households own a pet (232). Direct contact with animals at petting zoos may also play a role in disease transmission. Family and educational trips to petting zoos and farms have become popular outings in developed nations (213). On such outings, novel exposure to the pathogen and lack of acquired immunity increase susceptibility to disease.

Young animals (lambs, calves and puppies) are susceptible to colonization and shedding and are more likely to show clinical manifestations of the disease, thereby serving as a source of infection to humans (190, 210, 241). In Denmark , 29% of puppies carried *Campylobacter* spp. (76% *C. jejuni*, 5% *C. coli* and 19% *C. upsaliensis*) and 5% of kittens carried *C. upsaliensis* (66). A Swiss study showed that the highest prevalence was found in animals less than one year of age (dogs 67.6%, cats 52.4%) (65). The authors of a case-control in Australia showed that owners of domestic dogs less than six months of age were at increased risk of infection, OR 2.1 (95% CI:1.1-4.2) (196). A longitudinal study followed young domestic dogs (3-8 months old) to two years of age and found that prevalence increased from 60% to 100% (239). The species distribution in the study included *C. upsaliensis* (75%), *C. jejuni* (19.4%), *C. lari* (2.1%) and *C. coli* (0.7%).

In North-west England prevalence in canine fecal samples was 50% (from boarding kennels) and 73% (rescue kennels) (242). Of 130 canine and 51 feline samples, 46.9% and 37.3% respectively, were positive for *Campylobacter spp*. A U.S. study found prevalence to vary between 15-31% in four, separate populations of dogs (243). A cross-sectional study in healthy pets in Switzerland found a prevalence of 41.2% in dogs and 41.9% in cats (65). The study also showed that the only significant risk factor for *C. jejuni* carriage in dogs was 'regular contact with birds and/or poultry' (65).

A case-control study in Colorado found cases living in a household with a cat had 3.21 (95% CI:1.25-8.3) times the risk of infection that those without a cat (203). Outdoor

cats have higher carriage rates for C*.upsaliensis/C. helveticus* than strictly indoor cats (65) and a prevalence of *C. jejuni* in stray cats (16.8%) was found in 2008 in Italy (244). In that study, the prevalence of infection among cats over one year of age, and living near a harbor (47.4%) was higher than those residing in an urban environment (10.6%).

Excretion of pathogenic strains in pets may contribute to the overall disease burden, (242) yet the clinical status of the pet is not a strong indicator of presence or absence of campylobacters (239, 241, 245). One study found n significant difference in carriage rates between dogs with and without diarrhea and recorded higher prevalence in stray dogs and younger dogs (over 70% of positive dogs were under one year of age) (205). Authors of a Norwegian study found 18% of non-diarrheal and 16% of diarrheal samples from cats were positive for *Campylobacter spp.,* while 23% of non-diarrheal and 27% of diarrheal samples were positive in dogs (241).

Despite well-documented cases of direct contact as a cause of campylobacteriosis from pets, indirect transmission routes are considered to be more important and the frequency of zoonotic transmission remains difficult to quantify (190, 243). A study of 965 rural South Australia children (age 4-6 years) found that ownership of dogs and cats was protective [OR 0.71, 95% CI: 0.55-0.92] against gastroenteritis (232). While this study did not specify etiological agents, the authors hypothesize that low-level, chronic exposure to pathogens may induce acquired immunity.

15.0 Environmental epidemiology of campylobacteriosis

15.1 Domestic and wild animal disease reservoirs

Campylobacters known to cause human disease are widespread in animal and environmental sources (88, 167, 246). A primary mode of dispersal into the environment is through animal feces (4, 49, 64, 88, 247). The pathogen is excreted by a warm-blooded host, contaminates soil and aquatic systems and becomes a source of transmission to humans (100, 107). Known reservoirs include poultry, wild birds, dogs, cats, sheep, pigs, cows, and other exotic pets, thus an extensive potential for fecal contamination exists in both wild and domestic environments (135, 163, 205). Regions with high densities of cattle, pigs and chickens can have campylobacteriosis rates among humans that are 2-3 times higher than those in non-farming areas (225). An important difficulty in determining whether human disease arises from animals or the environment is the inability to accurately measure the contribution of indirect transmission from animals through soil and water.

Animal feces are a primary source of environmental contamination due to high carriage rates and shedding (162, 176, 214, 248, 249). Asymptomatic carriage of *C. jejuni* by dairy cattle is sufficient for maintenance of infection in the surrounding environment (64, 88, 114). In New Zealand, where agriculture is the primary industry, (164) over 45 million sheep and 10 million cattle providing a large *Campylobacter* reservoir, and year round grazing practices make fecal pollution an important source of transmission (250, 251). One New Zealand study identified *C. jejuni* positive fecal samples in dairy cattle (54%) rodents (11%) and flies (9%) supporting the hypothesis

that cattle, wildlife and their surrounding environment may be important reservoirs for disease (114).

15.1.1 Carriage and shedding in domestic farm animals

Farm level variation in the prevalence of campylobacters is due in part to differences in environmental and hygienic conditions (61). Factors such as diet, transport, stress, hormone levels, management practices, and parturition, combined with climatic and seasonal influences, cause levels of fecal shedding to vary in domestic animal populations (224). Shedding in sheep may be related to the quality and digestibility of grazing land as well as the location and type of pasture (252). Campylobacters can be isolated in sheep feces in the UK up to four days after excretion, during which time on-farm water sources, other animals and humans may be contaminated or infected (252). Jones et al. detected *Campylobacter* shedding rates in sheep to be lowest in November and December when fed hay and silage versus when grazing pasture during spring and summer seasons (252). Lambs negative for *Campylobacter* at birth may begin shedding just 3 days post partum (88, 252). The highest rates of shedding in sheep occur during lambing, weaning and movement to new pasture suggesting that stress induces shedding (252). The relative contribution of animal and environmental factors to the rate of fecal shedding and its impact on *Campylobacter* infection in humans remains unclear.

Scavenging of fecal pats, bedding and slurries by wild birds, rodents and insects has been implicated in the spread of *Campylobacter* on farms and in water sources (88). Sinton and colleagues found that 90% of *C. jejuni* in bovine fecal pats dies after

6.2 days and that bacterial counts decreased faster with desiccation on New Zealand pasture (251). While *C. jejuni* is unable to grow in fecal pats and only fresh pats are likely to be a significant reservoir, live detection of viable organisms was possible over two months in winter and one month of the summer during the study period.

A study in Barbados identified *C. coli* as the most common species isolated from pigs (90.5% of pigs sampled were positive for *Campylobacter spp.*)(205) Another study showed that while piglets were negative for *C. coli* at birth, the prevalence rose to 56.6% during the nursery period and two-thirds of all pigs sampled were shedding *C. coli* by the time they arrived in the fattening unit (61). Eleven of 1,474 samples taken around the pig farms were positive for *C. coli,* Including one water trough, one rodent and two flies.

Despite high carriage rates in domestic farm animals, studies suggest that only small proportions of a herd will shed at any given time, and the factors that affect fecal shedding are not well understood (88). Cattle can shed *Campylobacter spp.* over three months and longer in some cases, and shedding in young calves was comparable to that of broilers (64, 88). A Norwegian study found carriage rates to be higher in calves (46%) than in adults (29%) (162) and in the UK levels of *Campylobacter* in manure were higher when young farm animals (calves, lambs, piglets) were present (247). Direct transmission between wild birds and cattle is also suspected, and another UK study showed a higher probability of isolating *C. jejuni* from cattle feces in areas where bird feces were positive (176). This illustrates the interconnectedness between environmental reservoirs and animal-related factors for shedding and transmission.

15.1.2 Molecular evidence for animal reservoirs

Molecular typing has linked *Campylobacter* strains found in poultry, dogs, pigs, sheep, and cattle to sporadic human infections (159, 166, 246). Isolation rates using direct plating methods at slaughter for cattle and lambs in the UK were 26.7% and 47.8% respectively (88). Similar clonal complexes have been identified in isolates from farms and human stool samples (246). Wilson et al. measured the genetic differentiation between *Campylobacter* sources and estimated that chicken (56.5%), cattle (30%) and sheep (4.3%) were associated with the highest proportions of human infection (166). Johnsen et al. showed greater than 90% similarity using AFLP between human and bovine intestinal isolates in South-western Norway (162). Using molecular typing methods from samples taken throughout Denmark (59), researchers matched isolates between humans, cattle and poultry, and found agreement among six different genotypic and phenotypic markers. Kwan et al. collected samples from wildlife, cattle and environmental sources for MLST characterization in a rural dairy farming region of England (248). The most common *C. jejuni* genotypes identified (comprising over 60% of the data) contained clonal complexes (ST-21, 45 and 61) that are frequently associated with infection in humans.

Multiple species of *Campylobacter* can be carried by animal reservoirs (64, 162, 169, 205) and up to 13% of feedlot cattle fecal samples contain more than one species (64). Studies from Scotland and Germany suggest that the dominant strains in environmental reservoirs can change over time (61, 124). Similarly, a three year PFGE study in Norway showed that predominant genotypes differed each year between

chicken and human isolates and that some genotypes found in humans were not found in chickens and vice versa (168). This indicates that other sources of the disease are likely to circulate in the environment, especially during seasonal peaks. Systematic surveillance has not been sufficiently undertaken to determine how temporal trends in infections vary within livestock populations (36).

15.1.3 Wild Birds

Much speculation yet limited research has been devoted to understanding the impact of wild and migratory bird species on human incidence of campylobacteriosis. Between 2 and 50% prevalence has been reported in a variety of wild birds, yet their potential as a source of infection to humans and domestic animals remains unknown (253). Among wild birds known to visit grazing pastures, *C. jejuni* has been detected in crows, geese, pigeons, ducks and cranes (88). *Campylobacter jejuni* isolates have been identified in Antarctic penguins with molecular complexes similar to those isolated from humans and poultry in the U.S. (254) High *Campylobacter* carriage rates have been found in migratory birds in Barbados as well (205).

The majority of research concerning wild bird species and campylobacters is focused in the UK and New Zealand. Hughes et al. identified unique and genetically diverse campylobacters in magpies (*Pica pica*) and oystercatchers (*Haematopus ostralegus*) in Northern England (253). The authors also found that *C. jejuni* isolates in wild birds were unique from those in livestock. Kwan et al. identified wild bird MLST isolates that were highly prevalent in poultry sources (248). A UK molecular study found a range of *C. jejuni* genotypes in wild birds and Starlings that were genetically

associated with poultry and environmental sources (246). Starlings are common in the UK and free range chicken farms are an accessible habitat. In 2009, Colles et al. characterized shedding rates in wild European Starlings (n=967 fecal samples) at 30.6%, 0.6% and 6.3% respectively for *C. jejuni, C. coli* and *C. lari* (255). The majority of clonal complexes identified were host specific and differed from those historically isolated in humans and poultry. A direct link between these species and human infection was not established in the study.

In New Zealand, sparrows had a fecal *C. jejuni* prevalence of 40% in one farming area and 38% in an urban environment (114). In 2009, molecular complexes from avian fecal samples cultured from playgrounds in New Zealand city parks were similar to those found in UK starlings and indistinguishable from human cases in New Zealand (256). This suggests that children may acquire campylobacteriosis from fecal contamination of playgrounds and parks by wild birds. However, *Campylobacter* strains vary geographically among avian species, making it difficult to clearly link wild bird strains to human disease (257). The study of wild bird migratory routes and carriage rates of *Campylobacter spp.* is in its infancy, and the affect of this enormous mobile reservoir on the risk of infection, worldwide, in humans, livestock and poultry needs to be quantified.

15.2 Water as a source of infection

Campylobacters have commonly been identified in lakes, rivers, and other surface waters, yet the contribution of water as a source of infection has not been determined (164, 190, 214, 258). Contamination of water can be due to runoff from rain

or sewage system leaks (163). While consumption of untreated or inadequately treated drinking water has been responsible for rare disease outbreaks, this source is not commonly documented on an international scale (190). On occasion, outbreaks are traced directly to water sources by molecular comparisons between water and human samples (163, 248). Yet contrary molecular findings also exist (166) and inferences from typing studies must be made with caution as sources of contamination may vary regionally.

In the Eastern Townships of Quebec, one study showed that greater than 50% of reported cases of campylobacteriosis were seasonally and regionally linked to environmental sources such as drinking water (220). In Walkerton Ontario a waterborne outbreak occurred as a result of drinking water contamination from nearby farm runoff (157). When cattle samples were typed from surrounding farms, only two strains were traced to disease. The majority of environmental isolates differed from outbreak strains. It is not known if temporal variation, genetic plasticity, sample diversity and quantity or typing technique hindered the search for a genetic link between outbreak and source in this case.

In August 2006, in a small Japanese village, 71 cases of campylobacteriosis were traced to tap water contamination resulting from a week-long failure of the chlorination system (259). In Florida, an outbreak occurred from a chlorinated open-top municipal water system resulting in 865 cases of gastrointestinal illness, with 11 of 20 total fecal samples positive for *Campylobacter* (260). In this outbreak only one day of

chlorination failure occurred, suggesting a high likelihood for fecal contamination by wild birds.

In a Finnish outbreak, molecular complexes (ST-677) were the same in humans, natural water sources and wild birds (158). In Sweden, laboratory-confirmed campylobacteriosis in children was associated with drinking well water from home (OR, 5.3[95% CI, 1.9-14.8]) and exposure to river or lake water (OR, 5.8 [95% CI, 1.1-30]) (189). Risk of infection from water sources varied seasonally (cases associated with contaminated water were more frequent during summer). Continuous sources of contamination may be a substantial factor during the summer (95) and since *Campylobacter* does not replicate outside a mammalian host, isolation from water indicates recent contamination (250). Definitive associations between human cases and natural waters remain difficult to obtain in part due to varying environmental conditions (seasonal patterns, wild bird populations, temperature, farm run-off, etc.) (100).

In regions where river systems interface with farming, consumption of untreated water and outdoor recreation may contribute to higher risks of infection. In New Zealand, where campylobacteriosis rates are the highest in the world, agricultural runoff is frequently implicated in water contamination. Molecular studies using MLST have identified isolates associated with both river water and chicken meat (180) and Polymerase Chain Reaction estimated *C. jejuni* prevalence to be over 50% in river water samples (98). Furthermore, in rural areas, many residents drink untreated water and engage in water-related recreational activities (164).

Detecting *Campylobacter* in drinking water sources can be difficult due to the prolonged time between infection, clinical signs, physician visit, culture and notification of health authorities (163). These factors may preclude isolation of the bacteria from water samples that are transiently contaminated. The quantity of water tested is also an important factor for successful detection. Sampling for *E.coli* requires only 100 ml of water, yet between 1,000 and 8,000 milliliters may be necessary for the detection of campylobacters in some outbreak situations (163). The plasticity of the *Campylobacter* genome and difficulty in obtaining representative samples from large or moving bodies of water compound the problem (164).

15.2.1 Sewage and Water treatment

Exposure to raw sewage and sewage contamination of drinking water are risk factors for *Campylobacter* infection (58, 100). Enteric pathogens are documented in drinking water systems, groundwater from wells, surface waters and river systems following heavy rainfall and after systemic breakdowns or maintenance breaches in sewage systems (220, 258). In Brazil, *C. Coli* has been isolated in up to 25% of sewage samples (68). To effectively trace waterborne outbreaks to sewage sources, samples must be taken in replicates, include several possible contamination sites and include large volumes of water over long periods of time (163). Culture-based detection methods can be labor intensive and time consuming, and identification of the pathogen can prove difficult when prevalence is low and the pathogen is in the viable but nonculturable state (258). As a result, cases caused by exposure to sewage may be

grouped as “water-related” and accurate knowledge of the contribution to disease risk remains unknown.

15.2.2 Agricultural land use and surface water contamination

Farming and land use practices are major determinants of *Campylobacter* contamination in the environment (224, 249) and campylobacters can reliably be detected in drinking and recreational waters that contain known agricultural run-off and sewage (224, 261, 262). High levels of enteric pathogens have been recorded with concurrent increases in manure and precipitation on fields (224). Eighty five percent of cattle chronically shed campylobacters in feedlot production systems (64). Extensive livestock systems and manure application to soils also pose an increased risk of pathogen runoff in surface water (214, 224, 250, 258). *Campylobacter coli* has been isolated from river water in 26.5% of samples in Chile (68) and studies in the UK indicate that *Campylobacter* prevalence in surface waters can range between 40-57% (96, 100).

In a study of water samples taken from standing and running water sources in a dairy farming area of England, *Campylobacter spp.* were isolated in over 40% of water samples (100). The same study found *Campylobacter spp.* were twice as likely (P=0.04) to be isolated from clay versus non-clay soils. While direct transmission between farm animals and humans may occur, it is more likely that a common source of infection exists on either land or water (162).

15.2.3 The link between land and water use and recreational activity exposure to campylobacters

Surface water may be imbibed during recreational activities such as camping, swimming, or hiking (261, 263). In a rural UK area known for dairy production and recreational activities, PCR characterization demonstrated *C. jejuni* in 36% of cattle feces and 15% of all water samples (176). *Campylobacter coli* was found in 17% of water and 21% of sheep samples and *C. lari* was detected in 7% of birds tested and 5% of water samples. When fecal matter from domestic animals contaminates surface waters, especially during times of elevated rainfall, there is an increase in the human risk of recreational and occupational exposure (252).

Tracing surface water sources to suspected cases of disease isproblematic. Eyles et al. found greater genetic diversity in water samples than human clinical isolates, suggesting a wide variety of potential environmental sources (164). The same study, using a combination of Penner and PFGE typing methods, was unable to identify exposure to *C. jejuni* contaminated surface waters as a source of human infection. The effect and contribution of agricultural land use practices and surface waters on incidence of campylobacteriosis remains unclear and a current research challenge lies in quantifying direct links between agriculture, land use and the associated risk to humans (247).

15.3 Climatic Factors

15.3.1 Seasonality of campylobacteriosis

Temperate climate zones tend to have higher rates of campylobacteriosis and increases occur in a distinct spring-summer peak (9, 88, 264-266). In an international study, the seasonal peak occurred during the spring, with milder winters associated with earlier peak infection rates (267). In two time series analyses, (one performed in Scotland, the other in England and Wales) seasonal trends in campylobacteriosis were identified nationally and regionally (96, 265). Regional differences are hypothesized to be due to changes in transmission routes as well as urban verses rural residential status (9, 265). The causes behind seasonal variations in risk have yet to be elucidated.

15.3.1.1 Behavioral factors and seasonality

In temperate countries, seasonal increases in sunlight hours and warmer temperatures have a direct effect on human outdoor activities that increase exposure to *Campylobacter* sources (9). Known risk factors such as consumption of barbequed meats and untreated water, contact with farm animals (268, 269), and swimming in contaminated fresh water sources (261, 263) have been linked with seasonal risk. Seasonally driven increases in reported cases of campylobacteriosis may be due to changes in environmental sources of contamination or increased contact between humans and the environment (9). However, studies have not consistently demonstrated that seasonal variations in environmental source pathogen load correspond reliably with human infection (99, 261).

Distinct seasonal patterns in travel-related campylobacteriosis have also been recorded. Those who travelled in temperate regions had peak infection rates during the summer whereas minimal seasonal differences were detected in those who travelled to tropical regions such as Sub-Saharan Africa, Central and South America and the Indian Subcontinent (237).

Studies demonstrating a clear lack of seasonality should also be mentioned. The distribution of cases on the Caribbean island of Barbados was found to be even throughout the year (186). No correlation could be found between seasonality and human and water isolates in the Taieri River region of New Zealand (164). A study of *Campylobacter* prevalence on retail meats in Washington D.C. supermarkets found no seasonal difference in microbial contamination levels (192). A cross-sectional study in healthy dogs and cats in Switzerland found no seasonal difference in prevalence of campylobacters in stool (65). Similarly, Johnsen et al. found no seasonal difference in intestinal carriage rates for *C. jejuni* or *C. coli* in cattle and calves in South-western Norway suggesting that bovine reservoirs are a stable part of the transmission cycle (162).

15.3.1.2 Animal and environment-related seasonal factors

Seasonal prevalence of campylobacters vary in sewage, the environment and animal feces (88, 94). In the UK significant seasonal variations in the distribution of human infections were correlated with the prevalence of *C. jejuni* in fresh feces of dairy cattle (266). Vereen et al. found the highest bacterial counts in natural waters during the summer in Georgia (99), whereas studies in England and Washington state found

Campylobacter counts increased during the winter (261, 262). Researchers hypothesize that climatic factors are directly related to peaks in animal and environmental prevalence (88). However, it is unclear whether animals are being seasonally re-infected or subject to natural, biological, or managerial related fluctuations in *Campylobacter spp*. over time.

In the Taeiri River basin of New Zealand, summer is characterized by higher livestock density, increased rainfall and flooding, and pathogen levels are magnified as fecal material is transferred from land to surface waters (250). In Michigan, high-density poultry regions had an incidence rate 1.47 times higher than low-density counties during the winter season (120). Seasonal peaks in Wales showed that human infections were correlated with increased isolation rates from fresh retail chicken during early and late June (269). In Denmark, similar seasonal changes in prevalence were reported and both studies support the hypothesis that an environmental source is responsible for the nearly simultaneous increase in rates in both poultry and human populations (270). However, complex interactions between climate factors such as sunlight, temperature and humidity make it difficult to assess the direct impact of each (11). Furthermore, land use, environmental and farm animal reservoir ecology (including diet, grazing and housing strategies, hormonal changes, calving and water sources) are important factors in seasonal infection rates in livestock, yet the transmission, individual impact of reservoir hosts and routes of infection remain elusive and complex. While molecular typing has been undertaken to distinguish isolates from various potential sources on a

seasonal basis further research is warranted to clarify exactly how, and from where the organism is transmitted (271).

15.3.2 Temperature

The influence of ambient temperature on disease incidence varies between studies and countries. Tam et al. found that a 1^0C rise in temperature in England corresponded with a 5 % increase in reported cases, up to 14^0C (268). In Sweden, mean annual temperature was associated with increased risk of campylobactersis (RR 1.05 [95% CI:1.03-1.07]) (212). In Denmark, high temperatures four weeks prior to infection were good predictors (R^2=68%) of human incidence (11). In contrast, in a study encompassing Europe, Canada, Australia and New Zealand, only temperature increases occurring 10-14 weeks prior to elevations in human infection were statistically significant (P=.05) (267). In an Australian study, temperatures in subtropical Brisbane were positively correlated with campylobacteriosis cases whereas in Adelaide, a temperate city, temperature was inversely correlated (272).

Temperature has a strong influence on survival of *Campylobacter* in fecal pats and in the environment (251), as well as carriage and contamination rates in poultry, wild birds, insects and rodents (11, 70, 251). The effect of temperature on *Campylobacter* prevalence in broilers may play a role in human rates of disease (270). Flies also play an important temperature-dependent role in transmission of disease (113, 117). For example, Guerin et al. modeled the association between minimum temperatures at which flies become active and flock prevalence during the summer season in Iceland (117). Results indicated that at temperatures unsuitable for fly activity,

risk of flock colonization was low, whereas sustained high temperatures have a higher proportion of positive flocks. The correlation between temperature and prevalence might also be associated with the presence of other insects and migratory birds whose effects on transmission have yet to be identified (117, 272).

15.3.3 Precipitation

Environmental contamination, survival and transmission of *Campylobacter* resulting from rain and slurry runoff may increase the human incidence of disease (117, 176, 273). A statistical study found that over 50% of waterborne disease outbreaks in the U.S. between 1948 and 1994 were preceded by unusually heavy rainfall events (274) and historically, excessive rainfall has been associated with waterborne disease outbreaks worldwide (273, 275). The direct consequences of such events can be complex and difficult to quantify.

Campylobacters are sensitive to desiccation, therefore conditions of high rainfall and humidity are thought to improve survival of the bacteria in the environment (11). Rainfall can improve survival in fecal pats while simultaneously increasing leaching of bacteria (251). A study in Nigeria indicated that isolation rates were higher during the rainy season (233). Similarly, researchers speculate that hot, dry summer temperatures in the Helsinki area between 1996 and 1998 resulted in a lower incidence of disease than those during years of higher rainfall (168).

The prevalence of *Campylobacter* in environmental samples can be significantly lower following rainfall events, though the finding may be due to dilution (176). In 2004, investigators showed that precipitation explained only 6% of the variation in human

incidence in Denmark, and neither precipitation or humidity were explanatory with regards to prevalence in broilers (11). No association could be found between human incidence of disease and rainfall, humidity or temperature in two studies from Barbados (186, 205). Differences in the epidemiology of *Campylobacter* in nations located in tropical latitudes may be the reason for lack of association (186). More studies are needed in both temperate and tropical environments to understand how precipitation effects *Campylobacter* survival, transmission and human incidence of disease.

16.0 Time series modeling of campylobacteriosis

Studies show that variation in disease risk may be due to the temporal effects of temperature, rainfall, or humidity on the survival and reproduction of campylobacters in either the environment or on foods sources (11-13). Time series models, including multivariable methods that incorporate environmental variables, have been applied to surveillance data over the past twenty years as a means of accurately forecasting disease risk (12, 14, 15, 20, 21, 276, 277). Modeling the timing and shape of the seasonal peak may provide a better understanding of the drivers and constraints of temporal patterns, thereby aiding in the identification of potential sources of *Campylobacter* infection (8).

Temporal molecular studies from the UK show that important *Campylobacter* strains may persist over several years (136). French et al. performed MLST typing of fecal and environmental samples for *C. jejuni* within a narrow spatiotemporal window, providing an accurate characterization of the population structure and potential sources of infection (249). Karenlampi et al. examined the temporal and geographic distribution

of isolates by PFGE in Finland from July to September 1999, and found a 46% overlap among chicken and human strains (209). When comparisons between samples accounted for time and space, only 31% of the strains matched. The authors suggest that the timing and location of environmental sources affect infection rates for humans and chicken during the seasonal peak.

A time series study in Germany showed significant cross-correlations between human incidence, monthly temperature and rainfall (17). In the study, peak prevalence of human campylobacteriosis preceded a peak in broiler flocks, suggesting that the influence of environmental exposure was an important contribution to disease risk. Inclusion of temporal and/or spatial parameters into disease models may help to pinpoint sources and reservoirs of this genetically diverse pathogen. Comparing seasonal trends among regions with different environmental characteristics may help identify different risk factors and transmission routes, making time series modeling of great benefit to epidemiologists (14-17). Risk forecasting could also provide public health officials with an early indication of potential changes in disease incidence and act as an epidemic alert system (14, 19, 20).

17.0 Spatial analysis and Geographic Information Systems

According to Green and colleagues, "*Campylobacter* may be occurring in different places for different reasons" (225). This premise provides a foundation for the emerging field of geographic epidemiology. Geographic Information Systems (GIS) have become an invaluable tool for elucidating the epidemiology of disease and improving disease management (278). Spatial analyses help to optimize our

understanding of the dynamics of local and global disease transmission and are now common in academic research and health departments (279).

17.1 Spatial analysis as a tool for public health

Technological advances have placed spatial and temporal simulation models at the forefront of policy and decision making and are helping to bridge the gap between theoretical modeling and applied surveillance and prediction (279-281). As databases become more complete and accessible, the tools of GIS such as geo-referencing of farm and animal populations, and other spatial scan statistical methods have become increasingly used in surveillance systems (282). Advanced statistical methods are also being honed for the express purpose of accurate identification of zoonotic disease risks, including campylobacteriosis.

Predictive spatial models that incorporate environmental variables are a current focus of regional control strategies (279). Modeling tools can be used to understand disease patterns in wild and domestic animal populations, environmental exposures, vector-borne diseases, and for economic assessment of disease burden. For example, during the 2001 foot and mouth disease epidemic in the United Kingdom, over 70 full-time GIS staff were assembled to generate risk maps and analyze the disease spread potential of farms (280). Models were constructed rapidly enough to be effective for strategic policy making to curtail the epidemic. In a U.S. spatial study in 2010 strong relationships that were identified between air pollution and mortality were used to improve air quality standards and regulations. This trend, linking the scientific

knowledge base with public policy is likely to continue as GIS systems and databases become more tractable and integrated.

17.2 Spatial distribution of campylobacteriosis and campylobacters

In the United States, campylobacteriosis incidence rates have been shown to vary between states (differences between 6 and 32 per 100,000 persons), and true regional differences in risk may exist (3). Swedish investigators examined travel-related incidence throughout European countries over a seven year period (1997-2003) and found a distinct north-south gradient across southern countries (Spain, Portugal, Romania, Turkey) (238). Results showed higher incidence in southern versus Nordic regions. Differences in geographic patterns of campylobacteriosis have also been observed when data were analyzed at different spatial levels (ie. county versus municipality in Sweden) (212).

A recent focus of campylobacteriosis research aims to understand how environmental and demographic factors are associated with disease risk in different geographic regions. Spatial scan statistics and smoothing techniques have identified significant geographic variability in *Campylobacter* incidence in Manitoba, Canada (225). In Denmark, spatial analyses on over 11 years of registry-based data showed that odds ratios were highest for children (age 0-14) in rural areas (OR=1.65), people living in farmhouses (1.24) and low population density areas (1.28) (177). The researchers found that campylobacteriosis varies significantly with respect to municipality, housing type, drinking water supply and population density, supporting the

need to further investigate sporadic sources of infection, as well as different risk factors in different areas.

Survival of *Campylobacter* in the environment also varies geographically (250). A study by Garrett et al. showed similarities between human *Campylobacter* infection isolates and ruminant feces in rural settings (235). Another spatial study used MLST to test 172 samples from water, wild and domestic animal feces and soil taken over ten weeks on a 100 km^2 area of cattle farmland in the UK (249). Results identified samples from cattle and rabbit as genetically similar, suggesting that the transmission cycle is specific to pastures in the study area. Isolates identified from wildlife and water had molecular complexes unique to the study area yet not associated with human disease. Importantly, the primary clonal complex (ST-61) detected in cattle samples had been previously linked to human infection.

17.3 Limitations

One problem in assessing the spatial distribution of campylobacteriosis cases is the possibility that case reporting differs due to differences in access to health care and efforts to confirm the diagnosis across regions (225). Secondly, spatial scan statistics and smoothing methods can create bias from the “edge effect”, whereby models can be inaccurate at the borders of a study area due to the number of neighbors involved in calculations (225). Problems in analytical accuracy can also occur when there are errors in case data or it is assigned improper location identifiers (58). Spatial analyses are often subject to the modifiable areal unit problem (statistical bias resulting from arbitrary

or artificial boundaries), ecological fallacy, and problems with small numbers. These factors must be addressed to insure accurate identification of geographic risk patterns.

17.4 Trends in spatial analysis

The trend in GIS is towards increasingly sophisticated methodologies such as spatio-temporal models, GPS, real-time, remote sensing and other multi-level modeling approaches. These techniques allow for the extraction of meaningful relationships with greater precision. Bayesian geo-statistical approaches are also gaining ground in predictive disease mapping and modeling (283) and principal component analysis can be used to understand the relatedness between characteristics and disease risk factors in neighborhoods and other close knit regions (281).

According to Jerrett et al. “The largest challenge to the expanded use of GIS and allied methods for health surveillance is related to data availability, consistency and cost.” He further states that, “while knowledge and the technology are available to utilize spatial analysis in Public Health, the institutional structures for data collection , management and dissemination are lagging.” (281) Epidemiologists are still in the early stages of understanding the spatial constraints of *Campylobacter* survival and transmission in the environment and the effect on human incidence.

18.0 Control and prevention of campylobacteriosis

18.1 Poultry industry

The majority of food-borne infections associated with poultry in developed nations are caused by *Salmonella* and *Campylobacter* (224). While control efforts have successfully decreased rates of salmonellosis, human *Campylobacter* infections have

steadily risen over the past 20 years (224). In the 1990's many countries undertook quality assurance measures that included strategies for decreasing risk of contamination on retail meat products, reducing pathogen load on the farm, increasing hygiene practices during processing and slaughter and increased consumer education (192). However, despite the widespread acceptance of poultry as a primary reservoir and potential transmission source for *Campylobacter,* little has changed in the broiler industry to reduce contamination and colonization of flocks (84).

On-farm and slaughterhouse hygiene improvement is cited as a primary means of control and prevention in the poultry industry (166). Biosecurity measures, including proper design and location of farms and staff education are a main concern (284). Poultry are likely to be infected by bird fecal matter as well as environmental sources surrounding the farm such as water, other animals, and wild birds (112, 124, 206, 208). Fly control on poultry farms (screening, light traps, sticky fly traps, destroying maggot habitats) has been recommended to decrease flock prevalence at the farm level (117, 118). Good management practices such as controlling the moisture content of poultry bedding and treating poultry litter with aluminum sulfate or sodium bisulfate can also significantly minimize bacterial counts (131). *Campylobacter jejuni* has also been found to survive overnight on slaughterhouse processing equipment surfaces even after cleaning and disinfection (285). Methods to reduce or prevent colonization in birds and disinfect birds post processing continue to be priorities in controlling disease (190).

Pioneering molecular efforts for control of *Campylobacter* aim to reduce bacterial levels in the gut through the use of antimicrobial alternatives and by increasing host

resistance (151). The identification and use of anti-C*ampylobacter* bacteriocins (antimicrobial peptides) are a promising new strategy for reducing *C. jejuni* colonization in poultry (286). While a cost effective vaccine for reduction of *Campylobacter* in poultry is yet to be developed, the potential is currently under exploration and thought to be a promising strategy in reducing overall bacterial load (56, 131, 132).

18.2 Farm-level

Exposure to contaminated meat, poultry and food animals constitute major sources of human disease, suggesting that improved biosecurity and on-farm hygiene could reduce disease incidence dramatically (166, 196). Farm-level interventions such as improving isolation measures during rearing is one approach currently recommended (207). Implementation of on-farm sampling and testing of sheep and cattle is also recommended where feasible (88, 252). Reducing gastrointestinal tract colonization in bovines has been considered, yet practical measures to achieve this goal have not been devised (64). Another strategy is to isolate or cull high shedding animals in feedlot situations, although this approach is economically prohibitive (64, 88).

Controlling on-farm waste products may reduce the prevalence of *Campylobacter* and other pathogens such as *E. coli*, *Salmonella* and *Listeria* (131, 247). This strategy may also help to control colonization in livestock, as infection with one bacteria has been shown to predispose the animal to coinfection with other potentially pathogenic organisms (247).

18.3 Slaughterhouse

Slaughterhouse procedures such as scalding and chilling are often insufficient in removing campylobacters (207). Minimizing cross contamination at the level of the slaughter house by using counterflow water systems during processing and improving washing procedures for equipment has also been suggested (214, 285). Other methods of prevention include super-chlorination of water, carcass decontamination and carcass drying (207). Efforts should also be applied to the pig industry, as pigs harbor very high levels of *C. coli* prior to slaughter and slaughter hygiene is important to avoid carcass contamination (61). The role of specific sources of cross contamination at the level of the slaughterhouse (transport crates, equipment, different flocks) has yet to be quantified (207, 285). In summary, reducing pathogens from the farm environment, feed and water systems and improving sanitation during slaughter and transport are considered key intervention strategies (131).

18.4 Public health

The objectives of national programs such as the Hazard Analysis Critical Control Points (HACCP) are to ensure that all aspects of production, processing and distribution contribute to the microbiological safety of the product (167, 214). Risk reduction points created by the HACCP include six major categories defined in detail by Leifert et al. in 2008 (224). These include animal husbandry practices for reduced fecal shedding, outdoor livestock management, manure storage and processing, soil management, irrigation and crop handling and harvest methods.

Addressing food hygiene and preparation issues are widely acknowledged as effective strategies in the control and prevention of campylobacteriosis. Behavioral strategies for decreasing contamination include both consumer and retail level education programs that focus on proper food handling and preparation (167, 214). Adequate cleaning of utensils and hands are considered to be some of the most important hygiene strategies (216). Prolonged exposure (up to 10 seconds) to hot running tap water has been shown to reduce *C. jejuni* by destruction and physical removal of cells (216). The effectiveness of cooking is time dependent as well, and pan frying inactivates *Campylobacter* after five minutes at 70-80^{o}C (201). Realistically, simple behavioral modifications such as proper cooking, avoiding consumption of raw milk, untreated water and seafood will inevitably reduce incidence of disease (167, 214).

18.5 Surveillance

Improving and standardizing national and international surveillance strategies and prioritizing rigorous investigation of *Campylobacter* infections would also improve our understanding of the disease epidemiology. In England and Wales, *Salmonella* and *E. coli* cases have much higher investigatory follow-up than does *Campylobacter,* which scored lowest for follow-up out of 20 food-borne causes of disease (169). Auld et al. recommend the use of 'alert systems' capable of identifying and predicting the impact of heavy rainfall and severe weather events (273). Meteorological surveillance could serve as a complementary method to reporting to warn the general population, public health sector and water quality personnel about the potential for environmental contamination due to weather events.

18.6 Other avenues of prevention and control

Improved farm and slaughterhouse level management practices and behavioral changes may not completely control *Campylobacters* (224). Genetically selecting animals that are resistant to colonization is another proposed strategy (131). Molecular matching between human, animal and environmental sources may also provide a cornerstone for better understanding and control of this disease (164, 287).

In addition, safe and cost-effective human and/or animal *Campylobacter* vaccines would be an enormous contribution. Prevention and control of campylobacteriosis requires interdisciplinary approaches that include human and animal medical fields, farm workers, and public health officials (213). A priority for decreasing the economic and health-related burden must include a combination of targeted surveillance and improved public education.

19.0 Summary, conclusions and future directions

19.1 Summary

After over thirty years of research into *Campylobacter* spp. and human infection, much of the epidemiology remains unclear. Multiple factors contribute to its complexity. The bacteria are ubiquitous in the environment, and have many advantageous biological mechanisms for survival, transmission and colonization. A combination of intensive animal farming practices, land use factors and weather patterns serve to elevate and disperse the bacteria. In addition, the slaughter and distribution of animal products, specifically poultry, provide mobile, wide ranging sources of infection and contamination. The challenge lies in distinguishing whether infection arises from

consumption of animal products, direct contact with animals, or contact with shared environments and surrounding water sources.

The multifaceted relationship between ecological factors and bacterial adaptation make it difficult to identify individual environmental contributions to disease burden. Currently there is a need to identify how sources become contaminated, what factors influence environmental loading, the role of reservoir hosts and the modes of transmission from the environment to humans. In addition, the genetic plasticity of campylobacters and the occurrence of multiple strains, or coinfections make definitive identification a challenge. Difficulty in detection and comparison of isolates and their sources is amplified by a lack of standardization of molecular typing methods used by scientists and laboratories worldwide.

Human cases of campylobacteriosis are often sporadic, and the lengthy incubation period prior to clinical disease decreases the possibility of identifying a definitive source. Point source campylobacteriosis cases may remain hidden due to low likelihood of reporting–the majority of cases are self-limiting and reporting is often based on the severity of disease. Difficulties in assessing the disease burden may be a function of underreporting, combined with differences in surveillance methods between states, regions and countries.

Geographic and socioeconomic factors play an important role in the risk of disease, as do occupational and environmental exposures, notably in rural regions. Spatial associations between human campylobacteriosis, environmental and demographic determinants have not yet been systematically and predictably

established. Furthermore, exposure and infection do not guarantee clinical disease, and a better understanding of how host factors affect variation in individual susceptibility and clinical signs would improve our ability to identify important risk factors.

The effect of wild bird migratory routes and carriage rates of *Campylobacter spp.* on the risk of infection, worldwide, in humans, livestock and poultry is poorly understood and requires investigation. Furthermore, the increasing popularity of raw (unpasteurized) dairy products and organic foods are a “wild card” in the epidemiology of human disease, as the health and safety of these products has not yet been determined.

19.2 Conclusions and future directions

While great strides have been made in understanding this complex organism, quantifying the contributions of seasonal, geographic and socioeconomic drivers to the risk of disease will further elucidate its epidemiology. There is a growing need to improve surveillance technology such that it becomes standardized and accessible between clinical, environmental and epidemiological data sources for research and Public Health purposes. Consistent typing of pathogenic *Campylobacter* strains over long periods of time are needed to provide genetic matches between disease cases, sources of infection and reservoirs. Combined improvements in surveillance and molecular typing will expand our knowledge at the intersection of *Campylobacter* ecology and human infection. Continued investigation into the transmission mechanisms for antibiotic resistant genes is also warranted and prudent use of antibiotics within the human and animal medical and production industries is needed in

order to curtail the development of resistant strains. Research should also concentrate on intervention strategies that improve biosecurity in food production and limit sources of environmental contamination that contribute to human disease.

Chapter 2

Comparison of three time series models for predicting campylobacteriosis risk in Georgia, Minnesota and Oregon

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1.0 SUMMARY

Three time series models (regression, decomposition, and Box-Jenkins Autoregressive Integrated Moving Averages) were applied to national surveillance data for campylobacteriosis with the goal of disease forecasting in three U.S. states. Data sets spanned 1998 to 2007 for Minnesota and Oregon, 1999 to 2007 for Georgia. Year 2008 was used to validate model results. Mean absolute percent error, mean square error and coefficient of determination (R^2) were the main evaluation fit statistics. Results showed that decomposition best captured the temporal patterns in disease risk. Training dataset R^2 values were 72.2, 76.3 and 89.9% and validation year R^2 values were 66.2, 52.6 and 79.9% respectively for Georgia, Oregon and Minnesota. All three techniques could be utilized to predict monthly risk of infection for *Campylobacter* sp. However, the decomposition model provided the fastest, most accurate, user-friendly method. Use of this model can assist public health personnel in predicting epidemics and developing disease intervention strategies.

2.0 INTRODUCTION

Campylobacter sp. bacteria are motile, spiral shaped, gram negative organisms found ubiquitously in the environment (47, 178). They have been identified as a leading cause of human gastroenteritis in developed nations, surpassing pathogens such as *Salmonella* sp. and *E. coli* (1, 2). An estimated one percent of the U.S. population (2,400,000 persons) are infected annually resulting in 13,000 hospitalizations and 124 deaths (3). *Campylobacter* sp. can be found in the gastrointestinal tracts of a wide variety of domestic and wild animals and birds (4-6). As a result, establishment of causative associations between human infection and contaminated food or water, animal contact and other environmental sources is a formidable task. Geographic region, climate patterns, drinking and recreational water, land use and human behaviour comprise some of the complex set of determinants which have been shown to affect the rate of gastrointestinal disease (8-10, 17, 265, 288, 289). The incidence of campylobacteriosis varies seasonally and geographically, and tends to be highest in summer months, specifically in temperate climate zones (1, 264, 268, 288). While the seasonality of the disease has been well documented worldwide, extensive studies have not been performed to predict the future risk of disease in different geographic regions in the United States. Comparing seasonal patterns among regions with different environmental characteristics may help identify transmission routes making reliable time series forecasting of great benefit to epidemiologists and public health officials (14-17).

A variety of modelling approaches have been applied to surveillance data over the past twenty years in an attempt to accurately predict patterns of infectious diseases (12, 14, 15, 20, 21, 276, 277, 288-293). Statistical time series modelling is appropriate since *Campylobacter* sp. disease surveillance data can be aggregated into equally spaced time intervals, exhibits autocorrelation, trend and seasonality (20, 294). The potential for emerging infectious disease patterns to change in response to anthropogenic climate and land use changes warrants the continual improvement and updating of current forecasting systems. Technological advances in forecasting software and program capability beget systematic review of methods and their applicability in the realm of public health. Recent interest in automated, real time detection techniques have met with varying levels of success (292). Our study incorporates a univariate methodological approach to forecast monthly disease risk using campylobacteriosis incidence from three U.S. states.

Finding the most accurate time series disease risk model at the state level holds numerous practical implications. Systematic analyses of multiple modelling techniques aims to create an optimal model to be used by public health officials with a state specific, accurate and user-friendly method for predicting disease risk. The best model could potentially be implemented by trained public health professionals. Risk forecasting could provide public health officials with an early indication of irregularity in disease incidence and act as an epidemic alert system (14, 19-22). Model application could subsequently result in more efficient and cost-effective control strategies (23).

The purpose of this study is to evaluate three time series models using data from three U.S. states, Georgia, Oregon and Minnesota, to forecast the monthly risk of campylobacteriosis one year in advance. We also aim to determine if current software is capable of accurately simplifying time series methods for practical use in the public health arena.

3.0 METHODS

3.1 Data Source and Study Area Description

The data utilized for this project were obtained from FoodNet, an active surveillance system implemented in 1996 by The Center for Disease Control and Prevention (CDC) (295). To meet the operational case definition of campylobacteriosis, samples of either stool or blood must be laboratory-confirmed as positive for *Campylobacter* sp.

Data from Georgia, Oregon and Minnesota were chosen for completeness and climatic diversity. Both direct and indirect disease transmission may be affected by weather conditions, therefore, it is important to predict disease risk for geographically diverse regions (288). Oregon experiences temperate climatic conditions characterized by nine months of consistent cloud cover and rain (296). Regional variation in annual precipitation (50 to 500 cm) occurs. During the summer months, July through September, there are approximately fifty days of clear sky with average daily temperatures between 30 and 38^{o}C. Georgia is characterized by a humid subtropical climate and receives approximately 114 cm of annual rain in the middle of the state and 180 cm in the northeast mountains (297). Summers are hot and humid with an average

daily temperature of 32°C. Minnesota climate is the most extreme, with average daily temperatures ranging in January between -14 and -11°C, and between 19 and 23°C in July (298). Average precipitation is 48 cm in Minnesota's northwest region and 86 cm in the southeast. We hypothesize that climatic differences between states may affect the characteristics of the campylobacteriosis risk curve over the course of the year. Subsequently, this may influence statistical forecasting methods, as well as prevention and control strategies.

3.2 Data Preparation

FoodNet surveillance data was aggregated into counts by month for each state over the study period resulting in 108 data points in Georgia and 120 data points in Oregon and Minnesota, equally spaced over time. The series lengths are statistically appropriate for the three time series methods (299). To ensure the regional integrity of the risk estimates, cases identified as travel-related were eliminated from the dataset. The years 1998 (1999 for Georgia) to 2007 were used to model each time series and the year 2008 was held out of the data set for model validation. Data manipulation was performed in SAS version 9.2 (300). Risks were determined using annual population estimates as denominators obtained from the U.S. Census Bureau (301). The risk estimates were presented as number of cases per 100000 persons. The statistical analyses were performed in NCSS-2007 (302). The forecasting methods used were time series regression, decomposition, and Box-Jenkins Autoregressive Integrated Moving Averages (ARIMA). Fit statistics and holdout R^2 values were calculated manually. Separate model forecasts were assessed for each state.

3.3 Pattern Analysis and Outlier Identification

Pattern analysis was performed on monthly risk data using autocorrelation (ACF) and partial autocorrelation plots (PACF). Kruskal-Wallis ANOVA was performed on monthly medians to verify seasonality ($P<0.05$). A simplistic strategy of identifying outliers as data points three standard deviations from the mean for time series data are invalid since this ignores the autoregressive or moving average patterns in the data. Instead, the time series outliers were identified by fitting a basic ARIMA model to the data series. The resulting residuals are saved and standardized by the root mean square error for the ARIMA (1,0,0)(0,1,1). These standardized residuals are then control charted. Observations outside three standard deviations from the mean of zero are then flagged as outliers in the time series data. This outlier identification procedure avoids over identification of outliers in time series (303).

Outbreak information on individual cases is incomplete in this data set. All cases were aggregated by month regardless of outbreak status. Outliers identified by control charting were individually checked for potential outbreak status. No association between outlier months and reported outbreak cases could be found.

3.4 Time Series Modelling Techniques

The models were quantitatively evaluated based on their predictive ability using mean square error (MSE), MAPE (mean absolute percentage error), R^2 on the training data, and a holdout R^2 based on 2008 data for all three modeling techniques. Outside of time series analysis, most people associate R^2 only with multiple regression. However, there is a pseudo R^2 that can be computed for any time series model as follows:

$$R^2_{pseudo} = 1.0 - \frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2} \quad (1)$$

This pseudo R^2 is simply the sum of the residuals squared divided by the total sum of squares in the model. For a holdout R^2, the calculation is basically the same as in equation (1), except that the model from the training sample is applied to the holdout, the sample size is only over the holdout, and $\bar{y}$ is the mean for the holdout time period. In essence, all models can be evaluated in the same way. Henceforth, all further comparisons will be addressed simply as R^2.

3.4.1 Time Series Regression

Ordinary least squares multiple regression models were evaluated using additive (untransformed) and multiplicative (logarithm transformation) risks. Predictors included trend, month, year and trend by month interactions. Variables were retained if they improved predictive value (R^2), produced globally significant ($P<0.05$) models with significant regression coefficients, and lacked collinearity (variance inflation index less than five). The basic time series regression model used was additive and shown in equation (2):

$$Y_t = \beta_0 + \beta_1 x_{trend} + \sum_{i=2}^{p=12} \beta_i d_i + \varepsilon_t \quad (2)$$

This model assumes linear trend and seasonality but no interaction between the two.

Residual time series plots were examined for all models and checked for normality using the Shapiro-Wilk's goodness of fit for normality. In addition, one wants to find white noise

(no pattern) in the residuals after fitting a time series model. Therefore, the Portmanteau test was used to assess white noise, with degrees of freedom adjusted according to the number of predictor variables (299). This test assures that the pattern has been fully extracted from the series and that the residuals are randomly scattered.

3.4.2 Automatic Decomposition

A decomposition macro available in NCSS and other software was applied (302). The series was decomposed into trend, seasonal, cyclic and error components. The decomposition model that worked best on this data was multiplicative as shown in equation (3):

$$Y_t = T_t \cdot S_t \cdot C_t \cdot E_t \quad (3)$$

Residual analysis for white noise and normality was performed as described for time series regression.

3.4.3 Box-Jenkins Autoregressive Integrated Moving Averages (ARIMA)

The ARIMA modelling was based on the techniques described by Box and Jenkins in 1976 and further explained by DeLurgio (299, 304). The ACF and PACF plots were used to identify starting orders. Exhaustive combinations of autoregressive (AR), moving average (MA) and differencing parameters were fitted up to the third order. Orders above three were not attempted due to the high likelihood of model overspecification. First order seasonal differencing resulted in the best models for all three states and compensates for non-stationarity in the mean (304). The best models were selected after various fit statistics were

evaluated. The best ARIMA model was ARIMA(1,0,0)(0,1,1), which is captured using backshift operators in equation (4):

$$(1 - \varphi_1 B)(1 - B^{12})Y_t = (1 - \theta_1 B^{12}) \quad (4)$$

Significant (P<0.05) coefficients were retained with correlations less than 0.8 between parameter estimates. Residual analysis, as to normality and white noise, was performed as described for time series regression.

4.0 RESULTS

4.1 Pattern Analysis

Monthly risks ranged from 0.236-1.191 per 100,000 persons (mean=0.593) in Georgia, 0.635-2.895 (mean=1.443) in Oregon and 0.333-4.655 (mean=1.435) in Minnesota. All three series demonstrate seasonality (Figure 2.1a, b, c) (Note: All figures can be found in Appendix B). The vacillating seasonal pattern in the ACF plots dominates and potentially masks AR and MA components. The ACF and PACF plots for Georgia are shown in Figure 2.2.a and 2.2.b. The exponential decay in of the seasonality in the ACF along with the singular PACF first order spike is indicative of AR (1). Further looks at regular and seasonal differencing hinted at a possible MA (1) for the seasonal component. The patterns were not clean, implying other model possibilities or potential outliers or both.

4.2 Outlier Identification

Outliers were not identified in Georgia using the ARIMA control process techniques, therefore, no further preprocessing or smoothing methods were applied to the Georgia time series. For the Oregon series, June 1998 was flagged as an outlier in both the raw and residual control chart analysis. The mean risk in June was 2.11 per 100,000 persons. For the June observations a running median of 5 consecutive June values was chosen to preserve the seasonal effect. The original outlier value of 2.864 per 100,000 persons was replaced with 2.017. The models performed consistently worse with smoothed data. As a result, all Oregon forecasting was applied to the original unsmoothed data.

Control charting of the Minnesota series indicated that June 1998 was out of range for both control charting techniques. The data point was above 3 standard deviations from center. To correct the outlier, a running median of 5 for consecutive June data was chosen for smoothing. The replacement median risk value of 2.372 (original value=4.65, June mean=2.420) was used in all further analyses.

4.3 Time Series Model Results and Comparisons

The results for the best models identified for each technique are summarized in Table 2.1(Note: All tables can be found in Appendix A). All ARIMA and regression models were significant ($P<0.05$) both globally and for individual model coefficients. Decomposition models do not rely on overall model significance testing to assess fit.

4.3.1. Regression

The best regression model for all three states was additive and contained statistically significant ($P<0.05$) trend and monthly estimates. The R^2 value in Georgia was 69.3%, in Oregon 71.0% and 83.5% in Minnesota. In all three states, normality of the residuals was achieved but not white noise.

4.3.2. Automatic Decomposition

The decomposition risk predictions for campylobacteriosis resulted in the highest fit statistics of the three methods. The R^2 value for Georgia was 72.7%, for Oregon 76.3% and for Minnesota 89.9%. Normality in the residuals was achieved for all three series. None of these models attained perfect white noise. The Georgia model was adequate for white noise only for lags 1 and 2. The Oregon model was inadequate overall. The Minnesota residuals were adequate for white noise on lag periods 1, 4, and 7-11.

The actual and predicted risk values for the decomposition validation year (2008) are shown in Figure 2.3a, 2.3b and 2.3c. The validation dataset R^2 values for Georgia, Oregon and Minnesota were 66.2%, 52.6% and 79.9% respectively.

4.3.3 ARIMA

For all three states the most parsimonious model with highest R^2 value (Georgia= 65.5%, Oregon= 72.4%, Minnesota= 84.1%) was the ARIMA (1,0,0)(0,1,1). Better R^2 values were possible using AR and MA components higher than two. However, complex models tend to over fit, demonstrate multicollinearity and frequently do not pass the assumptions of

normality or achieve white noise. The majority of the models had markedly lower R^2 values than decomposition models, but the ARIMA models had a higher holdout R^2, except for Minnesota.

The observed seasonal variation in campylobacteriosis identifies the months of June, July and August as the highest risk months of disease for all three states. However, the overall shape of the curve differs across series (Figure 2.4). The Minnesota's annual curvature has the sharpest, narrowest seasonal peak until 2004 at which time the shape coincides closely with Oregon. The seasonal peak in Georgia is more rounded and less severe.

5.0 DISCUSSION

The automatic decomposition procedure resulted in a 4-7% improvement in R^2 over the best regression and ARIMA models. Comparing the three methods, decomposition was the fastest and least technical, achieved normality in the residuals yet was uniformly unsuccessful at achieving white noise. Lack of white noise implies that there is a pattern in the residuals not accounted for by the model. Residual patterns may increase model uncertainty. However, good predictive performance can be achieved without perfect attainment of white noise (291).

The use of ARIMA modelling for disease risk data is well documented (14, 19, 20, 23, 305). It was originally expected that ARIMA methods would be favoured based on previously published use with surveillance data, versatility and available prediction intervals (14). These data show that ARIMA models were closer to achieving white noise in the residuals and improved holdout sample fit statistics in Georgia and Oregon.

Compared with automatic decomposition, this method is technically challenging, requiring significant statistical background for appropriate and accurate implementation. Regression had the poorest model R^2 results. Advantages of regression include the ease of interpretation, computation of prediction intervals, robust and bootstrapping possibilities. Therefore, this technique should not be ruled out for risk forecasting of campylobacteriosis. For all three methods, MSE and MAPE were comparable and indicate accurate forecasting. However, fit statistics for decomposition were uniformly better than the other two methods across states. Furthermore, constant 95% prediction intervals can be manually calculated for decomposition to demonstrate a range in predicted risk values.

A specific strength of automatic decomposition is that it produced accurate monthly campylobacteriosis risk predictions for all three states. A unique characteristic of this technique is that it can be taught to public health officials with minimal statistical background. By combining accuracy with ease of use, improvements in epidemic preparation and timely intervention are attainable at state, regional and national levels (22).

The distinct seasonal pattern of campylobacteriosis may suggest climatic or environmental links to the risk of disease (264, 265, 268). Climate affects the survival and reproduction of *Campylobacter* sp. in the environment and on food sources and previous studies have shown that climatic factors influence disease incidence over time (11-13, 288). Hartnack et al. found significant cross correlations between human incidence, monthly temperature and rainfall (17). The study showed that peak

prevalence in human campylobacteriosis preceded that in German broiler flocks, further implicating environmental versus food borne components to disease risk. Seasonal risk variation may also be due to human behavioural factors such as picnics, barbeques and other outdoor activities (8, 294). Such behaviours may vary depending on the climatic and socioeconomic constraints of a geographic region. The timing of seasonal peaks in our study was comparable across states. This was in contrast to a recent study in Scotland which showed that the prominence of seasonal peak in incidence varied regionally (265). However, both studies demonstrated differences in the shape of the seasonal curve by region or state. Future studies are needed to elucidate the impact of these factors on disease risk by dividing states into unique climatic zones for time series analysis using environmental variables specific to different geographic regions.

Considerable variation was observed in validation data R^2 results across models and states. This may be a reflection of the model's predictive accuracy, shifts in disease patterns or reflect irregular values or outliers in the dataset. In Oregon and Minnesota, aberrant risk values were evident in the year 2006 seasonal peaks (Figure 2.4). These data were not flagged by control charting and were not smoothed prior to the analysis. The presence of outliers, change points or interventions can alter patterns and invalidate forecasts. We believe our results would have improved in these states had the year 2006 followed the typical seasonal curvature. Secondly, surveillance systems can underestimate actual disease risk, and reporting may vary between states. As a result, predictions based on surveillance data should be interpreted with caution.

Over the past twenty years active modern surveillance systems have been implemented in developed nations that offer more accurate statistical prediction capacity than was previously possible (22, 293). Risk data from surveillance systems can be modelled as a means of assessing associations between disease risk and epidemiological factors over time (17, 22). Detecting aberrant disease incidence can signal an impending epidemic (19). Currently, advanced software offers forecasting methods that are applicable for use by public health officials (14, 20, 292). These statistical computing techniques allow interdependence of observations in both time and space to be incorporated into epidemiological models. As a result the temporal structure of risk data may assist epidemiologists in modelling biological, environmental and behavioural factors of disease with greater accuracy than the classical one-dimensional regression framework (306). As demonstrated in this study, these techniques may provide health officials with practical, user friendly and accurate predictive warning systems based solely on previous risk data (20). The models can be implemented and validated monthly for the practical purpose of predicting the risk of campylobacteriosis. This information may be useful for public health professionals in early epidemic alert systems as well as add to our knowledge of seasonal disease patterns over time.

CHAPTER 3

The importance of climatic factors and outliers in predicting regional monthly campylobacteriosis risk in Georgia, USA

This chapter is a manuscript that will be submitted for publication in the International Journal of Biometeorology. The authors include: J. Weisent[1*] W. Seaver[2] A. Odoi[1] B. Rohrbach[1]

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My contribution to the paper includes conception of the research idea and review of the literature, data preparation and analysis, interpretation of results, drafting and editing the manuscript.

1.0 ABSTRACT

Incidence of *Campylobacter* infection exhibits a strong seasonal component and regional variations in temperate regions. Forecasting the risk of infection regionally may provide clues to identify direct and indirect sources of transmission affected by temperature and precipitation in different geographic areas. The objectives of this study were to (i) assess temporal patterns and differences in campylobacteriosis risk among nine climatic divisions of Georgia, USA (ii) compare univariate forecasting models with those that incorporate exogenous climate variables and (iii) investigate alternatives to supposedly random walk series and non-random occurrences that could be outliers or interventions.

Methods: Temporal patterns of campylobacteriosis risk in Georgia, USA were visually and statistically assessed. Univariate and multivariable forecasting models (which included temperature and precipitation) were used to predict the risk of campylobacteriosis and the coefficient of determination (R^2) was used for evaluating

training (1999 to 2007) and holdout (2008) samples. Statistical control charting and rolling holdout time periods were investigated to better understand the effect of outliers and improve results of divisional time series forecasts.

Results: Both state and division level campylobacteriosis risk exhibited seasonal patterns with peaks occurring between June and August, and there were significant regional associations between campylobacteriosis risk, precipitation and temperature. State and combination level forecasts were better than divisions, and forecast models that included climate variables were comparable to univariate methods. While rolling holdout techniques did not improve predictive ability, statistical control charting identified high risk time periods in each division that require further investigation. These findings are important in (i) determining how climatic factors affect environmental sources and reservoirs of *Campylobacter* spp. and (ii) identifying regional spikes in the risk of human *Campylobacter* infection and their underlying causes.

2.0 INTRODUCTION

Campylobacter has been identified as a leading cause of human gastroenteritis in developed nations (1, 2) with an estimated one percent of the U.S. population infected annually (3). The bacteria are found throughout the natural environment, as well as in the gastrointestinal tracts of animals and birds (47, 178). Due to geographic diversity in land use, drinking and recreational water sources and human behaviour, establishing associations between campylobacteriosis risk and definitive environmental sources is difficult (8-10, 17, 265, 288).

The incidence of campylobacteriosis varies seasonally and geographically in temperate regions, and tends to be highest during summer months (1, 264, 268, 288). Variation in disease risk may be due to the effects of temperature and rainfall on the survival and reproduction of campylobacters in the environment or on foods sources (11-13). Disease forecasting may provide clues as to how direct and indirect sources of transmission are affected by climate variation in different geographic regions (288). Specifically, investigating associations between temperature and precipitation patterns with the incidence of human campylobacteriosis may help focus efforts to identify the sources of infection and transmission, improve the accuracy of local forecasting, and provide an early warning system to alert epidemiologists and public health officials of potential epidemics (14-17). Research has shown that the seasonal patterns in campylobacteriosis risk vary among geographically diverse regions, yet are consistent within regions over time (10, 307, 308). Investigators have modelled the effect of climate on campylobacteriosis in Europe, Canada, Australia and New Zealand (9, 10, 12, 272, 309). However, regional models that incorporate temperature and precipitation have not been investigated in the United States.

Infectious disease patterns may change or behave erratically in response to biological and anthropogenic factors. Such changes can result in outliers or interventions—non-random deviations from the typical time series pattern. Interventions can take the form of a pulse (outlier), step or ramp, decay, or signify a more complicated directional change in the series. In surveillance data, change points may indicate outbreaks, geographic variation in reporting, and policy or prevention procedural

changes. Outliers can greatly affect forecasting accuracy, and are particularly problematic for prediction when they occur near the end of a series or in the holdout (310). Control charting can be used to detect outliers, and previous interventions can be incorporated into models to understand and improve process performance, as well as improve specification in forecasting and epidemic alert systems (18, 303). In this case study, some suggestions are made to provide practical ways of dealing with outlier issues in surveillance data.

The objectives of this study were to (i) assess temporal patterns and variations in campylobacteriosis risk in Georgia's nine climatic divisions (ii) compare univariate time series forecasting models with those that incorporate exogenous climate variables (precipitation and temperature) and (iii) investigate alternatives to supposedly random walk series and non random occurrences that could be outliers or interventions. We hypothesize that regional variations in temperature and precipitation affect campylobacteriosis risk over time. Study findings provide early indications of irregularities in disease incidence and therefore guide disease control programs that are based on empirical forecast results (14, 20, 22, 23).

3.0 METHODS

3.1 Study Area

The study was conducted in the state of Georgia which covers over 54,000 square miles and is characterized by a humid subtropical climate that varies greatly with topography (sea level to over 4,700 feet in elevation). Approximately 114 cm (40 inches) of rain fall in the central regions and 180 cm (75 inches) fall annually in the northeast

mountains (311). Summers are hot and humid with an average daily temperature close to 90°F (32°C) and winters tend to be mild, but are cooler in the mountains and piedmont than in the coastal and southernmost regions of the state.

Since the late 1950's the National Climatic Data Center (NCDC) has been recording and assessing temperature, precipitation and other climate indices based on geographic divisions across the United States (312). Georgia contains nine adjacent divisions (Figure 3.1.a, courtesy of the NCDC Climate Prediction Center). Division 3 had the lowest population (219,400) and Division 2, which includes Atlanta, the largest metropolitan area in the state, had the highest (3,875,000). The average population for the state of Georgia during the study years was approximately 8,828,600.

3.2 Data Sources and Preparation

Campylobacteriosis case data for the state of Georgia were obtained from the Foodborne Diseases Active Surveillance Network (FoodNet), a program implemented by The Center for Disease Control and Prevention in Atlanta, Georgia (313). A case was defined as a laboratory-confirmed stool or blood sample that tested positive for *Campylobacter.* Names of patients were deleted prior to release of data to investigators and cases identified as travel-related were removed from the dataset to ensure consistent regional risk estimates. The study was approved by The University of Tennessee Institutional Review Board.

Campylobacteriosis case data were aggregated using SAS version 9.2 into counts, by month per county, over the ten year study period (1999-2008), resulting in 120 equally spaced data points (300). *Campylobacter* case counts were then

aggregated at the division level (159 counties share exact boundaries with 9 divisions), and merged with annual NCDC division level climate data.

The measure of disease frequency used as the time series variable in this study was monthly campylobacteriosis risk. Risk was defined as the probability that an individual will develop campylobacteriosis during the study period (7). Divisional risks were created in SAS by combining county level U.S. Census Bureau annual population estimates as denominator data (301). The risk estimates were presented as the number of cases per 100,000 people, and a very small number (0.01) was uniformly added to the risk to account for a few zero values in the series.

3.3 Pattern Analysis, Cross Correlations and Outlier Identification

The divisional time series were plotted to visualise trend, seasonal patterns and potential outliers. Autocorrelation (ACF) and partial autocorrelation plots (PACF) were analyzed for seasonal, autoregressive and moving average patterns. Cross correlations were calculated between campylobacteriosis risk and precipitation and temperature for each time series. Cross correlations are a measure of similarity between X and Y as a function of a time lag (t=month) applied to Y ($Y_{t+k,}$ up to the k^{th} order). Kruskal-Wallis one-way non-parametric analysis of variance was used to verify seasonality ($P<0.05$) in a univariate way, and to identify potential outliers based on monthly medians. Control charts were also used to locate individual outliers (303). Case information on potential outliers was checked for outbreak status. While there is always a potential for outliers to be outbreak-related, outbreak reporting for campylobacteriosis surveillance data was not reliably collected for the study period.

3.4 Time series analysis

A flow chart was used to organize the time series analysis in a logical sequence (Figure 3.2). Time series regression, decomposition, Box-Jenkins Autoregressive Integrated Moving Averages (ARIMA) and Winter's Exponential Smoothing were initially used to analyse all datasets in the Number Cruncher Statistical System (NCSS) (302). Models were quantitatively evaluated for predictive ability and goodness of fit using standardized pseudo R^2, calculated as one minus the sum of the residuals squared divided by the total sum of squares:

$$R^2_{pseudo} = 1.0 - \frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{n} (y_i - \overline{y})^2}$$

The focus was then narrowed to those models with the best fit.

An automated multiplicative model decomposed the series into trend (T_t), season (S_t), cyclic (C_t) and error (E_t) components in NCSS (302) as follows:

$$Y_t = T_t \cdot S_t \cdot C_t \cdot E_t$$

Ordinary least squares regression was used to evaluate the series parameters of trend, month and year for each division. The best fitting additive time series regression model included the intercept, linear trend, month (seasonal component) and error terms shown respectively below:

$$Y_t = \beta_0 + \beta_1 x_{trend} + \sum_{i=2}^{p=12} \beta_i d_i + \varepsilon_t$$

External variables (precipitation and temperature) were entered and compared. Two and three month moving averages for precipitation were investigated for the effect of accumulated rainfall on campylobacteriosis risk. The SAS forecasting system was then used to compare models. For example, ARIMA patterns or high end outliers (interventions) were checked for significance in the model residual. For regression models, variables were retained based on model significance and significance of regression coefficients ($P<0.05$), improvement of predictive value (R^2) and lack of collinearity.

Division combination datasets were created by averaging division case counts, population data and climatic data to compare and improve upon forecasting results. The combinations included divisions 1-3, 4-6 and 7-9, and were based on proximity and topographical features (Appalachian mountain range, piedmont and lowland plains, coast and swamp) (Figure 3.1.b, courtesy of the U.S. Geological Society)(311, 314). Modeling and control charting were applied to these series as previously described.

Years spanning 1999 to 2007 were used to model each series and the year 2008 was held out of the data set for model validation, since it was the most recent, and important (310). Residual time series plots were checked for normality using the Shapiro-Wilk's goodness of fit test. The Portmanteau test was used to assess if patterns in the data had been fully extracted during the modeling process and resulted in "white noise", or random scatter, in the residuals (299).

3.5 Statistical control charting & holdout sample variation

Conservative ARIMA (0,0,0)(0,1,1) models were fit for all time series, as process control does not require precise, individual model specification (303). For these data, non-seasonal parameters were not significant. The raw residuals were divided by the root mean square error and scatterplots of the standardized residuals used to identify points that were greater than three standard deviations from zero, or "out of control" (303).

For divisions with known outliers in or near the holdout, or those with poor holdout performance, a moving holdout period that spanned the last 24 months of data (2007-2008) was constructed. Coefficient of determination (R^2) values were calculated and averaged for five holdouts with 12 month horizons (holdout months 1-12, 4-15, 7-18, 10-21 & 13-24). The training period was evaluated up to the beginning of each of the five holdout periods and the average compared with the holdout results from 2008.

4.0 RESULTS

4.1 Temporal Patterns of Campylobacteriosis Risk

The time series for Georgia campylobacteriosis risk is scaled and displayed with temperature and precipitation and shows a slight downward trend ($P<0.01$) (Figure 3.3). Monthly risks ranged from 0.236-1.191 per 100,000 (mean=0.593) (Table 3.1). Division risks were scaled for display with precipitation (Figure 3.4 a-i), and ranged from 0 (in all divisions except Division 2) to 4.97 per 100,000 (Division 3) (Table 3.1). Divisions 3, 5, and 7-9 showed no significant trend whereas Division 2 and 4 had slight downward trends ($P<0.01$) and Division 1 trended slightly upward ($P<0.05$). Autocorrelation Function Plots (not shown) indicated a strong seasonal pattern with potential

autoregressive and moving average components. The patterns were not clean, implying that there could be outliers, unidentified patterns in the series, or both. Kruskal-Wallis results verified seasonality in all series except Division 3, where the median risk between months did not vary significantly ($P>0.05$).

A consistent seasonal peak between June and August, (highest risk in July for most series) was identified in all divisions, including Georgia. In some divisions, for some years, the peak began early. For example, in the year 2000, risks were higher in May than any other year. The seasonal peak extended into September, sporadically, across divisions and years. Division 3 seasonality did not vary significantly by month, yet the June through August peak is visually evident, although less pronounced, than the other divisions.

To illustrate an example of the unique relationship between campylobacteriosis risk and precipitation, we compared Divisions 1 & 9 (Figure 3.4a & i) from January 2005 to 2008. In 2005 when precipitation levels were “normal” (drought categories in quotes determined by U.S. Drought Monitor indices (315)), risk and precipitation mirror one another in shape and magnitude. In April 2006, Georgia entered a period of “abnormally dry” weather that increased to “moderate drought” by mid-June. By the end of August drought conditions were severe in a majority of the state, and stayed dry, with ranging severity, until the end of 2008. The time series patterns of 2005 may be reflective of normal rainfall. This was followed by risk and precipitation patterns that were muted and divergent, but still cohesive, during the beginning of the drought in 2006. A distinct increase in risk is evident in 2007 that continues throughout 2008 following two years of

persistent drought conditions. This general pattern is also identified in Divisions 2, 7 & 8 (Fig. 4b, g & h) and at the state level (Figure 3.3). There is no simple visual or statistical way to confirm the exact nature of the relationship between campylobacterioisis risk and precipitation based on monthly variations in risk. However, it is possible that the relationship follows a complex cyclic or parabolic curve or is regionally dependent and requires further investigation that is beyond the scope of this study.

4.2 Cross Correlations & Outlier Identification

Cross correlations between risk and temperature were strongest at time zero, lags 1, 6 and 12 on all series (Table 3.1). Cross correlations for precipitation were weak and highly variable with the highest positive correlation for Georgia at time zero (0.31) and the highest negative correlation in Division 9 (-0.35, lag 6). The strong seasonality of campylobacteriosis risk contributed to the strength and timing of the cross correlations with temperature, and the weak correlations between risk and precipitation may be due to erratic regional patterns.

Outliers were not identified in the overall Georgia time series. Between one and two outliers were flagged in each division and combination series using Kruskal-Wallis monthly median analysis and/or control charts. All of the divisional series except Division 2 contained zero values, notably Division 3 (19/120, 15.8%), Division 6 (25/120, 20.8%), and Division 7 (18/120, 15%) (Table 3.1). However, these low-end values were not statistically significant outliers.

4.3 Forecasting Results and Comparisons

Time series regression models were significant ($P<0.05$) globally and for individual model coefficients. The best prediction results were obtained from the aggregated datasets (Georgia, then the combinations) followed by Divisions 2 & 6 (Table 3.2). Decomposition and time series regression forecasting results and holdouts were comparable (within R^2=10%) (Table 3.2), suggesting that one can forecast campylobacteriosis using a classical univariate modeling approach, or by incorporating external climate variables, with similar results. In Divisions 3, 4, 7 and combinations 1-3 and 4-6, models with external variables, inclusive of interventions showed marginal improvement over decomposition.

Temperature was significant ($P<0.05$) in univariate regression models with impact ranging from R^2=10% in Division 6, to 51% for the whole state (Table 3.2). Precipitation had a significant positive relationship with campylobacteriosis risk in Georgia, the three combinations, and Divisions 2, 3, 6, & 9. The R^2 contribution of precipitation was less than 10% when modeled alone, but improved model significance when included in multivariable models for Georgia, combination 1-3, and Divisions 2, 3, 6, & 9. The three month moving average for precipitation tested the cumulative (three month) effect of rainfall on campylobacteriosis risk and tended to decrease the predictive performance (R^2) of the divisional models by 3-10%, but improved performance for Georgia by 3% and combination 4-6 by 5%.

While the Georgia model did well on the holdout for both decomposition and time series regression, holdout R^2 was low for most divisions (R^2<40%). Decomposition models had more issues attaining white noise than did time series regression (regression models all

achieved white noise in the residuals). Most models had residuals with normal distributions ($P<0.05$).However, in some cases (Table 3.2), the presence of a few outliers hampered the achievement of normality, which may distort the regression fit and inflate significance tests.

4.4 Statistical control charting and holdout variation results

Control charting for outlier detection served two important purposes: (i) outliers were able to be incorporated into forecasting models to improve prediction and (ii) "out of control" months could be further investigated for regional outbreak potential. Control charts indicated at least one outlier in all divisions except Division 9 and Combination 7-9. The flagged observations are as follows: State of Georgia (July 2003), Division 1 (July 2007), Division 2 (June 1999), Division 3 (June 2005), Division 4 (February 1999 & January 2007), Division 5 (May 2000), Division 6 (August 2001 & June 2003), Division 7 (August 2001 & August 2003), Division 8 (December 2002), Combination 1-3 (June 2005), Combination 4-6 (May 2000 & June 2003). All outliers were high-end (data point above 3 standard deviations from zero) and most (except those in Divisions 4 & 8) occurred during the seasonal spring-summer peak. The moving holdout variation only marginally improved forecasting for Division 4, and results for Divisions 1 & 9 were worse than those from the initial 2008 holdout sample (Figure 3.5). While moving holdout trials proved unsuccessful in improving results, incorporating control charting may be useful for outbreak detection as well as improving forecasting.

5.0 DISCUSSION

Although the seasonality of campylobacteriosis has been well documented worldwide, no studies have compared univariate vs. multivariable forecasting

approaches in the United States. Our study confirmed a distinct seasonal peak in campylobacteriosis risk between June and August. However, unique variation that was consistent over time was identified among the nine divisions. Risk may differ regionally as a result of differences in sources of infection and urban verses rural residential status of individuals (9, 265). Regional differences in weather conditions, temperature and precipitation impact the surrounding environment, as well as contamination and carriage rates in animal and water reservoirs. Studies suggest that environmental sources are responsible for the nearly simultaneous increase in rates in poultry and human populations (269, 270). Similarly, the seasonal distribution patterns of campylobacters found in sewage and animal feces have been correlated with human infections (88, 94, 247, 266). In a Georgia ecological study, the highest *Campylobacter* counts were found in natural waters during the summer (99) coinciding with high summer time disease risk in our study. The role of regional factors on disease reservoirs and risk has yet to be elucidated, and it is unclear whether animals and the environment are being seasonally contaminated, re-infected or subject to natural, biological, or management-related fluctuations in *Campylobacter* over time.

In our study, models that forecast campylobacteriosis risk using temperature and precipitation were comparable to models based solely on inherent patterns in the data. However, the time series regression models that included climatic factors improved model specification, as demonstrated by attainment of white noise in the residuals. We expected climatic factors to be important as previous studies have shown these variables to influence disease risk, as well as affect the survival and reproduction of

Campylobacter spp. in the environment and on food sources (11, 12, 268, 288). Seasonal increases in sunlight hours and warmer temperatures have a direct effect on human activities that increase exposure to *Campylobacter* sources (9). Known risk factors such as consumption of untreated water, contact with farm animals (268, 269), swimming, camping, barbecuing and other outdoor recreation (261, 263) have been linked with increased seasonal risk and may also be due to changes in environmental sources of contamination (9).

5.1 Temperature

In univariate models, 10 to 50% of campylobacteriosis risk was attributable solely to temperature (at time zero). These results are consistent with previous research that links warmer temperatures with increases in campylobacteriosis risk (10, 12, 309). Tam and colleagues found that a 1^0C rise in temperature corresponded with a 5% increase in reported cases, up to 14^0C in England (268). In Sweden, mean annual temperature was associated with a slightly increased risk of campylobacteriosis (RR 1.05 [95% CI:1.03-1.07]) (212). Patrick et al. found that high temperatures four weeks prior to infection were good predictors (R^2=68%) of human incidence in Denmark (11). The timing and the extent to which temperature affects campylobacteriosis risk vary considerably worldwide. In a study that included Europe, Canada, Australia and New Zealand, only temperature increases 10-14 weeks prior to elevations in human infection were statistically significant (P=.05) (267). In an Australian study, temperatures in subtropical Brisbane were positively correlated with campylobacteriosis whereas in Adelaide, a temperate city, temperature had an inverse correlation (272). In our multivariable

models, temperature at time zero, or at the six month lag, outperformed the one month lag and improved results, suggesting that regional differences play an important role in the effect of temperature on risk. Complex interactions between climate factors such as sunlight, temperature and humidity make it difficult to assess the direct impact between different regions (11). While there is no absolute standard to quantify, or pinpoint a time frame for the importance of temperature, we were able to do so, regionally, in Georgia for the study period.

Correlations observed between temperature and disease risk may be associated with the presence of migratory birds, insects and flies, that play an important temperature-dependent role in transmission of disease (113, 117, 272). Temperature has a strong influence on carriage and contamination rates in wild birds, insects and rodents, as well as survival of *Campylobacter* in the environment (11, 70, 251). We suspect that the importance of temperature on campylobacteriosis risk may be dependent on the influence of regional environmental disease reservoirs, be they natural (i.e. water, wild animals) or anthropogenic (i.e. agriculture, land use). Further investigation is warranted to determine if the seasonal patterns identified in our study, on a regional level, provide useful insight into transmission routes, as well as pathogen and host specific interactions (308). Local public health officials must become knowledgeable about respective regional environments in order to identify and investigate important variations in risk factors for campylobacteriosis.

5.2 Precipitation

Precipitation patterns for the divisions correspond visually with campylobacteriosis risk during isolated sets of months, despite wide, erratic variations over the study period (Figure 3.4 a-i). This suggests that precipitation may be an important baseline factor and that divergent patterns are due to unknown local factors and spikes in campylobacteriosis risk. Precipitation was not strongly predictive in our study (<10% R^2). Similarly, authors of a study in 2004 found that precipitation explained only 6% of the variation in human incidence in Denmark (11). To further understand this relationship, other seasonal and regional factors and outliers that may distort or diminish the association between precipitation and campylobacteriosis risk should be investigated.

As indicated by our case study of years 2005-2009 (Division 1 & 9), climatic factors may combine to invoke a “threshold” at which the cumulative impact of summer induces an exponential biological or behavioral increase in the risk of campylobacteriosis, or causes outbreaks that are undetected by current surveillance. Our data showed an icrease in risk during drought. While rainfall has been shown to improve survival of the bacteria in the environment and in fecal pats, and increase leaching (11, 251), drought conditions may concentrate bacterial loads. In regions where summer is characterized by increased rainfall and flooding, pathogen levels are magnified as fecal material is transferred from land to surface waters (233, 250). A study in Germany found significant cross-correlations between human incidence, monthly temperature and rainfall, with peak prevalence in human campylobacteriosis

preceding the peak in broiler flocks (17). This suggests that environmental sources, as opposed to food/poultry were an important contribution to disease risk. For example, the northeast region of Georgia is well known for animal production, specifically intensive poultry farms. Therefore, understanding the regional cause of increased risk should include investigation of the animal production industry, its effects on the surrounding environment, and the populations at risk.

Results of the three month moving average for precipitation (improved predictions for Georgia as a whole vs. worsened divisional predictions) suggest that the predictive sense may be unique to each division. i.e., smoothing diluted the association between campylobacteriosis risk and precipitation at the division-level. The regional relationship between precipitation and campylobacteriosis risk may explain why studies that use data over large areas do not observe an association (10). Analogous to a magnifying lens, whereby “zooming out” (smoothing or aggregating data), conveys a broader sense of the relationship, “zooming in” captures the detail, as well as the anomalies. Public health officials should objectively and qualitatively assess time periods where campylobacteriosis risk diverges from typical regional precipitation patterns and investigate the potential causes. In doing so, decisions for control and prevention are based on empirical evidence.

5.3 Statistical Control Charts and outliers

In our study, control charts served to focus attention on specific time periods of high campylobacteriosis risk. While it may be useful to identify erratic points in an aggregated dataset (state or combination level), the division level control charts narrow

the focus of investigation. For example, May 2000 and June 2003 were flagged in the combination 4-6 series, and further identified in Divisions 5 & 6 respectively. Traditional models often incorporate smoothing, robust methods or model interventions to meet assumptions, thereby sculpting a state of statistical control. According to Alwan and Roberts, improving our ability to distinguish between special causes and common causes (in this case, for example, outbreaks vs. known seasonality) justifies the effort to identify an “out of control process” (303). Alternatively, statistical control charting can be used as a guideline to assess expected vs. observed level of risk (18) and serve to augment predictive models when patterns are erratic.

In the poorest performing divisions with outliers (Divisions 1 & 4) and without outliers (Division 9) we were unable to identify the point of tension in the series whereby the analysis could be improved or refined with a moving holdout. Excessive variation, randomness or outliers in the 24 month holdout period, holdout or horizon lengths, or a combination of these may have contributed to the lack of improvement. While outliers near the end of a series or in a holdout can have severe effects on forecasting ability and accuracy (310), incorporating moving holdouts may be impractical in the realm of public health when model improvement is not a guarantee, and the process requires expertise and can be time consuming. We recommend that these methods be reserved for time series with forecasts that are significantly improved (>10% R^2) as a result of accounting for such outliers.

6.0 Limitations

Considerable variation was observed in results among divisions and irregular patterns, randomness or outliers decreased forecasting accuracy (316). The presence of outliers, change points or interventions can alter patterns and invalidate forecasts and the effect of zero values in these series is unclear. Low or high disease counts and potential outbreaks should be verified and addressed using multiple techniques and sources of information when available. In addition, the use of external variables for forecasting increases model uncertainty. In these situations, a decision must be made as to what objectives are most important—accurate predictions or understanding biological and epidemiological patterns.

The divisional datasets illustrate potential real life dilemmas such as small number problems, unidentified outbreaks, variations and inconsistencies inherent in surveillance data. Surveillance systems tend to underestimate actual disease risk, and reporting may vary within states. As a result, predictions based on surveillance data should be interpreted with caution.

The models and divisions varied in the ability to achieve normality and/or white noise in the residuals. Lack of white noise may increase model uncertainty or indicate misspecification, as patterns in the residuals are not accounted for. However, models without perfect attainment of white noise can have valid results (291), and poor predictive performance was likely due to outliers as opposed to issues of model specification.

7.0 Conclusions

Regional climatic factors and statistical control charting improve models for predicting monthly campylobacteriosis risk, particularly when there are erratic, non-random patterns in the time series. Control charts can serve as an early warning system by detecting irregularities in campylobacteriosis risk at the division level. While forecasting on aggregated data (Georgia, or combinations of divisions) resulted in improved explanation of the variance in risk in decomposition models and multivariable time series regression, the patterns and climatic associations (temperature and precipitation) identified at the division level highlight how important information can be gleaned from finer levels of analysis. Public health officials can more readily address specific “out of control” risk estimates in a timely manner by focusing investigations on the causal factors that relate directly to their regions. Climate information can be rapidly and freely obtained, therefore assessing the baseline impact of temperature and precipitation, may be a useful “real time” alternative for those who do not have ready access to surveillance data. The descriptive information, temporally housed in empirical *Campylobacter* disease risk data may also improve our understanding of biological, environmental and behavioural drivers of disease. Furthermore, modeling and comparing campylobacteriosis patterns in regions with different environmental characteristics may help to identify regional differences in transmission routes, sources and reservoirs with greater accuracy.

CHAPTER 4

Detection of high risk campylobacteriosis clusters at three geographic levels

This chapter is a manuscript that has been published in the journal Geospatial Health (Geospat Health; 6 (1), 2011,65-76). The authors include: J. Weisent[1] B. Rohrbach[1] J.R.Dunn[2] A. Odoi[1]

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My contribution to the paper includes conception of the research idea and review of the literature, data preparation and analysis, interpretation of results, drafting and editing the manuscript.

1.0 Abstract

Background: Campylobacteriosis is a leading cause of bacterial gastroenteritis in the United States and many other developed countries. Understanding the spatial distribution of this disease and identifying high risk areas is vital to focus resources for prevention and control measures. In addition, determining the appropriate scale for geographic analysis of surveillance data is an area of concern to epidemiologists and public health officials. The purpose of the study was to (i) compare standardized risk estimates for campylobacteriosis in Tennessee over three distinct geographic scales (census tract, zip code and county subdivision), and (ii) identify and investigate high risk spatial clustering of campylobacteriosis at the three geographic scales to determine if clustering is scale dependent.

Results: Significant high risk clusters ($p<0.05$) were detected at all three spatial scales. There were overlaps in regions of high risk and clusters at all three geographic levels. At the census tract level, spatial analysis identified smaller clusters of finer resolution

and detected more clusters than the other two levels. However, data aggregation at zip code or county subdivision yielded similar findings.

Conclusions: The importance of this line of research is to create a framework whereby economically efficient disease control strategies become more attainable through improved geographic precision and risk detection. Accurate identification of disease clusters for campylobacteriosis can enable public health personnel to focus scarce resources towards prevention and control programs on the most at risk populations. Consistent results at multiple spatial levels highlight the robustness of the geospatial techniques utilized in this study. Furthermore, analyses at the zip code and county subdivision levels can be useful when address level information (finer resolution data) are not available. These procedures may also be used to help identify regionally specific risk factors for campylobacteriosis.

2.0 Introduction

Campylobacter are motile, spiral shaped, gram negative bacteria found ubiquitously in the environment (47, 178). The organisms have been identified as a leading cause of human gastroenteritis in developed nations (1, 2). *Campylobacter* is estimated to cause illness in over 1,300,000 people in the United States annually resulting in approximately 13,000 hospitalizations (317). *Campylobacter* species are found in a wide variety of sources and can be isolated from foods, sewage and water. As a result, causative associations between sporadic human infections and environmental sources are difficult to ascertain (4). The risk of disease has been linked

to a complex set of determinants, including geographic region, climate patterns, human behavior, and recreational water and land use (8-10). While the incidence of campylobacteriosis varies seasonally and geographically, the reasons behind this variation are not clearly understood (264, 307). In the United States, Foodborne Diseases Active Surveillance Network (FoodNet) identifies marked geographic variations in the incidence of *Campylobacter* infection (24). Incidence also differs considerably between countries based on health care seeking behavior and laboratory culturing practices (184). In 2009, the U.S. incidence of *Campylobacter* infection was 13.0 per 100,000 population, with a rate in Tennessee of 8.2 cases per 100,000 population(24). Despite the lower overall incidence, Tennessee public health officials seek a better understanding of why and where this disease is occurring. Studies have been performed in Denmark, Norway and Canada to characterize spatial patterns of campylobacteriosis (8, 225, 318). For example, Green and colleagues (2006) detected the highest incidence in rural and agricultural regions with high densities of farm animals. The use of Geographic Information Systems (GIS) and spatial statistics to identify high risk regions serves to support and expand current knowledge on the mechanisms of transmission and geographic risk factors for this disease. Furthermore, geospatial tools provide epidemiologists and public health officials with a starting point from which to assess potential geographic associations and risk factors.

A major goal in the application of spatial statistics is to make meaningful inferences from available geographic data (26, 27). For this reason, understanding the impact of spatial scale on the assessment of health outcomes is a topic of great

importance. A number of studies exist which utilize cluster detection and mapping techniques to identify high risk regions for infectious diseases (31-33). Recent studies have also compared techniques for ecological analyses and employed a variety of geographic scales for understanding health outcomes and disparities (29, 319, 320). However, few studies compare the differences in spatial patterns of disease at more than one level. Tian et al. (2010) explored the impact of geographic scale on breast cancer mortality among ethnic groups in Texas and found that the location of racial disparities changed depending on geographic level. This suggests that risk factors play different roles at different levels of aggregation. Odoi et al. (2003) compared giardiasis rates in Ontario Canada at two levels. Results showed spatial clustering of high rates at the smaller Census Sub-division scale, but not at the Census Division. Results of similar studies vary and there is no consensus on what geographic scale is most appropriate, precise or useful for surveillance data (28-30).

Despite inconsistency encountered in the literature, the choice of spatial scale may be crucial when investigating spatial patterns of disease. This choice is subject to the modifiable areal unit problem (MAUP) as well as the potential for ecological fallacy as described by Openshaw (321). The MAUP applies to potential problems in both spatial scaling and zoning whereby conclusions and inferences may differ depending on the spatial level of analysis and type of areal divisions. From a public health standpoint, the utility of surveillance data is oftentimes limited by the level to which it can be aggregated and analyzed. Consistent analytical results over multiple geographic scales serve to validate spatial statistical techniques as well as the reliability of the data used

for a given study area. To our knowledge, no studies to date have been conducted to explore the direct impact of geographic scale on results of spatial cluster investigation using aggregated campylobacteriosis data.

3.0 MATERIALS & METHODS

3.1 Study Area & Data Sources

The study area encompassed the entire U.S. state of Tennessee which has a land area 66,332.5 square kilometers and is the 36th largest out of 50 states. The estimated population in the year 2000 was 5,689,276 (approximately 63 people per square kilometer) (301). Campylobacteriosis surveillance data covering the period from September 1, 1991 to December 31, 2008 were provided by the Tennessee Department of Health. A case of campylobacteriosis was defined as culture-confirmed infection from a clinical specimen, typically stool or blood. Species and strain information were not reported. Participating laboratories are part of both a national and state-wide active surveillance system(24). This study received The University of Tennessee Institution Review Board approval, and all cases (n=4,723) were de-identified to maintain anonymity and confidentiality.

Cartographic boundary files were obtained at the census tract (n=1261), zip code (n=612) and county subdivision spatial levels (n=462) from the U.S. Census Bureau Tiger Files (2000)(322). These three spatial units are delineated as ‘statistical geographic entities’ by the U.S Census Bureau. Census tracts are the smallest units used in this study. They typically contain between 2,500 and 8,000 people and are

relatively homogeneous with respect to population characteristics and socioeconomic conditions. The 5 digit zip code areas are categories used by the U.S. Postal Service to group mailing addresses. Cartographic boundaries, specifically zip codes boundaries and numbers are subject to change over time. Unlike census tracts and county subdivisions, which are both subunits of counties, zip codes may cross county boundaries and occasionally cross state lines. For instance, Tennessee contains one small zip code (42223, area142.4 km^2), located on the northern, central border which crosses the state boundary with Kentucky. All population denominator data, including the U.S. standard population used for risk standardization were obtained from the year 2000 U.S. Census.

3.2 Geocoding and Data Aggregation

The complete Tennessee dataset consisted of 4,723 campylobacteriosis cases. Initial data exploration for errors, repeat specimens, normality and outliers, as well as preparation and formatting for geocoding, was performed in SAS version 9.2 (300). A total of 130 (2.75%) of the cases were identified as travel-related, 40 (0.85%) which lacked address information, and 24 (0.51%) repeat specimens were excluded during the cleaning and geocoding process. To allow for age and sex standardization, an additional 4 cases with missing sex information and 135 with missing age information were excluded from future analysis. A total of 967 (20.5%) cases were excluded from the dataset. From a geographical standpoint, these included 128 (2.7%) cases that contained information only at county level, 164 (3.5%) cases with only zip code level

information, 228 cases (4.8%) containing only Post Office Box numbers and 244 cases (5.2%) with addresses that were not geocodable.

Geocoding was performed using both Googlemaps (323) and Yahoo Maps Geocoder through BatchGeo (324). An iterative process was undertaken to accurately match location data to the lowest level, or finest possible scale. This included manually matching address data to its exact latitude and longitude. The dataset utilized in the final analysis included 3,756 cases with 2,638 cases at rooftop accuracy and 1,118 cases at street level accuracy. An observation coded to the 'rooftop' is precisely matched to its address on the ground. The 'street level', also referred to as 'range interpolated' accuracy, matched the case to an interpolated point on the road where the address would be located if the exact street number were unavailable. Each point was represented as a latitude and longitude coordinate and mapped in ArcGIS (325). A point-in-polygon join was then used to merge the campylobacteriosis data to the census tract, zip code and county subdivision cartographic boundary files.

To assess for systematic differences in spatial distribution of cases retained in the dataset and those excluded, the observations in the unused portion of the dataset, with the exception of the 40 cases lacking location information, were mapped separately to the highest available accuracy level and visualized. No visual difference in spatial distribution was detected between the dataset used for analysis and the observations that were eliminated. By maintaining only the cases at the lowest, most accurate level of geocoding (rooftop and street), we sought to maximize the strength of the relationship between the three aggregate geographic levels and disease risk (326).

3.3 Standardization

Campylobacteriosis typically demonstrates a bimodal age distribution (peaks occurring in children under 5 years of age and young adults), and it occurs more frequently in males (1, 76). In addition, gender specific incidence has been shown to vary considerably among a wide variety of age groups (122). To account for these differences in risk, the population was grouped by sex and subdivided into six age categories: 0-4, 5-19, 20-39, 40-59, 60-74, 75 and older. Age and sex standardization was then performed at all three geographic levels using STATA version 9.0 (327) with adjusted risk estimates presented as number of cases per 100,000 population. The estimates were classified into five categories using Jenk's optimization method, a common statistical technique available within ArcMap (325) which creates cut points based on inherent patterns in the data.

3.4 Spatial Analysis

3.4.1 Smoothing

Visualization of disease patterns can be distorted by spatial autocorrelation and excessively high variances often encountered in geographic areas with low disease counts and/or low background populations. To allow for better visualization, spatial empirical Bayesian (SEB) smoothing technique was implemented in GeoDa version 095i (328) using counts obtained by the standardization procedure. The SEB smoothing was used to adjust for spatial autocorrelation and mitigate the small numbers problem (329). The raw risk data were smoothed at each of the spatial levels, using first order Queen spatial weights. In keeping with Tobler's first law of geography (330), Queen

weighting gives greater influence to regions directly surrounding each areal unit of analysis. The SEB smoothing technique thereby maintains the integrity of the risk distribution without over-smoothing or creating a homogenous surface (26, 27).

3.4.2 Cluster Detection

Cluster detection was performed using Kulldorff's spatial scan statistic, implemented in SaTScan version 8.2.1 (331). The Poisson probability model was fit and estimates adjusted for sex and age in the analysis. Statistical significance was assessed at p-value <0.05 and performed using 999 Monte Carlo iterations. Scanning parameters included circular clusters of high risk only, with no geographic overlap. There is a lack of comprehensive knowledge as to effect of the scanning window size on disease cluster results. For this study, the maximum scanning window size incorporated 3% of the total population at risk and was chosen *a priori* with consideration to the low infectivity of the bacteria, geographic area and units of study. Window sizes of 5% and 10% were run post hoc to explore how cluster results vary by window size at the three geographic levels.

4.0 RESULTS

4.1 Risk estimates and smoothing

County subdivision campylobacteriosis raw risk estimates ranged from 0 (n=89) to 498 per 100,000 population (median=55.0). Zip code area estimates ranged from 0 (n=168) to 4,254 per 100,000 population (median=49.8). Census tract risk estimates were highly variable, ranging from 0 (n=241, median=53.8) to 13,122 per 100,000 persons, (raw risk maps not presented). Spatial empirical Bayesian smoothed maps

(Figures 4.1-3) demonstrate similar regions of elevated risk for the three spatial levels with the finest detail captured at the census tract level. From a broad perspective, these areas include the east Tennessee valley surrounding Knoxville, Cookeville and the region south of metropolitan Nashville-Davidson, in the central portion of the state. These regions are most clearly visualized at the county subdivision level (Figure 4.1), due to a combination of large size polygons and tighter range in risk estimates. The highest risk class category for both zip code and census tract spatial levels (Figures 4.2 & 4.3) contained outliers after smoothing (as detected by box plots, not shown). These included one small urban zip code area (37902) located beside the Tennessee River within Knoxville city limits (Figure 4.2 c) and the two highest census tract level risks located in the regions of Center Point (smoothed risk 12,808.5 per 100,000) and Philadelphia (smoothed risk 6196.7 per 100,000) as indicated in dark purple (Figure 4.3 a-d).

4.2 Cluster detection

According to cluster detection results, the high risk areas ($p<0.05$) surround Knoxville, Maynardville and Jefferson City, south of metropolitan Nashville-Davidson, Cookeville, Murfreesboro, Shelbyville, Franklin and Oak Ridge (Figure 4.4 a-c & Table 4.1). Regions east and west of the city of Knoxville were detected as clusters at all three spatial scales. Shelbyville, Kingsport and Lawrenceburg were identified as unique, singular, census tract clusters (Table 4.1) with very high relative risks (12.1, 5.8, 9.0 respectively). The Kingsport and Lawrenceburg areas were only identified as high risk clusters at the census tract level, with Kingsport being too small for visual identification

on the state map. In comparison, Kingsport ranked 29th and Lawrenceburg ranked 19th highest out of the 1261 tracts from the census tract unsmoothed, standardized risk results. The other single tract cluster is represented by the city of Shelbyville (ranked 12th highest in risk). The Shelbyville area is included as part of a larger cluster identified in zip code and county subdivision analyses.

An overlay map of the clusters (Figure 4.5 a-c) shows that the high risk clusters occur at consistently similar locations around Cookeville, east Knoxville and south Murfreesboro at all three spatial levels (labels excluded to aid visualization). The Franklin area, seen at the top most, western cluster contains a comparable overlay at the zip code and census tract levels, but not county subdivision. Other differences can be visualized in the easternmost cluster (Cluster 3, Table 4.1). At the zip code level this cluster encompasses a larger region of high risk, north and west of Oak Ridge (Figure 4.5). Furthermore, county subdivision analysis uniquely detected the Philadelphia area (Cluster 5, Table 4.1).

4.3 Alternate scanning window analysis

The larger scanning window sizes of 5% and 10% resulted in clusters covering similar regions, with some variation, at all three levels. As an example, consecutively larger cluster sizes (incorporation of greater number of areal units) were found at the county subdivision level as the window size was increased (Figure 4.6 a-c). Similar overall expansion of cluster size was found at the census tract and zip code levels (maps not shown). Regardless of the scan window size, the Lawrenceburg and Kingsport regions were not identified as clusters in the county subdivision analysis.

5.0 DISCUSSION

The results of both the smoothing and spatial scan analyses show the general regions surrounding Knoxville and Cookeville, and south of metropolitan Nashville-Davidson to be at high risk for campylobacteriosis in Tennessee, at all three spatial levels. Previous research suggests that the risk of acquiring campylobacteriosis may be higher in different areas due to the presence of different risk factors (8, 225, 318). For example, in metropolitan areas such as Knoxville, one might investigate centralized sources of contaminated poultry products. In rural areas exposure to domestic farm animals and local environmental sources might be of greater importance (33, 225). Addressing the impact of socioeconomic factors and quantifying potential health inequalities between advantaged and disadvantaged groups could be applied to high risk areas across the state. The specific reasons for the geographic differences found in this study are unknown and should be investigated further.

The fact that all three levels share comparable geographic clustering is a unique strength of this study. Other studies performing spatial analyses at more than one spatial scale demonstrate differences in patterns of health outcomes and associations across spatial scales (29, 321, 332). This may be a result of the MAUP, data errors and missing data, as well as variation in either the disease data or the population and ecological level data (26, 29, 30). While spatial variation in risk exists in some regions, these results highlight the overall similarity in spatial patterns detected between geographic levels using clustering and smoothing techniques simultaneously. Our study demonstrates that consistent results across spatial scales occur despite the inherent

limitations encountered in ecological analyses. While the results are specific to campylobacteriosis in Tennessee, these findings may lend credence to studies that utilize disease surveillance data at only one level (8, 225, 333).

The lower level (census tract) analysis produces the finest detail and is able to capture small, yet potentially important areas of high risk. This conclusion is supported by previous studies which found small area studies to be the most useful for measuring health disparities and reducing ecological bias (26, 332, 334). Where possible, analyses should be conducted at more than one level to assess the full spatial picture. This is particularly important for validating results from finer scale levels where positional inaccuracy can be more pronounced and lead to decreased precision (28). Furthermore, socioeconomic, demographic and environmental data are often unavailable at finer scales, and the process of geocoding can be costly and time consuming. When census tract analyses are either undesirable or not feasible, the zip code or county subdivision aggregations may provide sufficient information for cluster detection and prevention and control of campylobacteriosis in Tennessee.

In a recent comparison of cluster detection software methods, SaTScan was found to be a fast, user-friendly and well-developed for cluster detection (335). The spatial scan statistic allows the window to vary during the scan process. This methodology decreases the chance of pre-selection bias as clusters may be detected without prior knowledge of geographic extent (336). The fact that size, shape and number of clusters changed in our study when the scan window size was enlarged is a complication seen with use of this method. Understanding the implications of scanning

window size on clustering is beyond the scope of this paper. However, recent studies focus on result variability dependent on scan window size (333, 337). Further exploration into both the theoretical and practical implications of scan window choice would add to the growing body of knowledge on the spatial scan statistic. Consecutive analyses can lead to improved understanding of *Campylobacter* distribution over time and space (318). In our study, we interpret the SEB smoothed risk distribution in conjunction with cluster analysis at three levels to offer a more comprehensive visual and statistical approach to understanding campylobacteriosis disease patterns.

Cluster detection results suggest that environmental and/or socioeconomic factors contribute to disease transmission. A model-based approach may be applied to campylobacteriosis surveillance data as a means of assessing potential associations between disease risk and epidemiological factors (27, 225). Furthermore, mapping risk and cluster analysis results on all three levels with similar findings serves to statistically validate the location of high risk regions in Tennessee. Previous ecological studies have shown that associations between risk factors and disease may be dependent on the level of aggregation (33, 332). Modeling at more than one spatial level, therefore, serves to enhance current knowledge of disease patterns and provide a more accurate link between important disease predictors and a constantly changing environment. Temporal variations in campylobacteriosis risk may also be important in understanding transmission routes and changing distribution patterns (8, 318). In this study, time components were not examined, as partitioning the surveillance data into temporal units would result in insufficient data for meaningful analysis.

A number of factors may play a role in the accuracy and usefulness of this study. Many ill persons do not seek care, have a stool culture performed, or have the etiology identified. In addition, data reporting may vary and the impact of using census year 2000 as denominator data for cases spanning 17 years is not known. Underreporting is expected for campylobacteriosis as the disease is typically self limiting and very rarely fatal. These data include cases ascertained by active surveillance, and are thought to accurately represent the burden of culture-confirmed campylobacteriosis (317). Approximately 20% of the case data for this study were eliminated due to missing information or data address inaccuracy and the effect of this loss is difficult to quantify. The spatial distribution of cases with missing data was similar to those whose data were complete, implying that the missing data were randomly distributed. Misclassification of cases may also occur and may be a result of data inaccuracies, human migration and changes in geographic boundaries (26, 28). Furthermore, a large number of cases arising from a common source may result in a regional elevation in disease risk that distorts the location of clustering. Improved identification and documentation of cases due to an outbreak would help to eliminate this potential source of bias as well as add to our knowledge of the characteristics of the population at risk. Recently, FoodNet began collecting data on cases being outbreak-associated. This information was not available for all years analyzed. These inherent limitations add uncertainty and potential bias to spatial analysis research.

Patterns of campylobacteriosis may change over time in response to changing human and animal demographics as well as shifts in climatic factors and land use. Sequential analyses are warranted to improve our understanding of the disease. Our research is intended to enhance current knowledge of the human campylobacteriosis distribution and risk, and to create a foundation upon which ecological associations, including socioeconomic and environmental factors, can be superimposed on clusters to identify risk factors. Technological advances in statistical and mapping software and the growing availability of surveillance data justify continual review of spatial methods, the levels at which these methods are applied, and their applicability to public health.

6.0 Conclusions

These data demonstrate an overlap in spatial clustering of campylobacteriosis across three geographic scales in Tennessee. Some variation in the size and shape of clusters was present. However, the overall disease patterns were similar, leading to improved confidence in comparisons between levels. This finding is especially important in situations where address data are unavailable, making finer scale analyses impossible. Identification and superimposition of regional-specific risk factors would help generate hypotheses for the spatial differences in campylobacteriosis risk identified in these analyses. Visual comparison of smoothed risk estimates and cluster detection at multiple spatial levels has the potential to help public health officials effectively identify geographic, socioeconomic and environmental factors which may play an important role in the occurrence of campylobacteriosis. Subsequently, this knowledge could be used to

create a framework whereby future disease control strategies become more geographically precise and economically efficient.

CHAPTER 5

Socioeconomic determinants of geographic disparities in campylobacteriosis risk: a comparison of global and local modeling approaches

This chapter is a manuscript that has been published in the International Journal of Health Geographics (*Int. J. Health Geographics* (2012),11(1):45). The authors include: J. Weisent[1], B. Rohrbach[1], J.R.Dunn[2], A. Odoi[1*]

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My contribution to the paper includes conception of the research idea and review of the literature, data preparation and analysis, interpretation of results, drafting and editing the manuscript.

1.0 Abstract

Background: Socioeconomic factors play a complex role in determining the risk of campylobacteriosis. Understanding the spatial interplay between these factors and disease risk can guide disease control programs. Historically, Poisson and negative binomial models have been used to investigate determinants of geographic disparities in risk. Spatial regression models, which allow modeling of spatial effects, have been used to improve these modeling efforts. Geographically weighted regression (GWR) takes this a step further by estimating local regression coefficients, thereby allowing estimations of associations that vary in space. These recent approaches increase our understanding of how geography influences the associations between determinants and disease. Therefore the objectives of this study were to: (i) identify socioeconomic determinants of the geographic disparities of campylobacteriosis risk (ii) investigate if regression coefficients for the associations between socioeconomic factors and campylobacteriosis risk demonstrate spatial variability and (iii) compare the

performance of four modeling approaches: negative binomial, spatial lag, global and local Poisson GWR.

Methods: Negative binomial, spatial lag, global and local Poisson GWR modeling techniques were used to investigate associations between socioeconomic factors and geographic disparities in campylobacteriosis risk. The best fitting models were identified and compared.

Results: Two competing four variable models (Models 1 & 2) were identified. Significant variables included race, unemployment rate, education attainment, urbanicity, and divorce rate. Local Poisson GWR had the best fit and showed evidence of spatially varying regression coefficients.

Conclusions: The international significance of this work is that it highlights the inadequacy of global regression strategies that estimate one parameter per independent variable, and therefore mask the true relationships between dependent and independent variables. Since local GWR estimate a regression coefficient for each location, it reveals the geographic differences in the associations. This implies that a factor may be an important determinant in some locations and not others. Incorporating this into health planning ensures that a needs-based, rather than a “one-size-fits-all”, approach is used. Thus, adding local GWR to the epidemiologist’s toolbox allows them to assess how the impacts of different determinants vary by geography. This knowledge is critical for resource allocation in disease control programs.

2.0 Introduction

Campylobacter organisms are leading causes of human gastroenteritis in developed nations, affecting an estimated 13 million people in the United States annually (1, 2). Campylobacteriosis risk (defined as the probability that an individual will develop campylobacteriosis within a given time period (7)) is known to vary by geographic regions, climate patterns, human behaviors, and food and water sources (8-10). The Foodborne Diseases Active Surveillance Network (FoodNet) has reported substantial geographic variations in the risk of *Campylobacter* infections across the U.S.(24)(24,50) Currently, the reasons for these variations are unknown. Although the overall risk of disease in the U.S. population is 13 cases per 100,000, some areas in Tennessee have risks as high as 200 cases per 100,000 (25). Therefore, there is interest in identifying the determinants of the geographic disparities seen in disease risk so as to guide disease control efforts.

Understanding health disparities among racial, ethnic and across socioeconomic subgroups is a priority of public health personnel and policy makers (34, 35). In developed countries, the relationships between socioeconomic status (SES) and campylobacteriosis risk are complex and have been shown to vary geographically. For example, disease risks were found to be significantly lower in rural compared to urban areas in several studies (36-38). This is possibly due to continued exposure in rural areas, resulting in sustained immunity and hence lower disease incidence (79). However, in Manitoba, Canada the risk for young children was seven times higher in rural regions than in the City of Winnipeg (225). In a New Zealand study, urban adults

and rural children had higher rates of disease, signifying an age based disparity between these areas (36). The geographic differences in associations between SES factors and campylobacteriosis risk are a worldwide phenomenon, hence regression models need to account for the fact that regression coefficients, used to assess associations between risk factors and disease, might vary in space. Local modeling approaches enable investigators to more accurately estimate the true relationships between determinants and disease risk since they estimate regression coefficients for each location in the study area (39-41).

Geographically weighted regression (GWR) modeling techniques compute local regression coefficients thereby allowing the estimates of the associations between outcome and explanatory variables to vary spatially (42, 43). This flexible modeling strategy is necessary to improve our understanding of the determinants of geographic disparities of campylobacteriosis risk internationally. Recent interest in exploring geographic variation in the associations between socioeconomic factors and health outcomes has spurred studies in the US, UK and Taiwan. For example, local variations were detected in the occurrence of diseases such as obesity and breast cancer when modeled against socioeconomic factors (44-46). In these and other studies, global modeling approaches, that estimate one regression coefficient for each variable in the model, hide local variations in associations (40, 41). Since a local GWR model estimates a regression coefficient of an explanatory variable for each location in the study area (42), it allows health professionals to better assess how the effect of the explanatory variable changes by geographic location. Armed with this knowledge,

health planners can better identify the most important disease determinants for different regions and therefore better plan health programs, provision of services and resource allocation to meet the unique needs of different communities. Thus, this helps health professionals avoid using a one-size-fits-all approach but instead use empirical evidence provided by the local GWR models, to practice needs-based population health planning, enabling them to provide services based on the unique health needs of the different populations they serve. Thus the objectives of this study were to: (i) identify socioeconomic determinants of the geographic disparities of campylobacteriosis risk at the census tract level (ii) investigate whether regression coefficients for the associations between socioeconomic factors and campylobacteriosis risk demonstrate spatial variability and (iii) compare the performance of negative binomial, spatial lag and global and local Poisson GWR models.

3.0 MATERIALS & METHODS

3. 1 Study Area and Data Sources

The study was conducted in the state of Tennessee which consists of 1,261 census tracts, with an estimated total population of 5.7 million in 2000 (301). Census tracts are 'statistical geographic entities' which typically contain between 2,500 and 8,000 people and are relatively homogeneous with respect to population characteristics and socioeconomic conditions (301).

Cartographic boundary files, population denominator data, including the U.S. standard population used for risk standardization, and socioeconomic variables were obtained, at the census tract level, from the year 2000 U.S. Census (301). Campylobacteriosis data were collected through the FoodNet active surveillance system (313) and obtained from

the Tennessee Department of Health. These data covered the period from September 1, 1991 to December 31, 2008. Cases of campylobacteriosis were defined as culture-confirmed *Campylobacter* infections from clinical specimens, the majority of which were stool samples. Culture results were reported at the genus level only. Names of patients were deleted from the database before it was released to investigators. The study was approved by Tennessee Department of Health and The University of Tennessee Institution Review Boards.

3.2 Geocoding and Data Aggregation

The dataset consisted of a total of 4,723 confirmed campylobacteriosis cases reported during the study period. Initial data exploration, preparation and formatting for geocoding, was performed in SAS version 9.2 (300). Cases that could not be geocoded for various reasons (see Table 5.1) or that were due to infections acquired outside of the study area (travel-related) were excluded from the dataset.

An iterative geocoding process was performed to accurately match location data to the finest possible geographic scale using both Googlemaps (323) and Yahoo Maps Geocoder through BatchGeo (324). Dot maps of the final dataset were generated in ArcGIS (325) and included 3,756 cases: 2,638 (70%) rooftop accuracy and 1,118 (30%) street level accuracy. The geocoding was classified as ‘rooftop’ accuracy when there was a 100% match to the address. The ‘street level’, also known as ‘range interpolated’ accuracy, referred to instances when interpolation was used to identify address location along a street. This was done in cases where the exact street address number was

unavailable in the geocoding database. The campylobacteriosis data were then aggregated to the census tract level for subsequent analyses.

3.3 Computation of Campylobactriosis Risk, Smoothing and Mapping.

The measure of disease frequency used as the dependent variable in this study was the campylobacteriosis risk. Campylobacteriosis risk was defined as the probability that an individual will develop campylobacteriosis within a given time period (7). For this study, campylobateriosis risk was computed as the number of campylobacteriosis cases reported in a census tract during the study period divided by the population of the census tract. However, due to the potential confounding effect of age and sex, campylobacterisos risk was age and sex standardized using STATA version 9.0 (327). This ensured that differences in geographic distribution of campylobacteriosis risks observed were not due to geographical differences in the distribution of age and/or sex of the population. Socioeconomic variables from the 2000 U.S. Census, at the census tract level, were then merged to the age and sex standardized risk estimates (301) and spatial empirical Bayes smoothing was performed in GeoDa version 095i (328). Jenk's optimization classification method was used to determine critical intervals for spatial display of maps in ArcView (325). The unsmoothed campylobacteriosis risk estimates and the socioeconomic variables of interest were then assessed for spatial autocorrelation at the census tract level using Global Moran's I in GeoDa (328).

3.4 Regression Analysis

3.4.1 Univariate Regression Analysis

Race and ethnicity variables investigated for potential association with geographic distribution of campylobacteriosis risk were the proportion of the population that were black, white, Asian, Chinese, Hispanic/Latino, and Native American/Alaskan. Employment related variables included the proportion of the population that were unemployed, and those whose occupations were in the farming, fishing, forestry or service industries. Variables related to marital status included the proportion of the population that was divorced, never married, separated, or widowed. Educational attainment factors included the proportion of the population with no high school diploma, and those with a bachelor's or graduate degree. Other socioeconomic variables of investigated were: the proportion of the population living in rural verses urban areas, those in the armed services, those who were disabled, living in poverty or those on public assistance.

Pairwise Spearman rank correlation coefficients were computed to identify highly correlated explanatory/independent variables. Using a cut-off Spearman rank correlation coefficient of 0.6, only one of a pair of highly correlated variables (i.e. with $r \geq 0.6$) was retained for further investigation. Univariate (or simple) ordinary Poisson models were fit to the data using the generalized linear model procedure, PROC GENMOD, in SAS (300). The dependent variable specified in the model was the number of campylobacteriosis cases reported in each census tract and the census tract population was specified as the offset variable. An assessment of overdispersion

revealed significant overdispersion of the univariate (simple) ordinary Poisson models implying that the ordinary Poisson models were inappropriate for the data. Therefore, negative binomial models were used for all subsequent multivariable modeling and final comparisons with other modeling approaches investigated in the study.

3.4.2 Multivariable Regression Analyses

3.4.2.1 Negative Binomial Models

As for the ordinary Poisson models, the dependent variables specified in the multivariable negative binomial models were the number of campylobacterisis cases reported in each census tract and the offset variable was the census tract population. The regression equation for the negative binomial model is:

$$\ln(\lambda) = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n$$

Where λ is E(Y)/n, Y is the dependent variable, the βs are parameter estimates (regression coefficients) and the Xs are the socio-economic variables under investigation.

Initial multivariable negative binomial model building was performed using the forward stepwise selection procedure in SAS, using likelihood ratio tests to assess significance of variables in the model. Throughout the selection procedure, previously removed variables remained eligible for reentry into the model, provided they were statistically significant ($p<0.05$) and improved AIC by three or more points. Only one of a pair of highly correlated variables, such as black and white race, and rural or urban locale were entered into the model in order to avoid issues of collinearity.

McHenry's All Possible selection method in NCSS (302) in conjunction with the SAS model comparison macro, %genmodsummary (300) were used to identify the two most parsimonious multivariable models. Two-way interaction terms on the variables included in the main effects models were assessed for significance and model improvement. Residual diagnostics were performed by investigating for outliers (using standardized Pearson's residuals) and influential points (using Cook's Distance). Both the raw and standardized Pearson's residuals were also assessed for spatial autocorrelation using Global Moran's I (328).

3.4.2.2 Spatial Lag Models

To account for spatial autocorrelation in the residuals (identified in the negative binomial models), a spatial lag model was fit to the data in GeoDa (328), specifying the log transformed campylobacterisis risk as the dependent variable. The equation for the spatial lag model is:

$$Y = \rho WY + \beta_o + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n$$

Where Y is the dependent variable (log transformed campylobacteriosis risk), ρ is the spatial autoregressive coefficient of the spatial lag model, W is the spatial weight, WY is the spatial lag for the dependent variable, the βs are parameter estimates (regression coefficients) and the Xs are the socio-economic variables under investigation. A significant ρ of this model means presence of significant spatial autocorrelation of the dependent variable implying that a non-spatial model (such as the negative binomial model) is inappropriate for the data. The autoregressive coefficient is also an estimate of the degree of spatial autocorrelation present in the data. Using the queen definition of

neighborhood contiguity, a correlogram was constructed to identify the most optimal spatial weight. The queen spatial weights assessed were from 1st to 5th order, with each weight construction including lower orders. For instance, 2nd order queen weights included both 1st and 2nd order neighbors and 3rd order weights included 1st, 2nd and 3rd order neighbors in the construction of the spatial weight. The 3rd order Queen weight resulted in the best model fit and eliminated residual spatial autocorrelation. Since the campylobacteriosis risk and the proportion of the population that were black required log transformation, a factor of 1.1 was added to all records of these variables to deal with zero values.

Model residuals were assessed for normality (using Jarque-Bera and White tests), homoskedasticity (using Breusch-Pagan test) and residual spatial autocorrelation (using Moran's I). Additionally, the statistical significance of the spatial autoregressive coefficient was assessed using the likelihood ratio test.

3.4.2.3 Global and Local Poisson Geographically Weighted (GWR) Models

Global models estimate one coefficient per explanatory variable, averaged over all locations, whereas local Geographically Weighted Regression (GWR) models estimate as many coefficients as the number of locations (in this case, census tracts) in the dataset. The equation for the local GWR model is:

$$Y_i(\boldsymbol{u}) = \beta_{0i}(\boldsymbol{u}) + \beta_{1i}(\boldsymbol{u})X_{1i} + \beta_{2i}(\boldsymbol{u})X_{2i} + \cdots + \beta_{ni}(\boldsymbol{u})X_{ni}$$

Where the $\beta_{ni}(\mathbf{u})$ are regression coefficients for the relationship between an explanatory variable and the dependent variable around a location **u** and is therefore unique to that location while the Xs are the different explanatory/independent variables included in the model.

The local GWR model allows the investigator to compute a different regression coefficient for each location when assessing the relationship between the dependent and independent variable. Thus, assuming a causal relationship, it enables the investigator to assess how the impact of a specific risk factor on the outcome changes by location. The Poisson distribution within the GWR framework is currently the most appropriate available strategy for analyzing areal disease counts, especially when low numbers are involved (42, 43). As in the negative binomial models, the dependent variables for both the global and local Poisson GWR models were the number of cases of campylobacteriosis in a census tract and the offset variable was population of the census tract. Both models were fit in a specialized spatial statistical software called GWR (338).

For the local Poisson GWR, the adaptive kernel method was chosen to account for differences in the density of census tracts across the study area. Accommodating irregularly shaped census tracts is particularly important, as shapes, sizes and density varies widely between metropolitan and rural regions. The kernel varies the size of the analysis window so as to incorporate the same number of census tracts in each local estimate. In each local regression analysis a zero weight value is applied to all other census tracts not included in the analysis window. A manual iterative approach identified 300 nearest neighbors (census tracts) as the optimal model bandwidth based on AIC (43, 338)..

To demonstrate spatial variability in the association between campylobacteriosis risk and the explanatory variables, the estimated regression coefficients from the local

GWR were displayed as choropleth maps using Jenk's optimization classification scheme. Assessment of goodness–of-fit of the negative binomial, spatial lag, global and local Poisson GWR models was done using Akaike's Information Criterion (AIC).

4.0 RESULTS

4.1 Spatial distribution of campylobacteriosis risk and socioeconomic factors

Age and sex adjusted campylobacteriosis risk estimates varied widely, ranging from 0 (n=241, median=53.8) to 13,122 per 100,000 population. The spatial empirical Bayes smoothed map of campylobacteriosis risk showed evidence of geographic disparities in risk across the study area (Figure 4.3, adopted from our previous study published in Geospatial Health;6(1):65-76, used with permission). Areas of high campylobacteriosis risk centered around the cities of Knoxville, Cookeville, south of metropolitan Nashville-Davidson and north of Chattanooga (25).

Summary statistics of the selected socioeconomic factors showed that the study area is predominantly inhabited by whites followed by black, Hispanic/Latino and Asian residents (Table 5.2). In one census tract up to 100% of the population was reported to be living in poverty and approximately 50% had no high school diploma. Spatial distributions of the socioeconomic factors under investigation showed evidence of spatial clustering based on the statistically significant (p=0.001) Moran's I statistics (Figure 5.1). Census tracts in and around Memphis had high proportions of black population (Figure 5.1a), whereas rural census tracts tended to have higher proportions of the population with no high school diploma (Figure 5.1b).

Significant positive correlations were found between the proportion of the population that were black and those that were never married ($r=0.73$; $p<0.001$) or that were separated ($r=0.61$; $p<0.001$) (Table 5.2). As expected, the proportion of the census tract population employed in the agricultural industry was highly correlated with the proportion of the population living in rural regions ($r=0.78$; $p<0.001$). Census tracts with a high proportion of those with a bachelor's degree tended to have lower proportions of those who were living in poverty ($r=-0.61$; $p<0.001$) and those with no high school diploma ($r=-0.79$; $p<0.001$).

4.2 Socioeconomic determinants of geographic distribution of campylobacteriosis risk.

The majority of the socioeconomic factors investigated for univariate (simple) associations with campylobacteriosis risk had highly significant ($p<0.001$) associations with campylobactriosis risk (Table 5.3). Due to the presence of significant overdispersion (implying that ordinary Poisson models were inappropriate for these data) and the fact that the negative binomial models fit the data better than the ordinary Poisson models (as evidenced by the lower AICs of the negative binomial models), only the results of the negative binomial models are presented. Presence of significant overdispersion implies that the ordinary Poisson models are inappropriate and therefore it was unnecessary to present the results thereof.

McHenry's All Possible variable selection, used to identify the most optimal parsimonious models, revealed an exponential drop and stabilization of the root mean square error with four variables in the model (Figure 5.2). This implies that the most

optimal and parsimonious models were those that had four explanatory variables. Thus, the final competing models included four explanatory variables. Models 1 & 2 had the best fit as evidenced by the fact that they had the lowest AICs. Therefore, these models were used to compare the 4 modeling approaches (negative binomial, spatial lag, global GWR and local GWR) investigated in this study. Both negative binomial models 1 and 2 showed that the risk of campylobacteriosis tended to be lower in census tracts that had higher proportions of blacks and unemployed populations (Table 5.5). However, campylobacteriosis risks were significantly higher in census tracts that had high proportions of the population with no high school diploma (Table 5.5). Additionally, in Model 1, census tracts with high proportions of the population living in urban areas tended to have higher risk of campylobacteriosis, whereas in Model 2, the risk of campylobacteriosis was lower in census tracts with high divorce rate.

Assessment of Pearson's standardized residuals from the negative binomial models showed evidence of residual positive spatial autocorrelation (Moran's I:0.083, p=0.001). This implies that, although the negative binomial model was better than the ordinary Poisson model, it still has residual spatial autocorrelation and thus did not totally eliminate spatial autocorrelation. The presence of significant spatial autocorrelation in the residuals requires use of an appropriate spatial model.

4.3 Comparison of the Modeling Approaches

Based the AIC goodness-of-fit statistic for comparing models, the model with the lowest AIC statistic is the one with the best model fit. Using this criterion to compare the 4 modeling approaches used to fit model 1, the local Poisson GWR model had the best

fit (AIC=3344) followed by the global Poisson GWR (AIC=4854), spatial lag (AIC=4908) and lastly negative binomial model (AIC=5962). A similar pattern was observed for model 2 with local Poisson GWR again having the best fit (AIC=3244), followed again by global GWR (AIC=4860), then spatial lag (AIC=4914) and finally negative binomial (AIC=5960). The overall best fitting model is local GWR model 2 which had the lowest AIC of 3244.

It is important to stress that both local Poisson GWR models 1 and 2 showed evidence of non-stationarity of all the regression coefficients. This is evidenced by the fact that the interquartile ranges of the local regression coefficients were all larger than twice the standard errors of the regression coefficients of the global Poisson GWR model (Table 5.5). This implies that the regression coefficients of each of the variables included in the local GWR models were not constant but changed across the census tracts in the study area. The implication of this is that, the strength of the associations between campylobacteriosis risk and each of the explanatory variables vary depending on the spatial location. Thus, assuming a causal relationship, the effects of the determinants are not constant across the study area but are heavily dependent on the geographical location (338).

The spatial autoregressive coefficients (ρ) of both the spatial lag model 1 ($\rho=0.622$; $p<0.001$) and spatial lag model 2 ($\rho=0.655$; $p<0.001$) were significantly greater than 0 confirming the fact that there was significant spatial clustering in the data. Statistical significance of the autoregressive coefficients also imply that the non-spatial models (i.e. the negative binomial models are inappropriate for these data). This

conclusion is further supported by the results of the assessment of the goodness-of-fit of the models which revealed that the negative binomial models had the worst fit.

The spatial patterns of the local GWR regression coefficients of the explanatory variables common to models 1 and 2 are shown in Figures 5.3 and 5.4, respectively, and the statistical evidence of their non-stationarity is shown on Table 5.5. On both local Poisson GWR models 1 and 2, campylobacteriosis risk tended to be higher in census tracts that had high proportions of individuals with no high school diploma and these census tracts were mainly located in southeast Tennessee. Areas where lack of high school diploma had the strongest positive association with campylobacteriosis, risk tended to have relatively high proportions of black population (18.4%). By contrast, areas where lack of high school education had the strongest negative association with campylobacterisis risk had relatively few blacks (only 8.4%). Moreover, areas where lack of high school diploma had the strongest positive association with campylobacteriosis risk tended to have a low (29%) proportion of the population living in rural areas (i.e. they were mainly urban areas). On the other hand, areas where lack of high school diploma had a negative association with campylobacteriosis risk tended to be more rural with as high as 61% of the population living in rural areas. Thus, assuming a causal relationship, it appears that low education has a more marked impact on increasing risk of campylobacteriosis in the urban than in the rural areas. It is also worth noting that that areas where lack of high school diploma had the strongest positive association (strong risk factor) tended to have relatively high mean income

($46,000) compared to those where it had the strongest negative association with campylobacteriosis risk where the mean income was $40,300.

The strongest positive association between high unemployment rate and campylobacteriosis risk was observed in the western third of the state (Figures 5.3c & 5.4c). These areas tended to be in the rural having, on average, 62% of the population living in rural areas. On the other hand, areas where high unemployment rate had a negative association with campylobacteriosis risk tended to be urban with only 36.9% of the population living in rural areas.

In some urban centers, such as Memphis and Knoxville, and their surrounding areas, campylobacteriosis risk was high, whereas in other urban centers, such as Nashville, the risk was low. With regard to the geographic disparities in distribution of the association between campylobacteriosis risk and urbanicity, the areas with the strongest positive association (Figure 5.3d) between urbanicity and campylobacteriosis risk tended to have a relatively higher percentage of black (38.9%) compared to areas which had the strongest negative association between urbanicity and campylobacteriosis risk (Figure 5.3d) that had only 2.5% of black population.

Areas that had the strongest positive association between campylobacteriosis risk and divorce rate (Figure 5.4d) tended to be in the rural where, on average, 75.1% of the population lived in rural areas. By contrast, areas that had the strongest negative association between campylobacterisis risk and divorce rate tended to be urban where only an average of 34.4% of the population was rural.

5.0 DISCUSSION

Although past studies have investigated associations between socioeconomic factors and campylobacteriosis risk and others have reported that campylobacteriosis risk varies geographically (25, 339), to our knowledge, no studies have used local GWR approaches to investigate the geographic variations of the association between campylobacteriosis risk and socioeconomic factors. Thus, the current study is, in part, intended to fill this knowledge gap. The modeling approaches used in this study (i.e. local GWR) are novel and provide powerful tools to epidemiological investigations and should therefore be applied to many diseases throughout the world. Although local GWR models offer insight into socioeconomic risk factors and their complex relationships with health outcomes not many studies have used them. We believe that these spatial variations in regression coefficients need to be investigated to ensure that appropriate disease control programs are used regardless of the disease of interest and the geographical areas concerned.

The global multivariable models in our study showed that census tracts with high proportions of the population that were black, unemployed and divorced tended to consistently have a lower risk of campylobacteriosis, whereas those with high proportions of the population living in urban areas, and with no high school diploma had a higher risk of campylobacteriosis. In contrast, local Poisson GWR models revealed a diverse range of regression coefficients for the associations between campylobacteriosis risk and the socioeconomic determinants across the study area. Thus, since the regression coefficients of the determinants ranged from negative to

positive over the study area, global models are inaccurate and unreliable. This complex spatial heterogeneity in the associations between socioeconomic factors and campylobacteriosis risk explains: (i) why local Poisson GWR models outperformed negative binomial, spatial lag and global Poisson GWR models and (ii) how global models mask the true nature of the relationships between determinants and campylobacteriosis risk. These findings imply that the strength of association between a determinant and disease changes by location and this needs to be factored in disease control programs since a factor may be a more important determinant of disease in some areas and not others.

Local Poisson GWR results identified spatial patterns for some of the spatially varying coefficients in this study. For instance, positive associations were observed between high campylobacteriosis risk and urbanicity in areas that tended to have low education attainment and high proportion of blacks. Similarly, areas which had positive association between high campylobacteriosis risk and high divorce and unemployment rates tended to be rural. These patterns support the hypothesis that the reasons for the differences in campylobacteriosis risk vary geographically across the study area. In fact, several studies have reported that variation in the risk of campylobacteriosis may be due to regional differences in the distribution of socioeconomic risk factors, as well as unknown or underlying regional characteristics (8, 225, 318, 339). For example, wealthy and highly educated populations might acquire campylobacteriosis through exposure to undercooked foods in restaurants or contaminated outdoor environments while vacationing, whereas populations living in poverty or with lower levels of education

attainment may be exposed through poor food handling at home. Regional variation in how underlying socioeconomic characteristics influence the parameter estimates of high risk areas warrant further investigation at a local level. Suffice it to say that the global models do not provide the true nature of the relationships which sometimes varies from negative association in some areas to positive associations in others. This has practical implications in disease control because a one-size-fits-all strategy (which would be used if results of global models are used) is not appropriate since local Poisson GWR reveals that certain determinants may be more important in some areas than others. Thus, health planning and service provision need to use a needs-based approach based on empirical data such as these.

The fact that areas where lack of high school diploma had the strongest positive association with disease risk tended to have relatively high mean income could be due to the income disparities between the urban and rural areas where the urban populations tend to earn slightly more than the very rural areas. On the other hand, the fact that areas where lack of high school diploma had a negative association with campylobacteriosis risk tended to be rural seems to suggest that low level of education may have a higher impact on risk of campylobacteriosis in urban than rural areas probably due to higher cost of living in the more urban centers that would potentially force low education and low income population to have much poorer living conditions in the cities than in the rural areas. Poorer living conditions would then inevitable increase the risk of campylobacter infections in these populations. The observed lower risk of campylobacteriosis in census tracts with high proportions of blacks and unemployed

population might be a reflection of under reporting rather than a lower disease burden in these communities.

The association between campylobacteriosis risk and the proportion of the census tract population living in urban areas showed the largest spatial variation, as evidenced by the coefficient range (-0.453 to 0.932) across the study area. Campylobacteriosis risk factors have been shown to differ between rural and urban environments due to different direct and indirect exposure opportunities (37). Typical rural exposures include poultry and farm animals, unpasteurized milk and contaminated surface waters (120, 212, 228, 234). Yet exposure to *Campylobacter* infection by poultry and farm animals is not limited to rural areas, as animal products are processed and distributed at varying distances from their source, and contamination may result from processing plants located in urban areas. This source of environmental contamination from the food industry is an underlying risk factor which should be investigated locally in rural and urban regions.

In our study, local Poisson GWR models had better statistical fit (lower AIC's) than the global models investigated. These findings are similar to those of several other studies that have compared the performance of local GWR and global regression methods in investigating associations between disease and risk factors (39, 40, 45, 340). For example, after modeling determinants of drug resistant tuberculosis, Liu et al. reported that the local GWR model had a much better fit (AIC=395) than the global regression model (AIC=471). Moreover, the GWR model had an increase of over 15% in explaining the variation in the outcome. Gilbert and Chakraborty and Cheng et al.

reported spatial variability in regression coefficients and found improvements of over 10% in R^2, and decreases in AIC (over 70 units), respectively, for local compared to global models (40, 340). Although local GWR modeling is relatively new, their results convincingly indicate that local spatial characteristics can have a profound effect on regression coefficients and statistical significance of variables (341). Geographic disparities in risk should therefore be investigated at local levels to: (i) capture regional differences in the nature of the relationship between risk factors and disease outcome, (ii) avoid misleading inferences and conclusions from global models, and (iii) better inform disease control programs.

One of the primary goals of the U.S. Department of Health and Human Services is to eliminate health disparities associated with socioeconomic status and geographic location (342). Accurate measurement and reporting of health disparities has important implications for decision-making and policy implementation at a local, national and international level (343). Quantifying the effects of socioeconomic factors should be prioritized and approached in an interdisciplinary and collaborative manner using methodologically sound techniques (34, 41). Therefore, advanced analytical techniques such as GWR, which incorporate geography into epidemiological studies in novel ways, need to be more widely accessible to researchers and epidemiologists globally.

The complexity of the relationship between geography and socioeconomic status creates a difficult task for public health professionals. Health disparities are likely to change empirically as societal conditions change over time and space (35). Populations move and become diverse, altering social and ethnic demographics and disease

patterns (34). Local GWR modeling strategies address these issues more efficiently by helping to identify differences in the strengths of association between determinants and health outcomes across areas (41). By adopting strategies that target known high risk socioeconomic groups, limited and precious resources can be more efficiently allocated and policy and planning can better target regional public health needs.

Although approximately 20% of the disease data in this study were eliminated due to missing information or inaccuracy of residential addresses, the spatial distribution of cases with missing data was similar to those whose data were complete. This suggests that the missing data were randomly distributed, and therefore not likely to have biased the results of our study. The impact of using U.S. Census year 2000 denominator data for cases spanning 17 years is not known, however, the 2000 census offered the best representation of both population and socioeconomic data available at the time of the study.

Suffice it to say that the local GWR methods used in this study are quite novel and would significantly add to the spatial epidemiologist's toolbox when investigating determinants of geographical disparities of health outcomes. Thus, although the specific results of this study may not be generalizable to other regions in the world, the methods used and results obtained are eye openers to spatial epidemiologists across the globe that deeper insights are obtained when local GWR models are used to investigate determinants of health since the magnitude of the impact of determinants vary by geographical location. This is important information that can be used by health planners and service providers to ensure that resources are better allocated to improve health

outcomes. There is no doubt that these tools need to be incorporated in routine investigations by epidemiologists and decision makers interested in addressing issues related to health disparities so as to improve health outcomes for all.

6.0 Conclusion

The international significance of the findings from this work is that they highlight the fact that global regression strategies, frequently used to investigate determinants of geographic disparities in disease distribution, generally tend to mask the true nature of the relationship between the outcome and explanatory variables. Since local GWR models estimate a regression coefficient for each location in a study area, they are able to more powerfully reveal the geographic differences in the associations between the explanatory variables and the outcome/disease. Thus, the information obtained provides critical empirical evidence to health planners and public health professionals to guide health planning and disease control programs. Since the regression coefficients change based on geographical location, it implies that a determinant of disease may be a more important risk factor in one location and not other locations. Incorporating this information in health planning and service provision ensures that health professions do not use a “one-size-fits-all” approach but instead the planning and provision of services would be guided by the needs of the areas as evidenced by the local regression coefficients of specific disease determinants. Thus, local GWR regression models should be an important addition to the toolbox of public health epidemiologists globally. This tool would allow them to assess how the impact of different determinants of disease outcomes vary by geographical location which information would greatly

improve the decision making process in relation to allocation of resources for disease control programs.

CHAPTER 6
Summary and Recommendations

1.0 Time Series Investigations

Despite the high incidence of campylobacteriosis in the United States, the time series investigations herein are the first, to our knowledge, that compare forecast modeling strategies for campylobacteriosis risk in this country. We show how surveillance data can be used to accurately predict monthly campylobacteriosis risk at the state level, as well as assess regional associations between climatic factors and disease risk over time. Specifically, decomposition models were able to reliably predict monthly disease risk in Minnesota, Oregon and Georgia for up to one year in advance. The temporal structure of risk data can therefore assist epidemiologists in anticipating high risk time periods and understanding the relationships between determinants of disease and campylobacteriosis.

Although the seasonality of campylobacteriosis is well documented, worldwide, no studies have compared the utility of univariate and multivariable forecasting approaches. Our investigation confirmed a distinct seasonal peak in campylobacteriosis risk between June and August in Georgia and identified individual variation that was consistent over time among the nine climate divisions. Regional differences in weather conditions, temperature and precipitation impact the surrounding environment, as well as contamination and carriage rates in animal and water reservoirs. Strong correlations observed in our study between temperature and disease risk may be associated with the presence of temperature-dependent transmission routes such as migratory birds, insects and flies. It is unclear whether animals and the environment are being seasonally re-infected or subject to natural, biological, or management-related

fluctuations in *Campylobacter* over time, and the role of regional factors on campylobacteriosis reservoirs and risk has yet to be elucidated. Further investigation is warranted to determine if the seasonal patterns identified in our study, on a regional level, provide useful insight into transmission routes, as well as pathogen and host specific interactions. Local public health officials must become knowledgeable about their respective environments in order to identify and investigate important variations in risk factors for campylobacteriosis.

Box Jenkins ARIMA control charts served to augment division level predictive models by focussing attention on specific temporal high points in campylobacteriosis risk. Control charting on the combination data sets supported division level results by identifying outliers in common. Statistical control charting can be used as a guideline to assess expected vs. observed level of campylobacteriosis risk and alert public health officials by identifying erratic patterns and interventions.

In our study, models that forecast campylobacteriosis risk using temperature and precipitation were comparable to models based solely on inherent patterns in the data. In univariate models, 10 to 50% of campylobacteriosis risk was attributable solely to temperature. In multivariable models, temperature at time zero, or at the six month lag produced stronger models than the one month lag found in studies from different areas of the world. This suggests that geographic differences play an important role in the effect of temperature on risk. These differences may be a result of seasonal increases in sunlight hours and warmer temperatures that subsequently increase human exposure to *Campylobacter* sources. However, complex interactions between climate factors such

as sunlight, temperature and humidity make it difficult to assess the direct impact between regions, and while there is no absolute standard to quantify the importance of temperature, we were able to do so in Georgia.

Precipitation patterns for Georgia's climate divisions correspond visually with campylobacteriosis risk during isolated sets of months over the study period. Therefore, precipitation may be important as a baseline climatic factor. Risk patterns that diverge from precipitation patterns over time may be a function of unknown local causes or weather-related spikes in campylobacteriosis risk. For example, a drought that occurred during the study period coincided with a seasonal spike in the risk of campylobacterisosis. This indicates that the cumulative impact of drought and other seasonal factors may induce a biological or behavioral-based increase in disease risk, or cause outbreaks that are undetected by surveillance systems.

Regional variation may be the key to unlocking and understanding the complex relationships between climatic factors such as temperature and precipitation on campylobacteriosis risk. The variation we observed between divisions in Georgia suggests that these factors may influence regional environmental disease reservoirs, be they natural (i.e. water, wild animals) or anthropogenic (i.e. agriculture, land use). Furthermore, environmental sources, as opposed to food/poultry may be an important contribution to campylobacteriosis risk in regions of intensive animal production, such as northeast Georgia. Understanding the cause of regional increases in risk should include investigation of the animal and poultry production industry and its effects on the surrounding environment, and populations at risk. We recommend that public health

officials be familiar with specific risk factors that are linked to regional environments. This would allow for objective and qualitative assessments on why and when atypical patterns of high risk campylobacteriosis occur.

2.0 Spatial Investigations

Identifying “hot spots,” or clusters of high risk, is a topic of international interest in the field of health geographics, as it allows public health officials to focus prevention and control efforts on regions with the highest population at risk for disease. Our spatial clustering results identified regions of high risk for campylobacteriosis surrounding Knoxville and Cookeville, and south of metropolitan Nashville-Davidson in Tennessee. The risk of acquiring campylobacteriosis may be higher in these areas due to the presence of different risk factors such as centralized sources of contaminated poultry products, exposure to domestic farm animals or unknown local environmental reservoirs of disease. This is the only investigation, to our knowledge, that validated the results of clustering at more than one spatial level, i.e. census tract, zip code and county subdivision. The fact that all three levels share clusters of high risk is a unique strength of this study, serving to statistically validate the location of high risk regions in Tennessee.

The lower level (census tract) analysis produced the finest detail and is able to capture small, yet potentially important areas of high campylobacteriosis risk in Tenessee. We recommend that where possible, analyses be conducted at more than one level to assess the full impact of the spatial picture. Furthermore, socioeconomic, demographic and environmental data are often unavailable at finer scales, and

geocoding (necessary for census tract analysis) can be costly and time consuming. When fine scale analyses are either undesirable or not feasible, our findings suggest that the zip code or county subdivision aggregations may provide sufficient cluster detection results for implementation of campylobacteriosis prevention and control measures in Tennessee.

Patterns of campylobacteriosis may change over time in response to changing human and animal demographics as well as shifts in climatic factors and land use. Sequential analyses are warranted to improve our understanding of the spatial distribution of this disease. Our research enhances current knowledge of the distribution of human campylobacteriosis risk in Tennessee, and offers an ecological framework upon which socioeconomic and environmental risk factors can be identified and tested.

To our knowledge, no studies have used local geographically weight regression approaches to investigate the geographic variations of the association between campylobacteriosis risk and socioeconomic factors. The global multivariable models in our second spatial investigation showed that census tracts with high proportions of the population that were black, unemployed and divorced tended to consistently have a lower risk of campylobacteriosis, whereas those with high proportions of the population living in urban areas, and with no high school diploma had a higher risk of campylobacteriosis. In contrast, local Poisson GWR models revealed a diverse range of regression coefficients for the associations between campylobacteriosis risk and the socioeconomic determinants across the study area. Thus, since the regression coefficients of the determinants ranged from negative to positive over the study area,

global models are inaccurate and unreliable. This complex spatial heterogeneity in the associations between socioeconomic factors and campylobacteriosis risk explains: (i) why local Poisson GWR models outperformed negative binomial, spatial lag and global Poisson GWR models and (ii) how global models mask the true nature of the relationships between determinants and campylobacteriosis risk. These findings imply that the strength of association between a determinant and disease changes by location and this needs to be factored in disease control programs.

Local Poisson GWR results identified positive associations in Tennessee between high campylobacteriosis risk and urbanicity in areas that tended to have low education attainment and high proportion of blacks. Similarly, areas which had positive association between high campylobacteriosis risk and high divorce and unemployment rates tended to be rural. The association between campylobacteriosis risk and the proportion of the census tract population living in urban areas showed the largest spatial variation. These patterns support the hypothesis that the reasons for the differences in campylobacteriosis risk vary geographically across the study area. Wealthy and highly educated populations might acquire campylobacteriosis through exposure to undercooked foods in restaurants or contaminated outdoor environments while vacationing, whereas populations living in poverty or with lower levels of education attainment may be exposed through poor food handling at home. Campylobacteriosis risk factors have also been shown to differ between rural and urban environments due to different direct and indirect exposure opportunities and typical rural exposures include poultry and farm animals, unpasteurized milk and contaminated surface waters.

Regional variation in how underlying socioeconomic characteristics influence the parameter estimates of high risk areas warrant further investigation at a local level in rural and urban regions.

In our study, local Poisson GWR models had better statistical fit (lower AIC's) than the global models investigated. Although local GWR modeling is relatively new, the results convincingly indicate that local spatial characteristics can have a profound effect on regression coefficients and statistical significance of variables. Geographic disparities in risk should therefore be investigated at local levels to: (i) capture regional differences in the nature of the relationship between risk factors and disease outcome, (ii) avoid misleading inferences and conclusions from global models, and (iii) better inform disease control programs.

Health disparities are likely to change empirically as societal conditions and populations change over time and space, altering social and ethnic demographics and disease patterns. When the nature of a relationship varies from negative association in some areas to positive associations in others disease control strategies must be tailored, geographically, to fulfill the goal of needs-based health planning. By adopting strategies that target known high risk socioeconomic groups, limited resources can be more efficiently allocated and policy and planning can better target regional public health needs.

A number of factors may play a role in the accuracy and usefulness of these studies. Many ill persons do not seek care, have a stool culture performed, or have the etiology identified. In addition, data reporting may vary and the impact of linking census

information to campylobacteriosis risk is not known. Underreporting is expected, as campylobacteriosis is typically self limiting and very rarely fatal, therefore predictions and associations based on surveillance data should be interpreted with caution. The presence of outliers, change points or interventions can alter patterns and invalidate models. Furthermore, a large number of cases arising from a common source may result in a regional elevation in disease risk that may distort temporal and spatial modeling results. Improved identification and documentation of cases due to an outbreak (a strategy recently implemented by FoodNet) would help to eliminate this potential source of bias as well as add to our knowledge of the characteristics of the population at risk.

Accurate measurement and reporting of health disparities has important implications for decision-making and policy implementation at a local, national and international level, and constitutes a primary goal of the U.S. Department of Health and Human Services. Over the past 20 years developed nations have implemented modern active surveillance systems that offer more accurate modeling and prediction capacity than was previously possible. Epidemiological and statistical software has advanced considerably, in line with our changing world. Specifically, advances in the utility and accessibility of temporal analysis tools broaden the investigative capacity of epidemiologists and public health officials in the field. SaTScan, a fast, user-friendly and well-developed cluster detection technique further adds to the growing body of knowledge on geographic distributions of disease. Similarly, local GWR approaches are novel, and we believe that the spatial variations in regression coefficients need to be

investigated. Advanced interdisciplinary analytical techniques that incorporate temporal and geographic parameters into epidemiological studies in novel ways, need to be more widely accessible to researchers and epidemiologists.

Conclusions

Forecasting campylobacteriois risk in Georgia using inherent temporal patterns and incorporating regional climatic factors improves our understanding of biological, environmental and behavioural drivers of disease. The addition of statistical control charting augments predictive models when erratic, non random patterns exist and serves as an early warning system at the division level. Forecasting on the state level improved our explanation of the variance in campylobacterioisis risk in decomposition and multivariable time series regression models. However, the patterns and climatic associations identified by division level analyses highlight how important information can be gleaned at finer levels of investigation. Public health officials can more readily address specific “out of control” risk estimates in a timely manner by focusing on the causal factors that relate directly to their regions. Furthermore, climate and weather information can be rapidly and freely obtained, and assessing the baseline impact of temperature and precipitation, in real time, may be a useful alternative for those who do not have ready access to surveillance data. Modeling and comparing campylobacteriosis time series patterns in regions with different environmental characteristics is an important step towards identifying transmission routes, sources and reservoirs with greater accuracy.

The overlap in spatial clustering of campylobacteriosis across three geographic scales in Tennessee improved our confidence in hot spot detection and attests to the validity of comparing between levels. This finding is especially important in situations where address data are unavailable, making finer scale analyses impossible. Identification and superimposition of regional-specific risk factors would help generate hypotheses for the spatial differences in campylobacteriosis risk identified in these analyses. Subsequently, this knowledge could be used to create a framework whereby future disease control strategies become more geographically precise and economically efficient.

Global regression strategies frequently used to investigate determinants of geographic disparities in disease distribution, generally tend to mask the true nature of the relationship between the outcome and explanatory variables. Since local GWR models estimate a regression coefficient for each location in a study area, they are able to more powerfully reveal the geographic differences in the associations between the explanatory variables and the disease. Thus, local GWR regression models provide a tool to understanding the true impact of health inequalities and improving the allocation of resources for disease control programs.

The level at which an investigation is conducted dictates the sense of the relationship identified between disease risk and temporal and spatial variables. A critical decision must be made *a priori*, as to the chosen level of study, based upon the practicality of predictive models, epidemiological and biological information and the importance of capturing minute details and anomalies. In a recent commentary,

epidemiologist R.S. Bhopal stated that “the absence of both clarity in conceptual reasoning and high-quality data are twin obstacles, arguably making the task [ethical and just policy] impossible” (344). By adopting more focused regional goals, public health officials can better identify the most important disease determinants, plan unique health programs, allot resources and provide timely and cost-effective services based on high risk populations.

A common thread that weaves these four diverse studies together resides at the interface between statistical and epidemiological modeling, technological advances in computing and software and practical issues of public health and policy. Every aspect of our planet and our lives are changing, in some cases exponentially. Our investigations into the temporal and spatial patterns of campylobacteriosis are a microcosmic glimpse into how epidemiological modeling can provide novel, accurate and quantifiable results that serve as an impetus for local and global change. The research community, government and non-government health organizations, worldwide, can no longer afford to observe the realm of public health through a narrow lens, but must embrace the tools and the mindset that address health disparities from a multidimensional and interdisciplinary perspective.

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Appendices

Appendix A: Tables

Table 2.1. Time series model comparisons for campylobacteriosis risk per 100,000 persons in GA (1999 to 2007), OR and MN (1998 to 2007).

State	Model	R^2, PRED*	R^2Holdout**	MSE	Normality	WN (adequate lags)	MAPE
	Regression	0.693, 0.602	0.733	0.014	Yes	No (5-7)	0.162
Georgia	**ARIMA(1,0,0)(0,1,1)**	0.655	0.757	0.016	No-close	Yes	0.197
	Decomposition	0.727	0.662	0.011	Yes	No (1-2)	0.147
	Regression	0.710, 0.633	0.588	0.073	Yes	No (28 on)	0.154
Oregon	**ARIMA(1,0,0)(0,1,1)**	0.724	0.620	0.070	No-2 off	Yes	0.177
	Decomposition	0.763	0.526	0.053	Yes	No	0.145
	Regression	0.835, 0.792	0.682	0.089	Yes	No	0.194
Minnesota	**ARIMA(1,0,0)(0,1,1)**	0.841	0.599	0.107	No-close	Yes-All except lag 1	0.219
	Decomposition	0.899	0.799	0.049	Yes	No (1,4,7-11)	0.156

* PRED is a prediction R^2 value (sometimes referred to as a Press R^2).
This statistic is used to internally validate the regression model using jackknife techniques.
** R^2 of 2008 validation sample
Mean square error (MSE)
White Noise (WN)
Mean Absolute Percent Error (MAPE)

Table 3.1 Descriptive statistics and cross correlations (temperature and precipitation) for monthly campylobacteriosis risk estimates per 100,000 population in Georgia, climate divisions 1-9, and combined divisions 1-3, 4-6 and 7-9**.**

Area Division/ combination	**Risk Range** per 100,000		**Mean Risk** (Standard Deviation)		**Zero values** (# in dataset)	**Trend** (P<0.05)	**[1]Cross Correlations**				
							Temperature				**Precipitation**
							Lag: 0	Lag:1	Lag:6	Lag:12	Lag, CC
Georgia	0.24	1.19	0.59	(0.20)	None	Down	0.71	0.57	-0.72	0.69	0, 0.31
1	0.00	2.97	0.78	(0.50)	2*	Up	0.44	0.47	-0.48	0.46	4, -0.15
2	0.13	1.38	0.63	(0.24)	None	Down	0.53	0.41	-0.51	0.52	0, 0.17
3	0.00	4.97	0.99	(0.78)	19*	—	0.34	0.26	-0.34	0.32	0, 0.28
4	0.00	1.09	0.37	(0.22)	3*	Down	0.40	0.33	-0.44	0.45	8, -0.18
5	0.00	1.58	0.45	(0.33)	8*	—	0.43	0.33	-0.50	0.42	0, 0.13
6	0.00	1.92	0.36	(0.32)	25*	—	0.31	0.15	-0.39	0.33	0, 0.29
7	0.00	3.90	0.69	(0.59)	18*	—	0.35	0.33	-0.31	0.32	0, 0.16
8	0.00	2.18	0.84	(0.49)	6*	—	0.38	0.30	-0.35	0.33	1, 0.19
9	0.00	2.03	0.67	(0.43)	4	—	0.50	0.40	-0.45	0.41	6, -0.35
1,2 & 3	0.22	2.13	0.79	(0.36)	None	—	0.58	0.48	-0.58	0.56	0, 0.31
4,5 & 6	0.07	1.17	0.40	(0.22)	None	—	0.54	0.38	-0.62	0.54	0, 0.19
7,8 & 9	0.08	1.58	0.72	(0.34)	None	—	0.60	0.50	-0.55	0.52	0, 0.22

[1]Strongest cross correlations for temperature (lag 0,1,6,12) and precipitation (varies) are reported.

* Some zero values are located in the last 24 months of data (2007-2008)

Table 3.2. Decomposition & time series regression with external variables and interventions for Georgia, USA, Divisions 1-9 and Combinations 1-3, 4-6 & 7-9

Division	Univariate Regression		Decomposition				Time Series Regression				
	PCP (P<0.05)	TMP (R^2)	R^2	Holdout	Norm	White Noise	Variables*	R^2	Holdout	Norm	White Noise
Georgia	Yes	0.51	0.73	0.66	Yes	No, close	T, PCP, TMP (lag 6)	0.64	0.66	Yes	No
1	No	0.26	0.44	0.13	No, close	Yes	TMP (lag 6) IV: July 2007	0.40	0.02	Yes	Yes
2	Yes	0.30	0.55	0.40	Yes	No	T, PCP, TMP	0.46 Robust[1]	0.48	Yes	Yes
3	Yes	0.12	0.27	-0.37	Yes	No	PCP, TMP IV: June 2005	0.36	0.03	Yes	Yes
4	No	0.17	0.37	0.19	No, close	Yes	T, TMP (lag 6) IV: Jan 2007	0.42	0.26	Yes	Yes
5	No	0.19	0.41	0.09	Yes	Yes	TMP (lag 6)	0.33 Robust	0.31	No, close	Yes
6	Yes	0.10	0.47	0.21	Yes	No	PCP, TMP (lag 6) IV: Aug 2001 IV: June 2003	0.47	0.45	Yes	Yes
7	No	0.13	0.31	0.24	No, close	Yes	TMP IV: Aug 2001 IV: Aug 2003	0.35	0.20	Yes	Yes
8	No	0.15	0.29	0.11	Yes	Yes	TMP IV: Aug 2002	0.27	0.00	No	Yes
9	Yes	0.25	0.36	0.12	Yes	No	PCP (lag 6), TMP	0.31	0.09	No, close	Yes

Table 3.2 (continued)

1,2 & 3	Yes	0.33	0.49	0.43	Yes	No	PCP, TMP (lag 6) IV: June 2005	0.52	0.46	Yes	Yes
4,5 & 6	Yes	0.27	0.55	0.43	Yes	No	TMP (lag 6) IV: May 2000 IV: June 2003	0.57	0.34	No, close	Yes
7, 8 & 9	Yes	0.36	0.51	0.22	Yes	No	TMP	0.36	0.28	Yes	Yes

*Variables: T=trend, PCP=precipitation, TMP=temperature, IV=Intervention
[1] Robust method improved normality and model results

Table 4.1. High risk campylobacteriosis cluster profiles at census tract, zip code and county subdivision spatial levels.

	Cluster (p-value)	General Region	Observed[1]	Expected[2]	RR[3]	Count[4]	Population
Census Tract	1*(p=.001)	Knoxville	289	106.5	2.86	32	161,266
	2 (p=.001)	Cookeville	97	28.3	3.43	14	42,008
	3 (p=.001)	Maynardville, Jefferson City	185	80.6	2.36	37	121,862
	4 (p=.001)	Murfreesboro	122	46.0	2.71	18	67,564
	5 (p=.001)	Shelbyville	16	1.3	12.06	1	2,010
	6 (p=.001)	Nashville-Davidson, Franklin	176	106.2	1.69	33	167,671
	7 (p=.001)	Kingsport	16	2.8	5.82	1	4,181
	8 (p=.003)	Lawrenceburg	10	1.1	9.00	1	1,775
Zip Code	1*(p=.001)	Knoxville	267	110.8	2.52	8	12,627
	2 (p=.001)	East Knoxville, Jefferson City	157	74.6	2.15	12	114,482
	3 (p=.001)	Oak Ridge	179	96.6	1.90	16	149,081
	4 (p=.001)	Cookeville	97	45.6	2.16	7	70,054
	5 (p=.001)	Nashville-Davidson, Franklin	182	110.0	1.69	6	168,288
	6 (p=.001)	Shelbyville, Murfreesboro	98	56.3	1.76	11	83,231
County Sub-division	1* (p=.001)	West Knoxville	207	98.2	2.17	11	151,917
	2 (p=.001)	Maynardville	104	43.5	2.43	10	66,116
	3 (p=.001)	Cookeville	89	38.9	2.32	4	59,547
	4 (p=.002)	Shelbyville, Murfreesboro	61	30.3	2.03	7	11,215
	5 (p=.009)	Philadelphia, Barnard	19	5.5	3.47	2	8,433

[1]The observed number of cases within the cluster
[2]The expected number of cases as calculated by the spatial scan algorithm
[3]The relative risk of campylobacteriosis for the cluster
[4]Denotes the number of areal units included in the cluster
*Denotes the primary cluster detected in the analysis

Table 5.1. Cases deleted from initial dataset and reasons for deletion.

Number of cases (total=967)	% Total	Reason for removal
130	2.75	Infection acquired outside study area (travel-related)
40	0.85	No address provided
24	0.51	Duplicate data/data entry error
4	0.05	Missing sex
135	2.80	Missing age
520	11.00	Missing street or address information
244	5.20	Incorrect address or typographical error

Note: One hundred twenty nine cases contained more than one of the above reasons for removal. (Total number of cases described above=1096, Total number eliminated=967)

Table 5.2. Summary statistics of the socioeconomic factors investigated for potential associations with campylobacteriosis risk.

Category	*Variable (% of census tract population)*	*Mean*	*Std Dev*	*Median*	*Min*	*Max*
Race & Nationality	Black or African American	19.2	28.4	5.6	0	99.7
	White	77.2	29.0	91.1	0	100.0
	American Indian or Alaskan household	0.3	0.4	0.2	0	11.1
	Asian	1.0	1.7	0.4	0	25.6
	Hispanic/Latino	2.1	2.9	1.2	0	34.2
	Native American/Alaskan	0.2	0.2	0.2	0	1.1
Employment	Unemployed	3.8	2.9	3.2	0	36.3
	Service occupation	14.9	6.7	13.6	0	82.6
	Agriculture: forestry, fish/hunt/mine	1.6	2.3	0.7	0	20.4
	Farming Industry	0.6	1.1	0.3	0	14.5
	Disability (age 21-64)	23.1	8.6	22.9	0	60.4
	Armed forces	0.3	2.7	0.0	0	70.1
Education	No high school diploma	15.7	7.7	15.8	0	50.2
	Bachelor degree	11.9	9.3	8.7	0	52.1
	Graduate or Professional Degree	6.4	6.2	4.3	0	39.6
Marital status	Never married	24.4	11.1	19.9	0	88.9
	Separated	2.3	2.2	1.7	0	21.7
	Divorced	11.6	3.9	11.3	0	51.1
	Widow	7.4	3.3	7.0	0	28.5
Poverty, Public assistance & Urbanicity	Poverty level	12.5	11.3	9.7	0	100.0
	Receive public assistance	4.0	4.4	2.8	0	57.1
	Urban	62.7	42.8	89.1	0	100.0
	Rural	37.1	42.7	10.0	0	100.0

Table 5.3. Spearman Rank Correlation Coefficients of variables investigated for potential association with campylobacteriosis in Tennessee.

	Black	White	Unemp[1]	Service Industry	Ag Employ[2]	Dis-ability	No HS Diploma[3]	Bach Degree[4]	Grad Degree[5]	Never Married	Sep[6]	Divorced	Poverty	Public Assist[7]	Urban	Rural
Black	1															
White	-0.97 (<.001)	1														
Unemployed	0.37 (<.001)	-0.35 (<.001)	1													
Service Industry	0.40 (<.001)	-0.39 (<.001)	0.57 (<.001)	1												
Ag Employ[2]	-0.49 (<.001)	0.52 (<.001)	-0.07 (<.001)	-0.17 (<.001)	1											
Disability	0.15 (<.001)	-0.11 (<.001)	0.51 (<.001)	0.55 (<.001)	0.19 (<.001)	1										
No High School Diploma	0.20 (<.001)	-0.15 (<.001)	0.50 (<.001)	0.51 (<.001)	0.24 (<.001)	0.76 (<.001)	1									
Bachelor's Degree	0.05 (0.08)	-0.06 (0.02)	-0.39 (<.001)	-0.35 (<.001)	-0.41 (<.001)	-0.72 (<.001)	-0.79 (<.001)	1								
Graduate Degree	-0.01 (0.86)	-0.01 (0.61)	-0.36 (<.001)	-0.32 (<.001)	-0.34 (<.001)	-0.62 (<.001)	-0.73 (<.001)	0.84 (<.001)	1							
Never Married	0.73 (<.001)	-0.74 (<.001)	0.40 (<.001)	0.48 (<.001)	-0.49 (<.001)	0.16 (<.001)	0.16 (<.001)	0.09 (<.001)	0.04 (0.12)	1						
Separated	0.61 (<.001)	-0.59 (<.001)	0.47 (<.001)	0.54 (<.001)	-0.24 (<.001)	0.48 (<.001)	0.48 (<.001)	-0.32 (<.001)	-0.30 (<.001)	0.55 (<.001)	1					
Divorced	0.27 (<.001)	-0.26 (<.001)	0.31 (<.001)	0.36 (<.001)	-0.21 (<.001)	0.36 (<.001)	0.32 (<.001)	-0.18 (<.001)	-0.17 (<.001)	0.36 (<.001)	0.42 (<.001)	1				
Poverty level	0.29 (<.001)	-0.26 (<.001)	0.61 (<.001)	0.63 (<.001)	0.12 (<.001)	0.75 (<.001)	0.73 (<.001)	-0.61 (<.001)	-0.53 (<.001)	0.37 (<.001)	0.58 (<.001)	0.36 (<.001)	1			
Receives public assistance	0.31 (<.001)	-0.28 (<.001)	0.58 (<.001)	0.58 (<.001)	0.06 (0.04)	0.70 (<.001)	0.69 (<.001)	-0.57 (<.001)	-0.50 (<.001)	0.30 (<.001)	0.55 (<.001)	0.38 (<.001)	0.76 (<.001)	1		
Urban	0.57 (<.001)	-0.60 (<.001)	0.17 (<.001)	0.26 (<.001)	-0.78 (<.001)	-0.10 (<.001)	-0.17 (<.001)	0.40 (<.001)	0.34 (<.001)	0.62 (<.001)	0.36 (<.001)	0.32 (<.001)	0.03 (0.24)	0.06 (0.03)	1	
Rural	-0.56 (<.001)	0.61 (<.001)	-0.16 (<.001)	-0.25 (<.001)	0.78 (<.001)	0.11 (<.001)	0.18 (<.001)	-0.39 (<.001)	-0.33 (<.001)	-0.61 (<.001)	-0.35 (<.001)	-0.31 (<.001)	-0.02 (0.40)	-0.05 (0.06)	-0.99 (<.001)	1

[1]Unemployed, [2]Agricultural employment, [3]No high school diploma, [4]Bachelor's degree, [5]Graduate degree, [6]Separated, [7]Receives public assistance

Table 5.4. Results of assessment of univariate (simple) associations between campylobacteriosis risk and selected socioeconomic factors.

Category	Variable (proportion of census tract population)	Estimate (95% Confidence Interval)	SE[1]	P-value	AIC[2]
Race & Nationality	Black or African American	-0.0133 (-0.0158,-0.0108)	0.0013	0.0001	5972
	White	0.0127 (0.0103, 0.0152)	0.0012	0.0001	5977
	Asian	0.0265 (-0.0143, 0.0672)	0.0204	0.2037	6072
	Hispanic/Latino	-0.0065 (-0.0278, 0.0148)	0.0109	0.5499	6074
	American Indian/Alaskan	0.3575 (-0.0490, 0.7641)	0.2074	0.0843	6071
Employment	Unemployed	-0.0902 (-0.1170,-0.0634)	0.0137	0.0001	6030
	Service occupation	-0.0291 (-0.0403,-0.0178)	0.0058	0.0001	6049
	Agriculture: forestry, fishing, hunting & mining	0.0585 (0.0317, 0.0854)	0.0137	0.0001	6055
	Farming Industry	0.0278 (0.0367,-0.0442)	0.0999	0.4485	6074
	Disability (age 21-64)	-0.0169 (-0.0253,-0.0086)	0.0043	0.0001	6058
	Armed forces	-0.0462 (-0.0779,-0.0144)	0.0162	0.0043	6065
Education	No High school diploma	-0.0097 (-0.0188,-0.0006)	0.0046	0.0373	6070
	Bachelor degree	0.0122 (0.0049, 0.0195)	0.0037	0.0011	6069
	Graduate/Professional Degree	0.0159 (0.0054, 0.0265)	0.0054	0.0031	6070
Marital status	Never married	-0.0134 (-0.0193, -0.007)	0.0030	0.0001	6055
	Separated	-0.1560 (-0.1909, -0.1211)	0.0178	0.0001	6000
	Divorced	-0.0390 (-0.0570, -0.020)	0.0092	0.0001	6056
	Widow	-0.0559 (-0.0776, -0.0342)	0.0111	0.0001	6049
Poverty, Public Assistance & Urbanicity	Below poverty level	-0.0198 (-0.0264,-0.0132)	0.0034	0.0001	6041
	Receives public assistance	-0.0458 (-0.0627,-0.0289)	0.0086	0.0001	6047
	Urban	-0.2573 (-0.4133,-0.1013)	0.0796	0.0012	6064
	Rural	0.2573 (0.1013, 0.4134)	0.0796	0.0012	6064

[1] Standard Error
[2]Akaike's Information Criterion

Table 5.5 Comparison of negative binomial, spatial lag, global and local geographically weighted Poisson models.

	Model type with Coefficient estimates (p-values)					
	Negative Binomial Model	**Spatial Lag Model**	**Global Poisson GWR[1] Model**	**Local Poisson GWR[1] Model**		
Model 1:				**Min**	**Max**	
Intercept	-7.164	(0.0001)	1.716 (0.000)	- 7.155	(0.000)	-8.187 -6.247
Black Race	-0.015	(0.0001)	-0.169 (0.001)	-0.014	(0.001)	-0.0487 0.0218
No diploma	0.021	(0.0004)	-0.012 (0.112)	0.003	(0.003)	-0.0553 0.0533
Unemployed	-0.041	(0.0141)	-0.030 (0.112)	-0.014	(0.009)	-0.1866 0.0851
Urban	0.235	(0.0154)	0.357 (0.014)	0.186	(0.055)	-0.4526 0.9321
Model 2:						
Intercept	- 6.73	(0.0001)	1.80 (0.000)	- 6.85	(0.000)	-7.71 -4.905
Black Race	-0.0129	(0.0001)	-0.093 (0.000)	-0.012	(0.001)	-0.0161 0.0311
No diploma	0.0175	(0.0009)	-0.018 (0.011)	0.000	(0.003)	-0.0650 0.0882
Unemployed	-0.0330	(0.0433)	-0.026 (0.179)	-0.010	(0.008)	-0.1847 0.0752
Divorced	-0.0260	(0.006)	-0.004 (0.733)	-0.016	(0.005)	-0.2485 0.0382

[1] Geographically Weighted Regression

Table 5.6. Assessment of the stationarity of the local Geographically Weighted (GWR) Model regression coefficients

Model 1:	**Global Poisson GWR[1] SE[2]**	**Global Poisson GWR[1] 2xSE[2]**	**Local Poisson GWR[1] IQR[3]**	**Is Regression Coefficient Non-Stationary?**
Black Race	0.001	0.002	0.015	Yes
No diploma	0.003	0.006	0.028	Yes
Unemployed	0.009	0.018	0.068	Yes
Urban	0.046	0.092	0.469	Yes
Model 2:				
Black Race	0.001	0.002	0.016	Yes
No diploma	0.003	0.006	0.015	Yes
Unemployed	0.009	0.018	0.067	Yes
Divorced	0.005	0.010	0.033	Yes

[1] Geographically Weighted Regression
[2] Standard error of the global GWR model
[3] Interquartile local coefficient estimate range

Appendix B: Figures

(a)

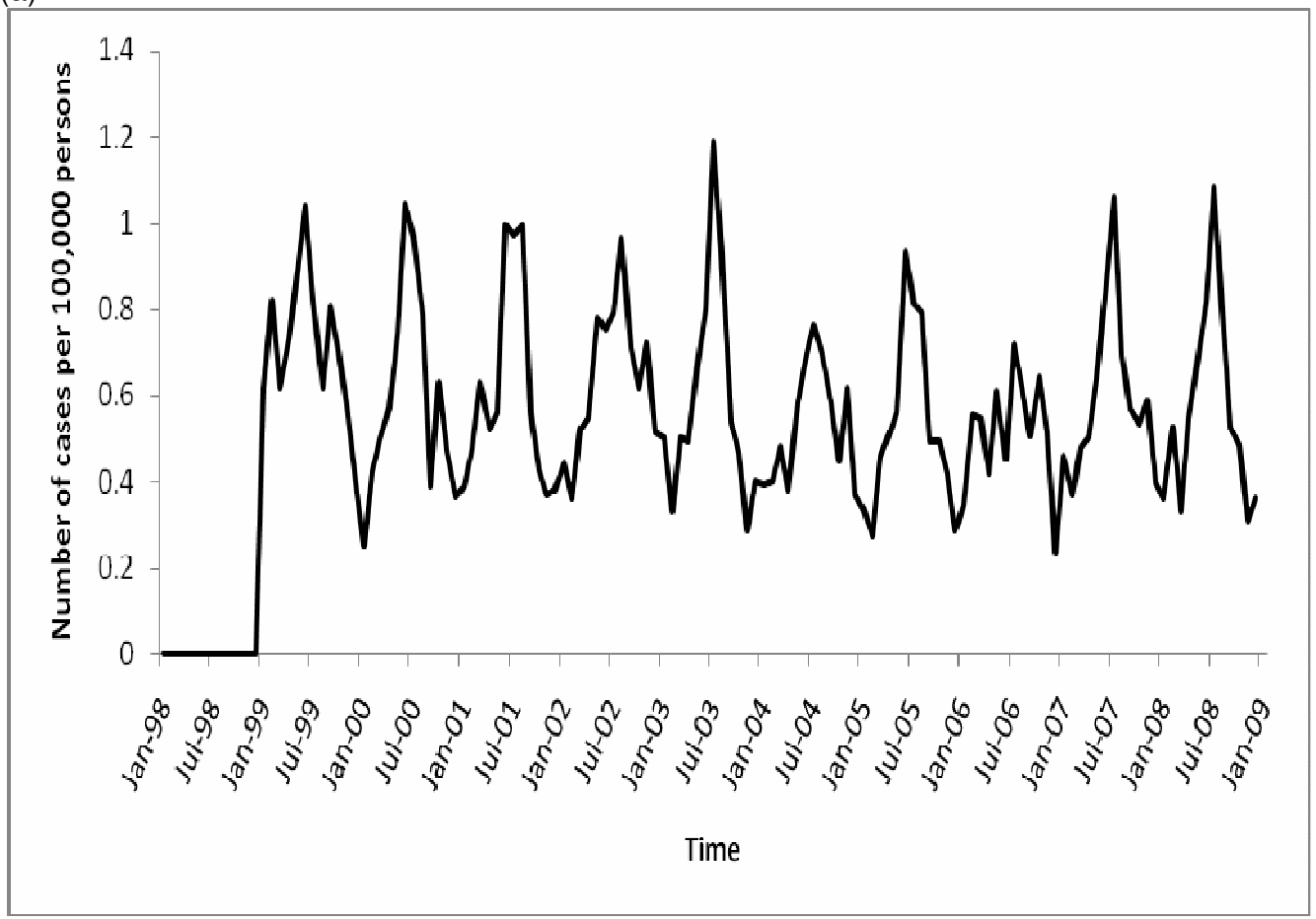


Figure 2.1 (continued)
(b)

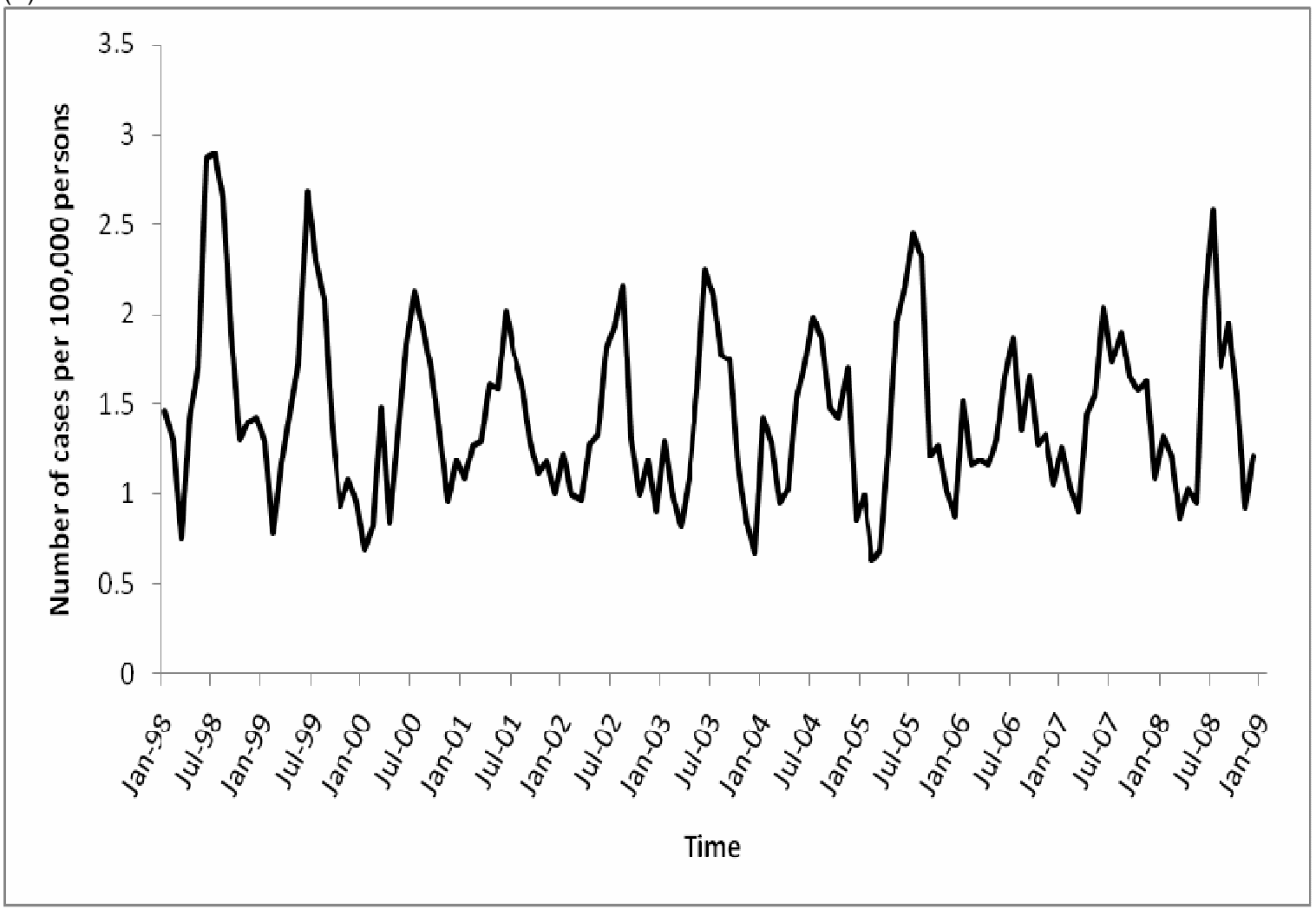


Figure 2.1 (continued)
(c)

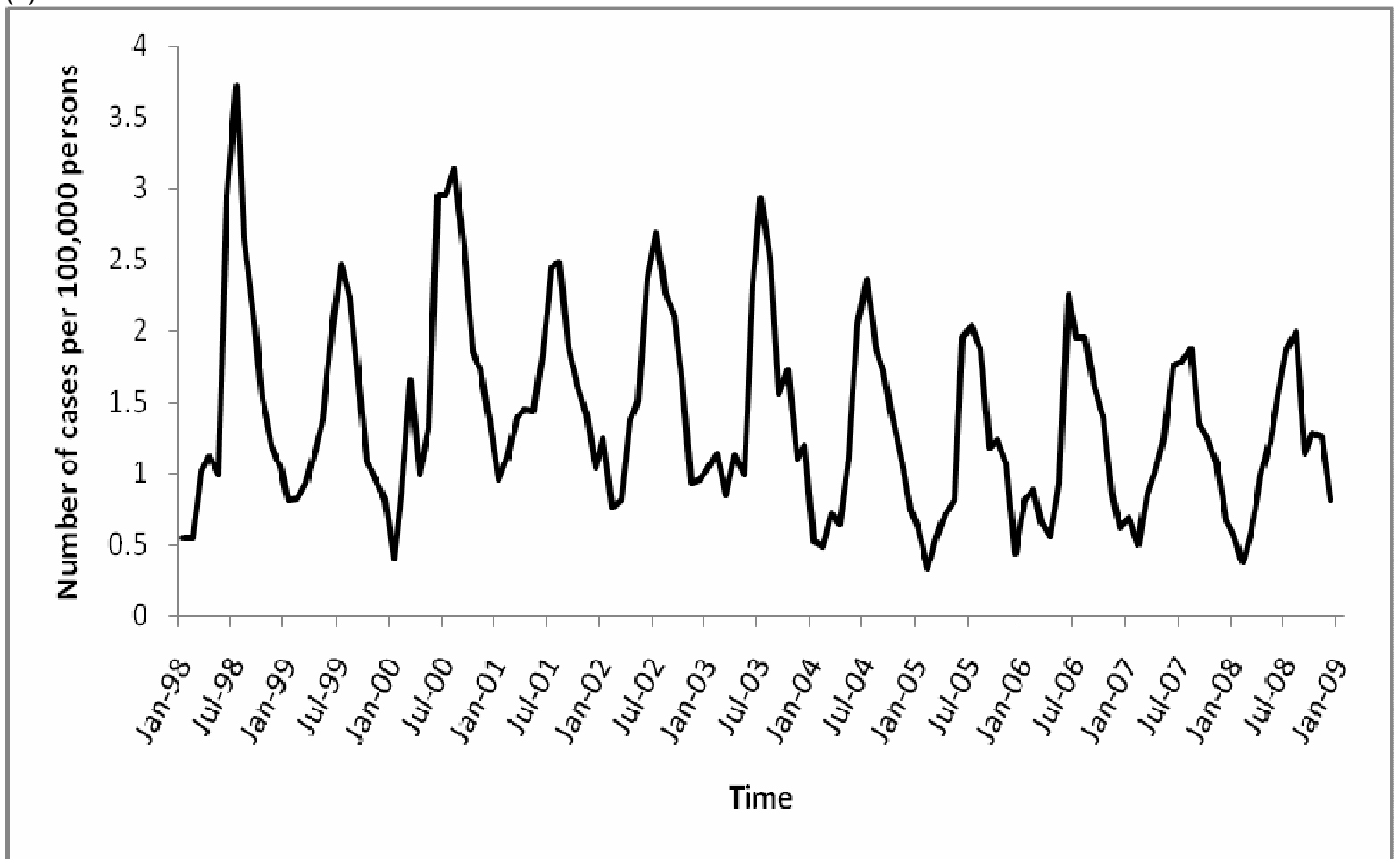


Figure 2.1. Risk of campylobacteriosis per 100,000 persons in (a) Georgia (1999-2007), (b) Oregon (1998-2007) and (c) Minnesota (1998-2007).

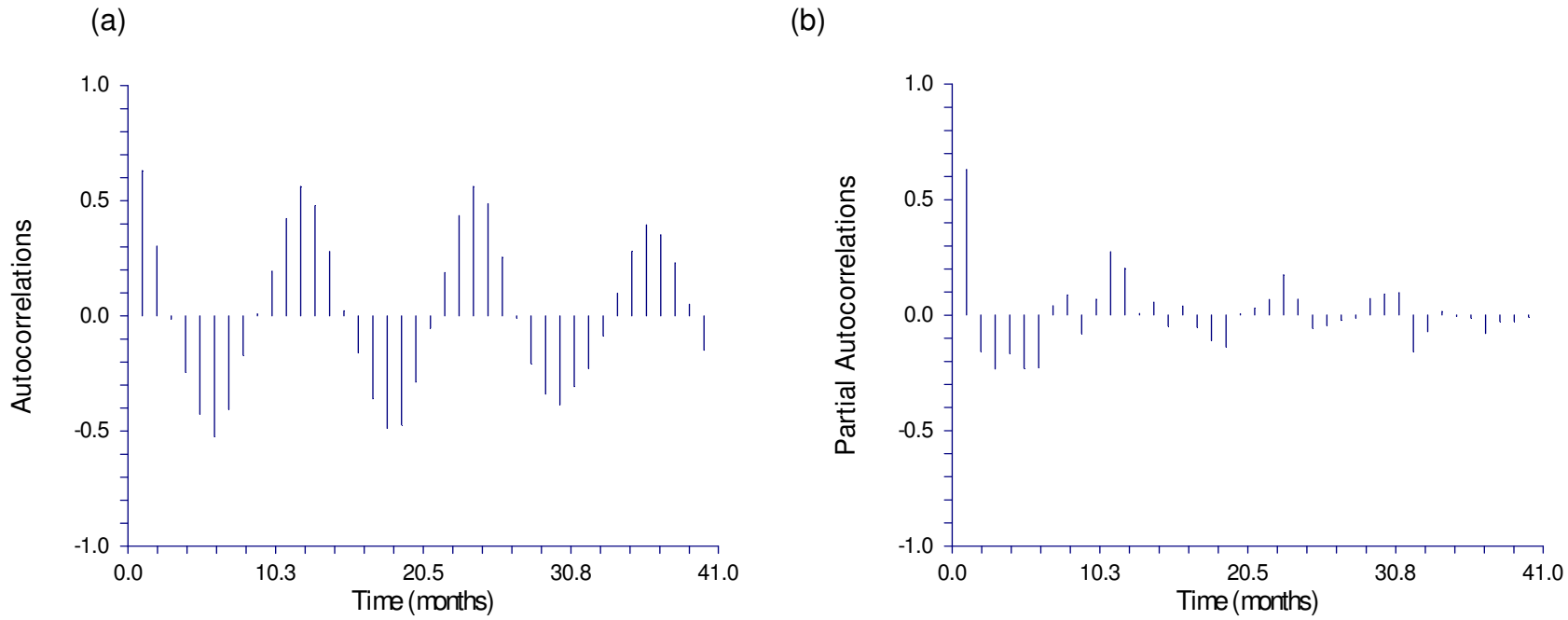


Figure 2.2. (a) Autocorrelation and (b) Partial Autocorrelation plots for Georgia campylobacteriosis risk per 100,000 persons.

(a)

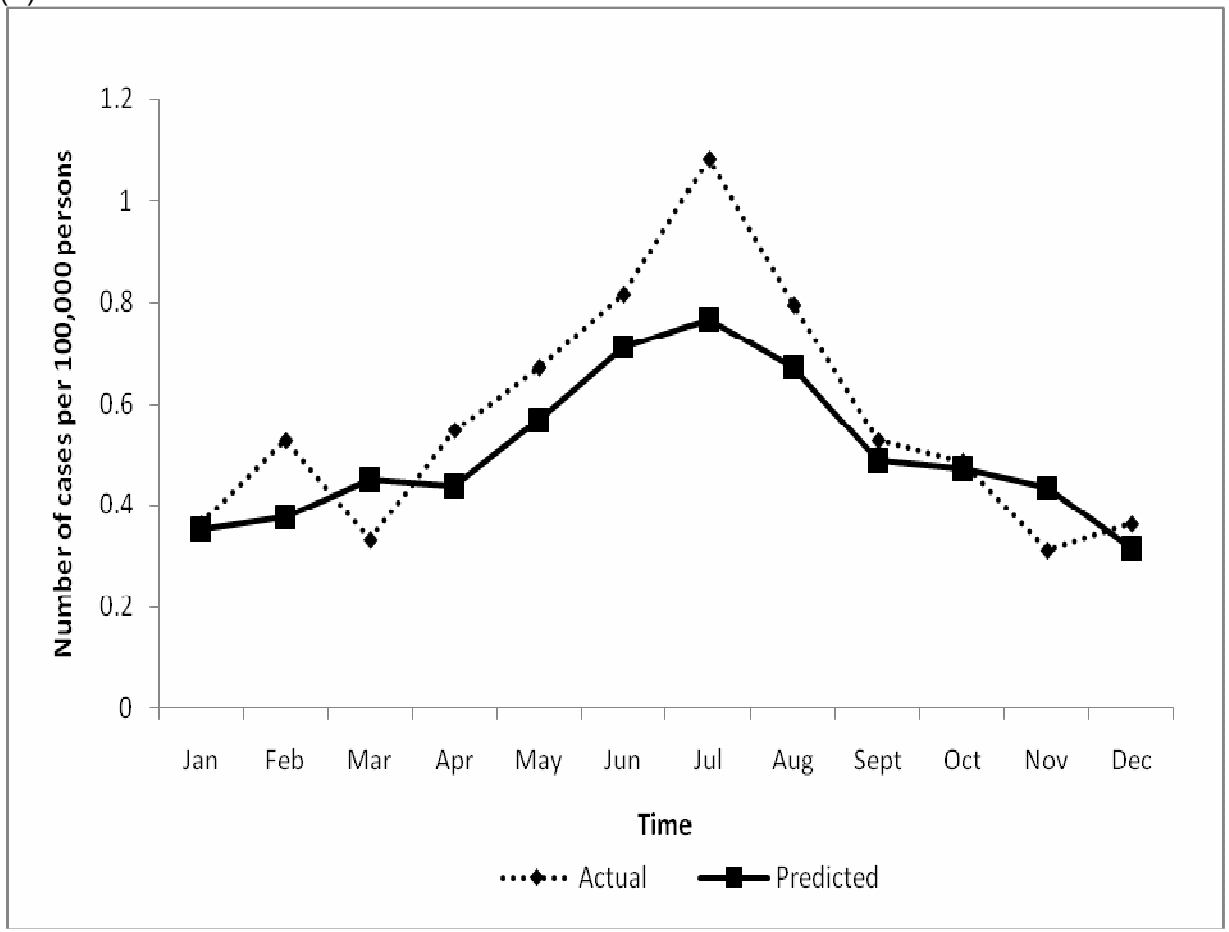

1.2
1
0.8
0.6
0.4
0.2
0
Number of cases per 100,000 persons
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Time
Actual
Predicted

Figure 2.3 (continued)
(b)

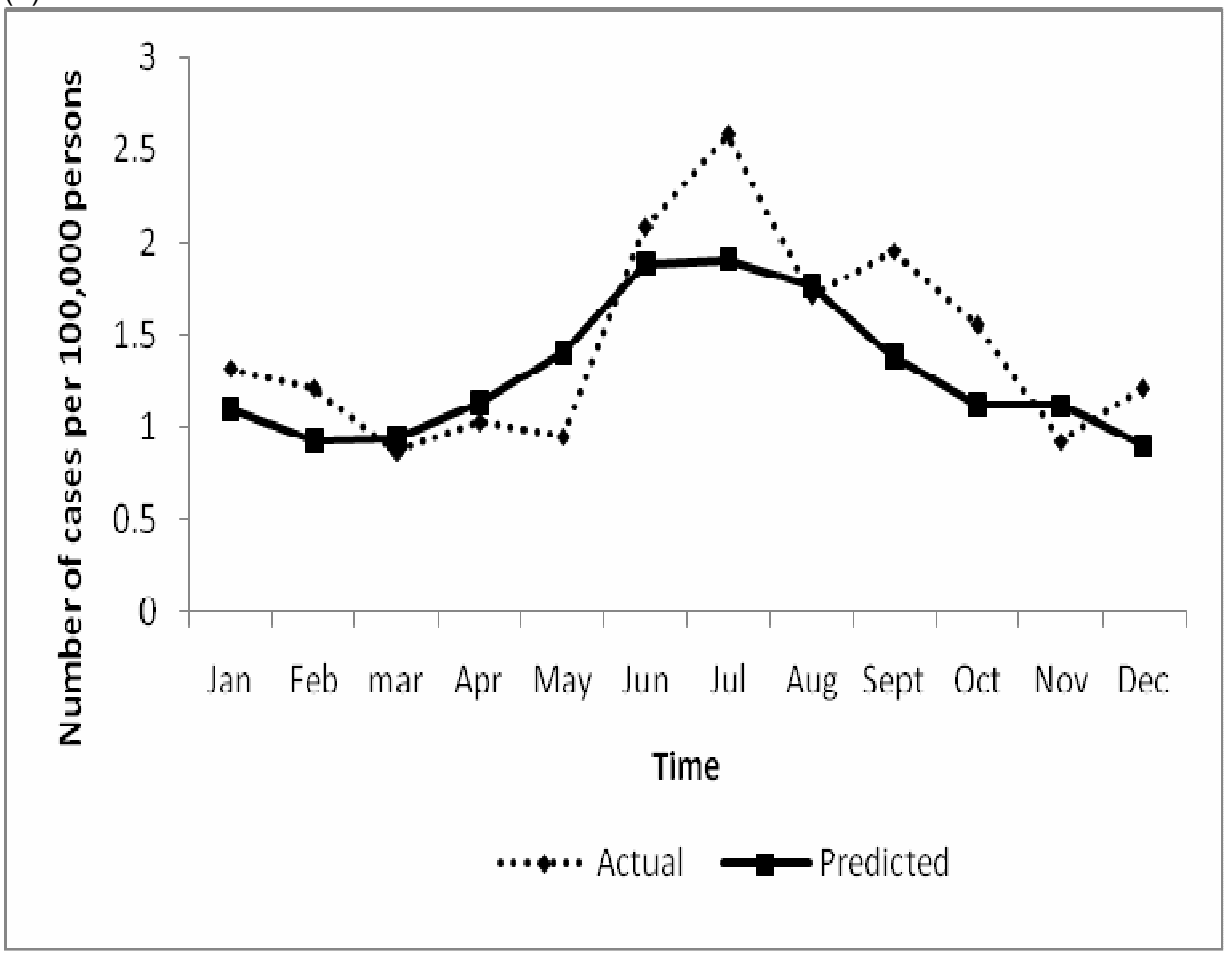


Figure 2.3 (continued)

(c)

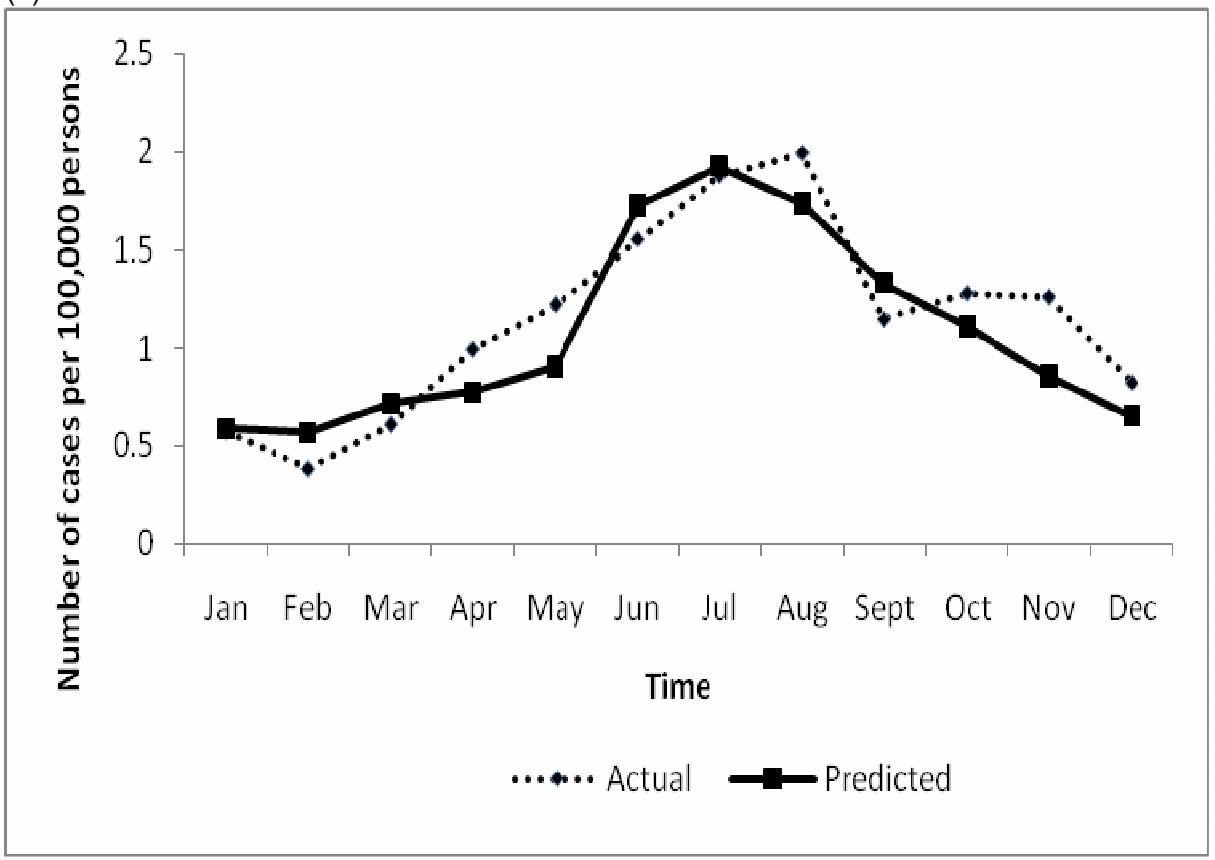


Figure 2.3. Validation year (2008) actual verses predicted risk of campyloacteriosis per 100,000 persons in (a) Georgia, (b) Oregon and (c) Minnesota

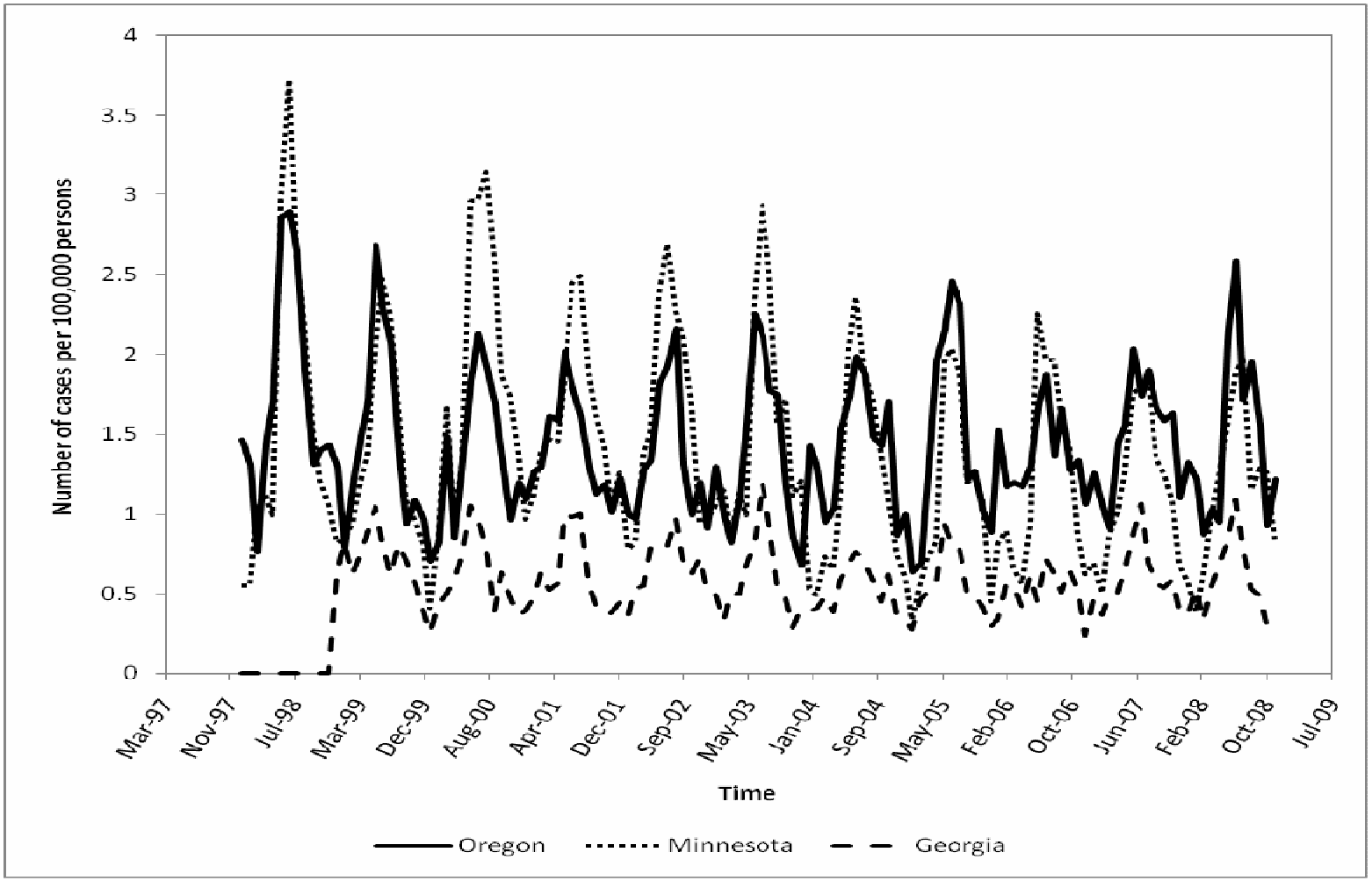


Figure 2.4. Comparison of temporal patterns in risk of campylobacteriosis in Oregon, Minnesota and Georgia.

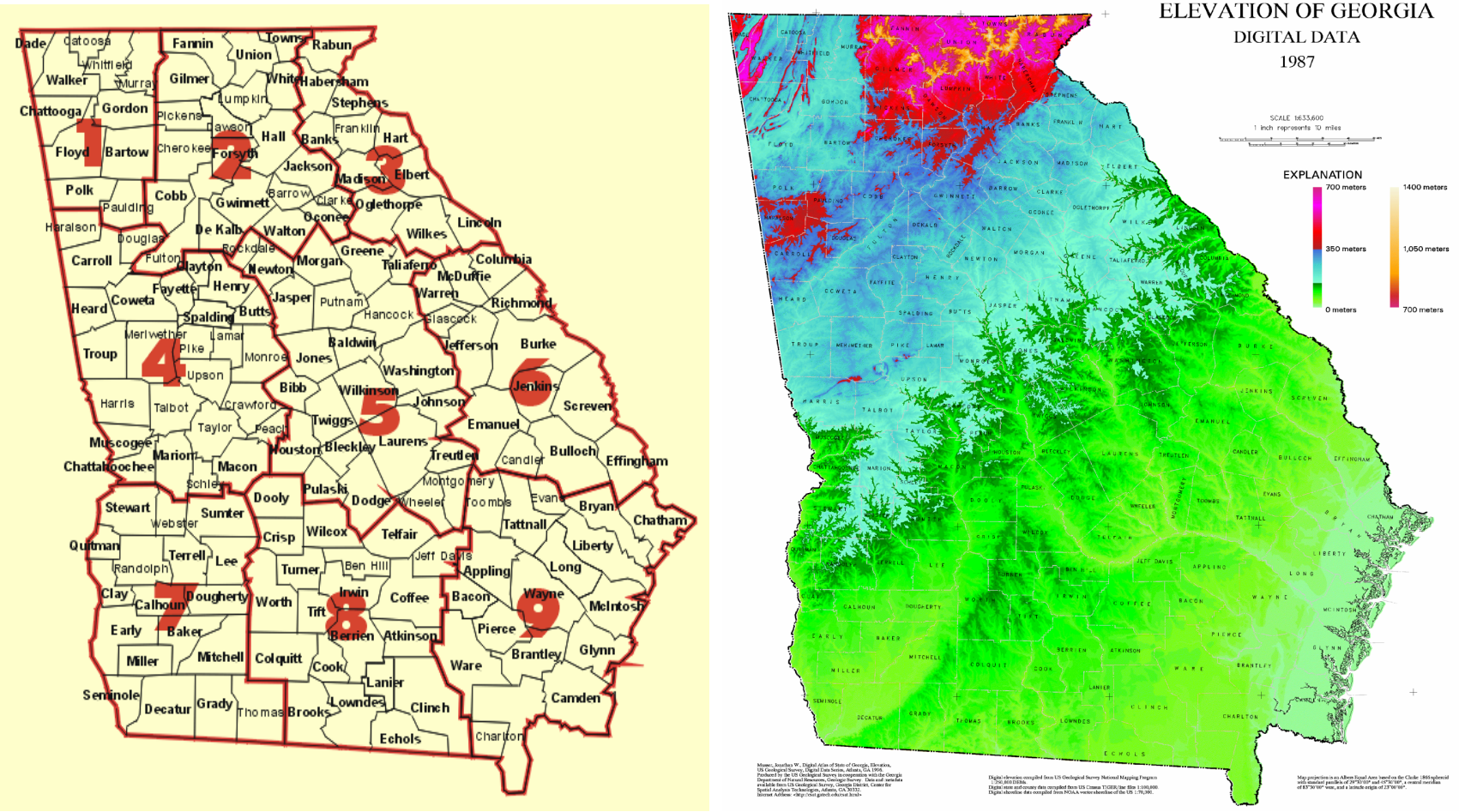


Figure 3.1. (a) Georgia state map including county (names) and climate division (numerical) boundaries and (b) Georgia diigital elevation map highlighting the regions that correspond to mountains (red, pink), piedmont (dark blue), coastal plain (blue to green interface) and swamp regions (yellow to light green).
Note: Maps are public domain and courtesy of (a) NCDC Climate Prediction Center and (b) U.S. Geological Society

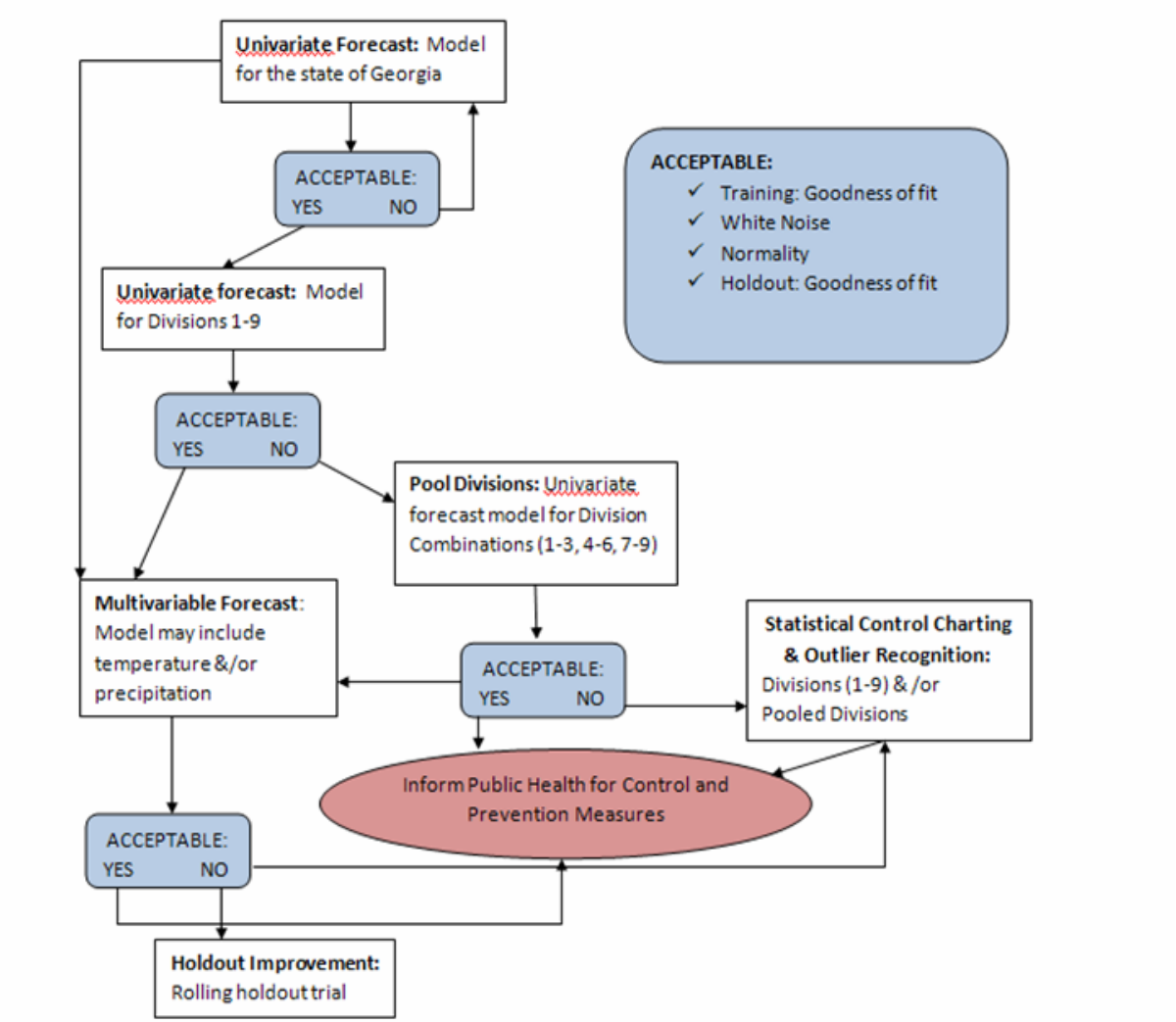


Figure 3.2. Procedural flow chart for forecasting campylobacteriosis risk in Georgia, USA

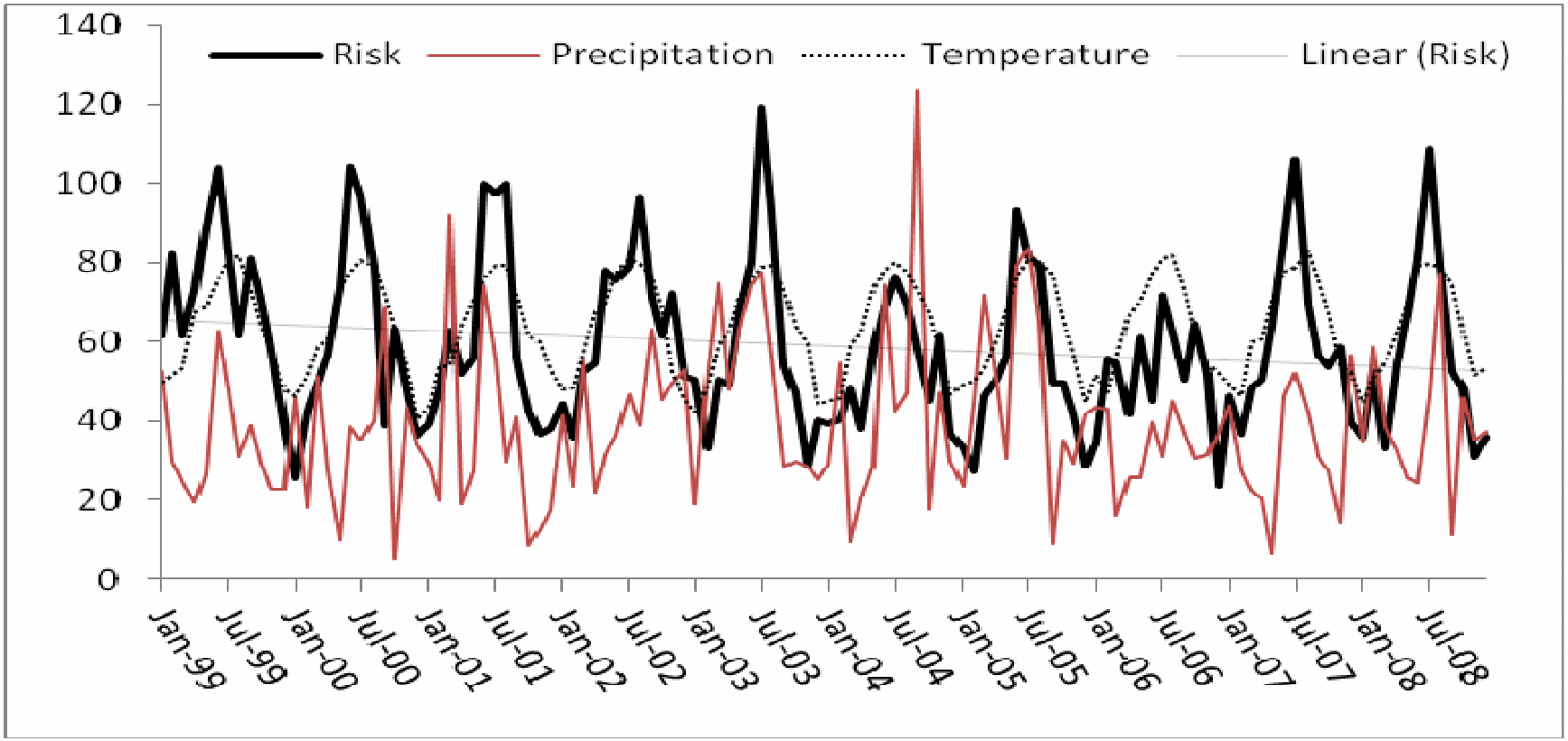
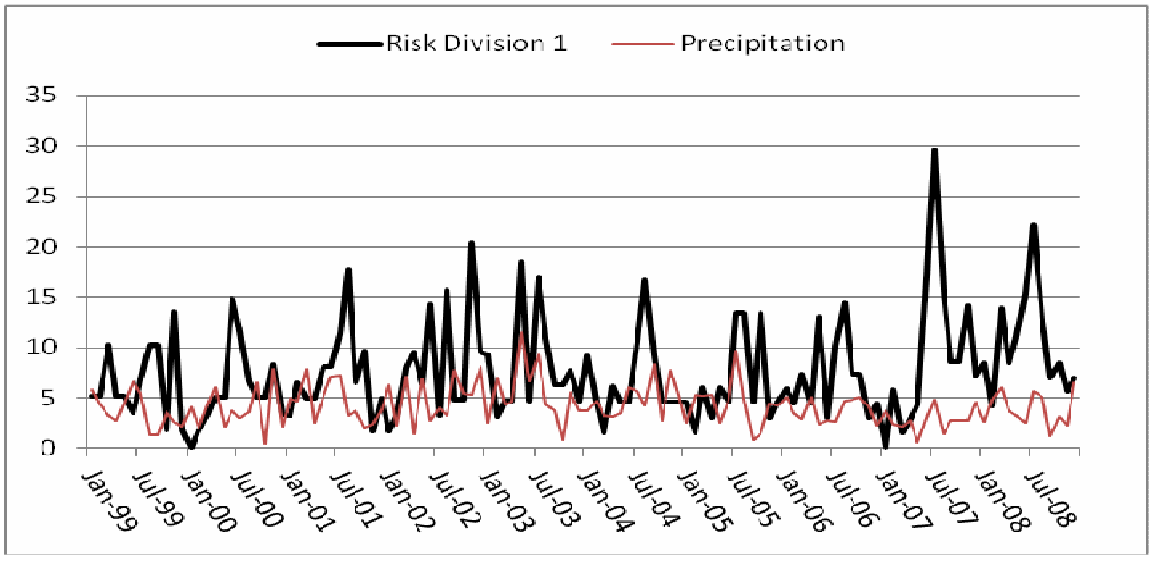
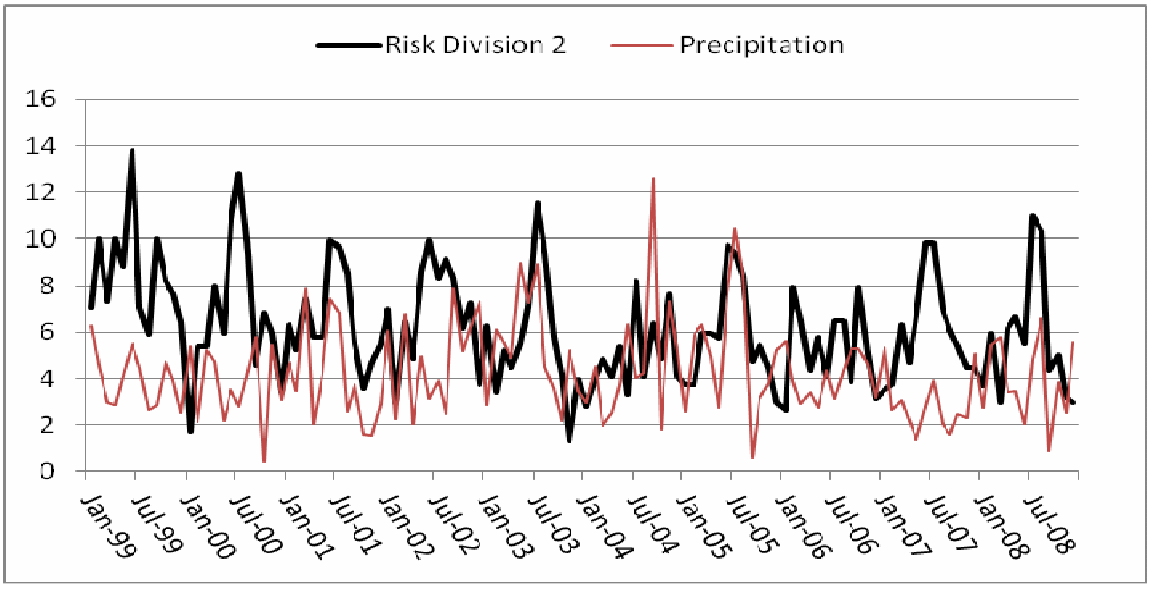


Figure 3.3. Risk (and linear trend) of campylobacteriosis (scale factor of 100), precipitation (scale factor of 10) and temperature in Georgia, USA from 1999-2008.


Risk Division 1
Precipitation
35
30
25
20
15
10
5
0
Jan-99
Jul-99
Jan-00
Jul-00
Jan-01
Jul-01
Jan-02
Jul-02
Jan-03
Jul-03
Jan-04
Jul-04
Jan-05
Jul-05
Jan-06
Jul-06
Jan-07
Jul-07
Jan-08
Jul-08

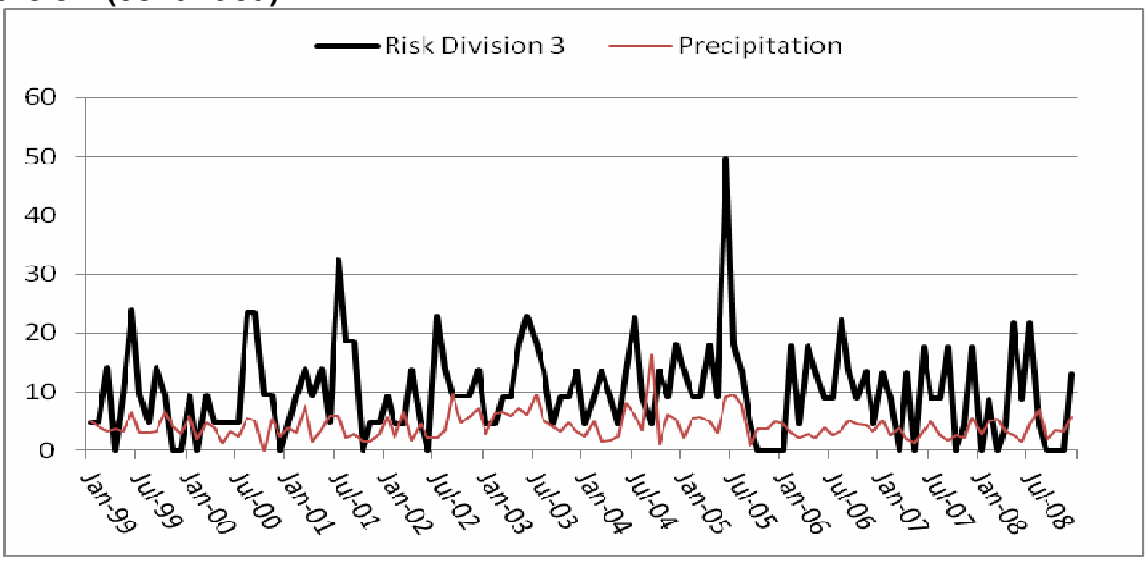
(a)


Risk Division 2
Precipitation
16
14
12
10
8
6
4
2
0
Jan-99
Jul-99
Jan-00
Jul-00
Jan-01
Jul-01
Jan-02
Jul-02
Jan-03
Jul-03
Jan-04
Jul-04
Jan-05
Jul-05
Jan-06
Jul-06
Jan-07
Jul-07
Jan-08
Jul-08

(b)

Figure 3.4 (continued)

(c)



(d)

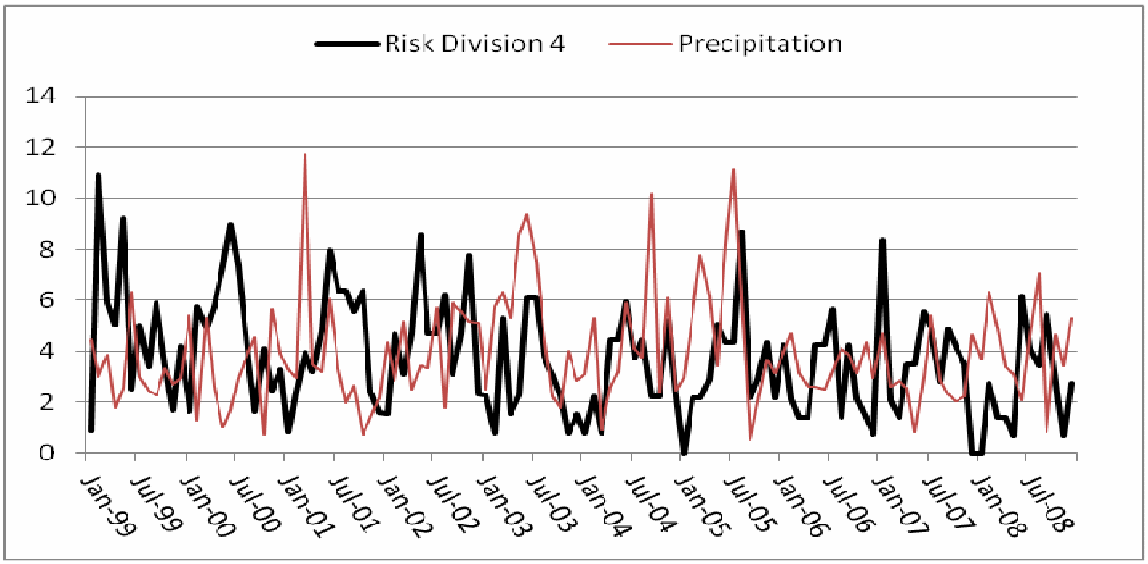
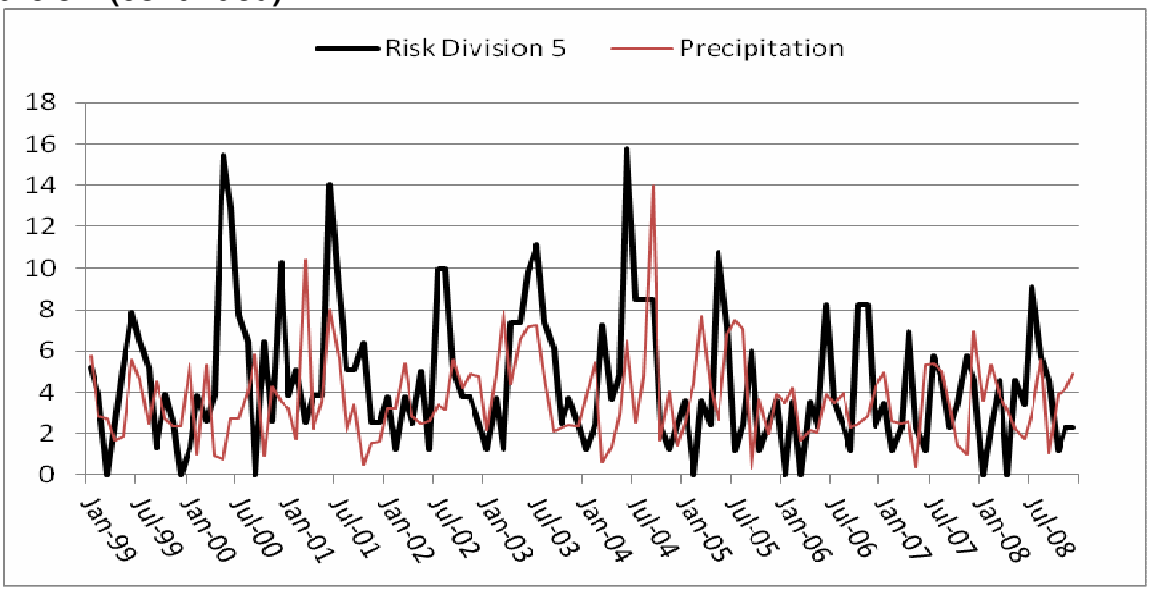


Figure 3.4 (continued)

(e)



(f)

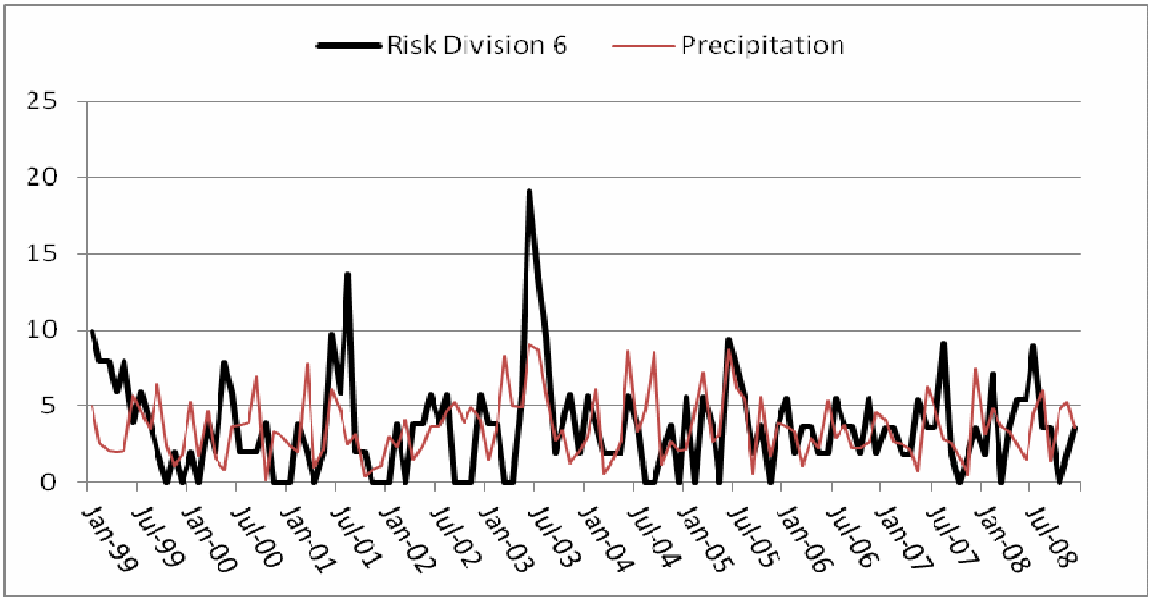
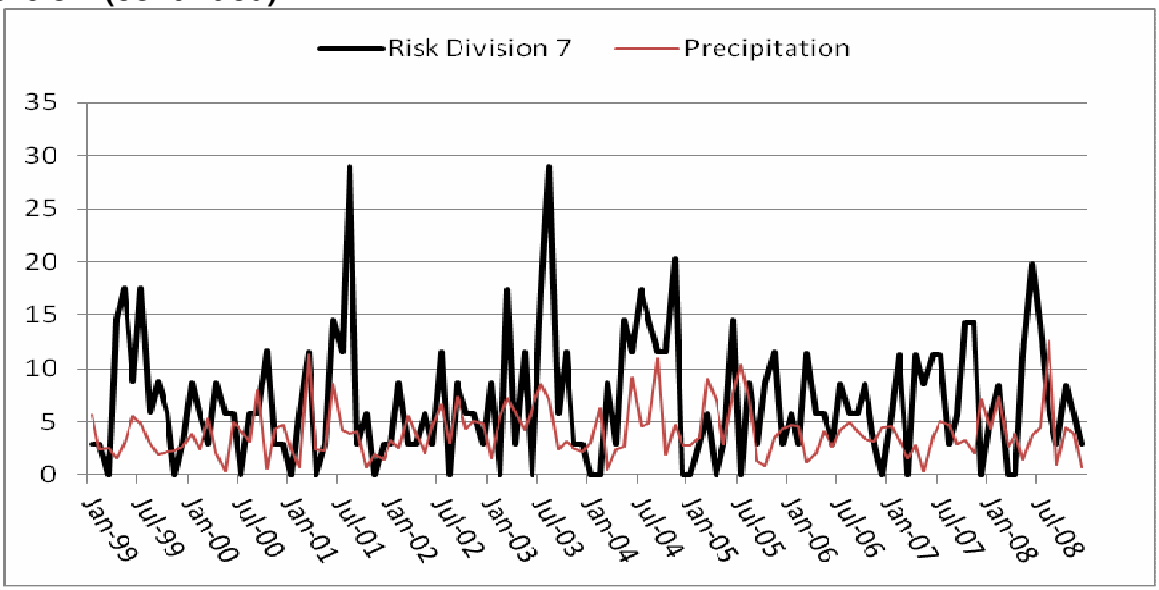


Figure 3.4 (continued)

(g)



(h)

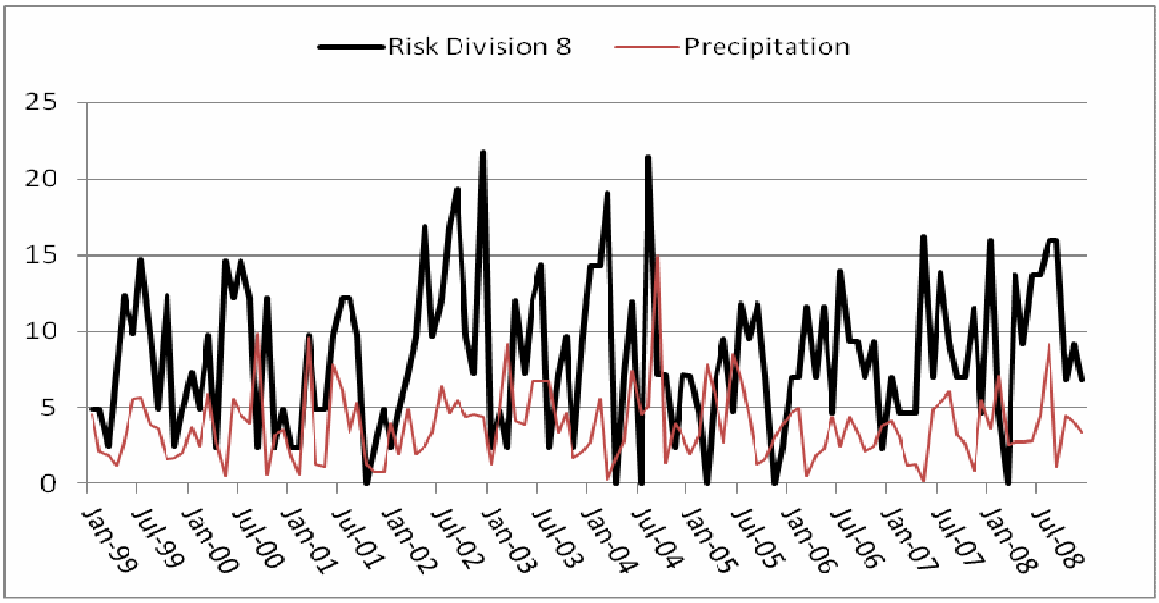


Figure 3.4 (continued)

(i)

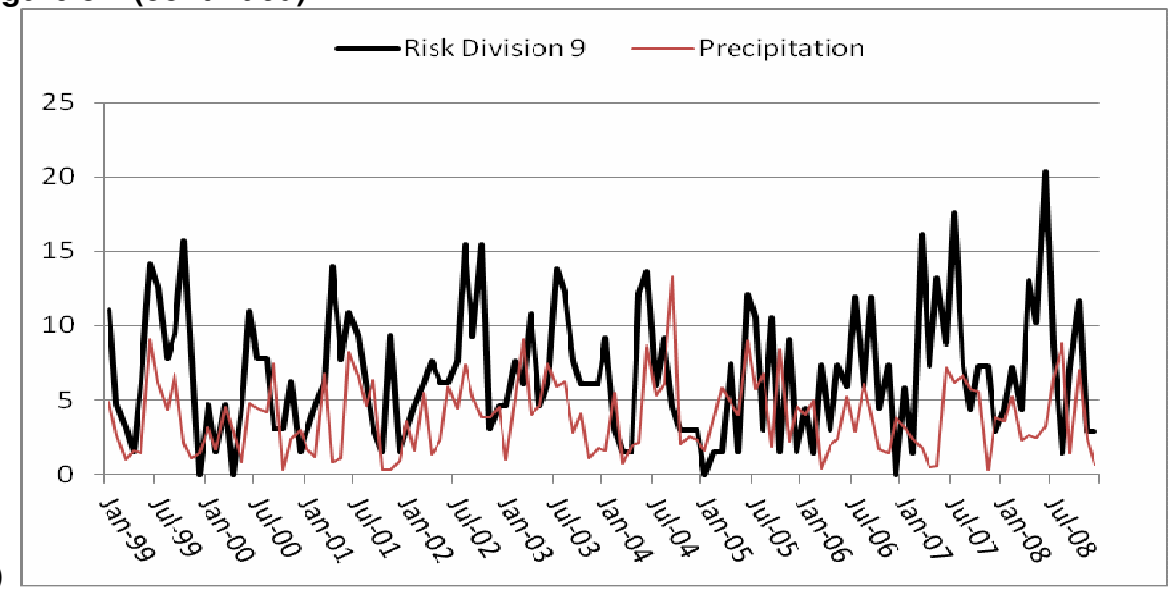
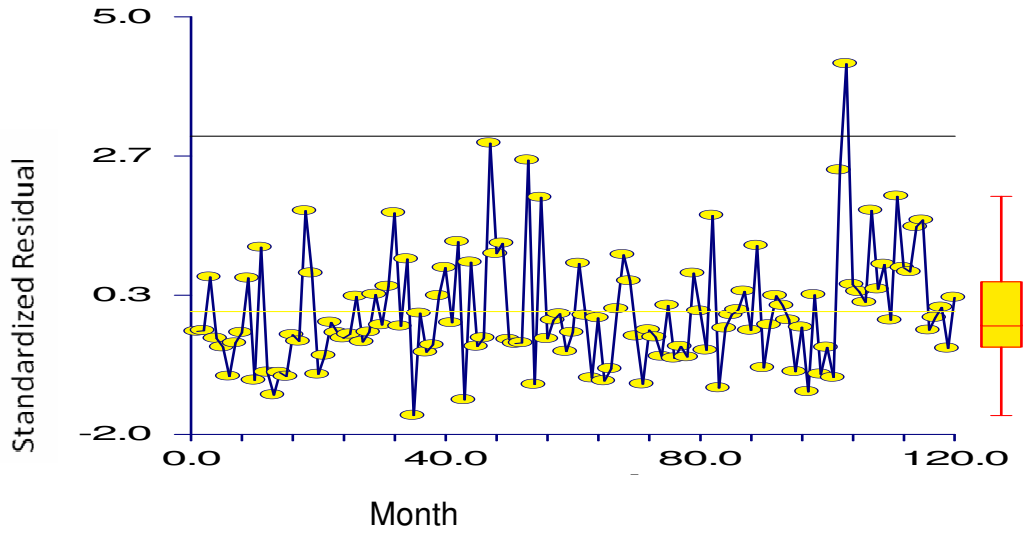


Figure 3.4. Risk of campylobacteriosis (scale factor of 10) and precipitation for Georgia, USA climate divisions 1-9 (a-i) from1999-2008.

(a) Mean training R^2: 0.37 (0.43), Holdout R^2: 0.10 (0.13)



(b) Mean training R^2: 0.39 (0.37), Holdout R^2: 0.24 (0.19)

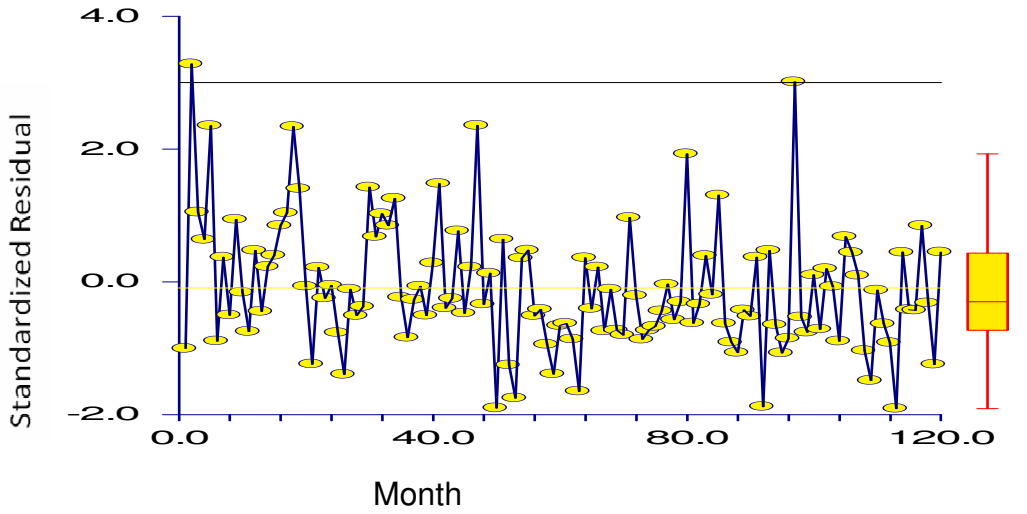


Figure 3.5. ARIMA (000 011) Control Charting and Moving holdout results for Divisions 1 (a) & Division 4 (b). Included are the standardized residual scatter plots and mean training and holdout R^2 results for Moving holdout analyses using decomposition (compared with 2008 results).

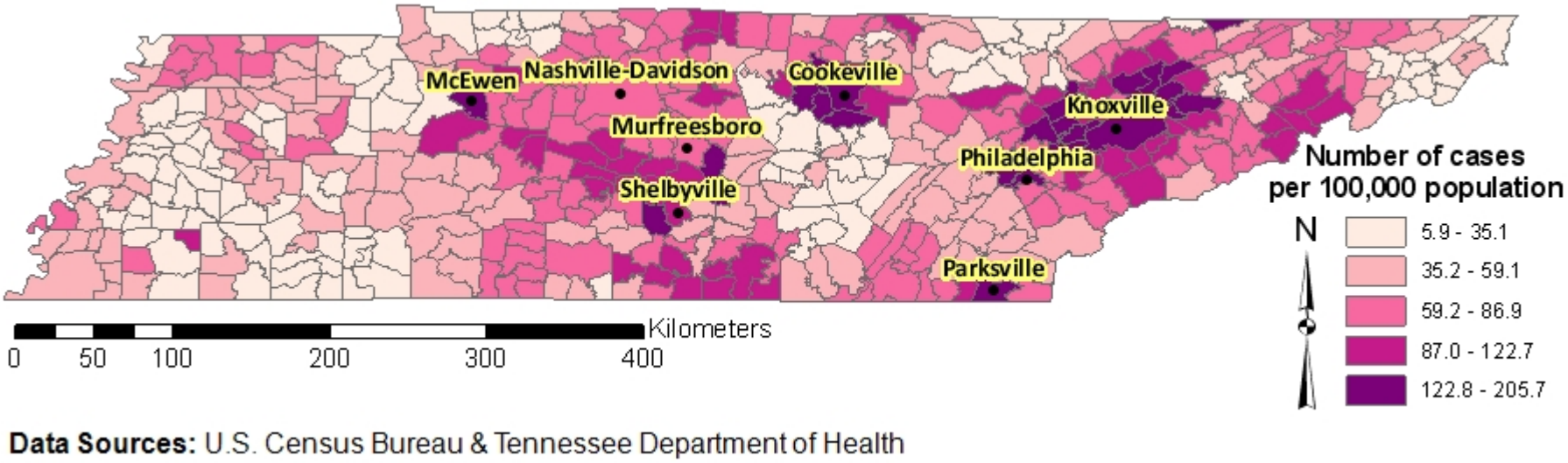


Figure 4.1.. Spatial empirical Bayesian smoothed risk of campylobacteriosis in Tennessee during the study period September 1, 1991 to December 31, 2008 at the county subdivision spatial level. The risks are standardized by age and gender.

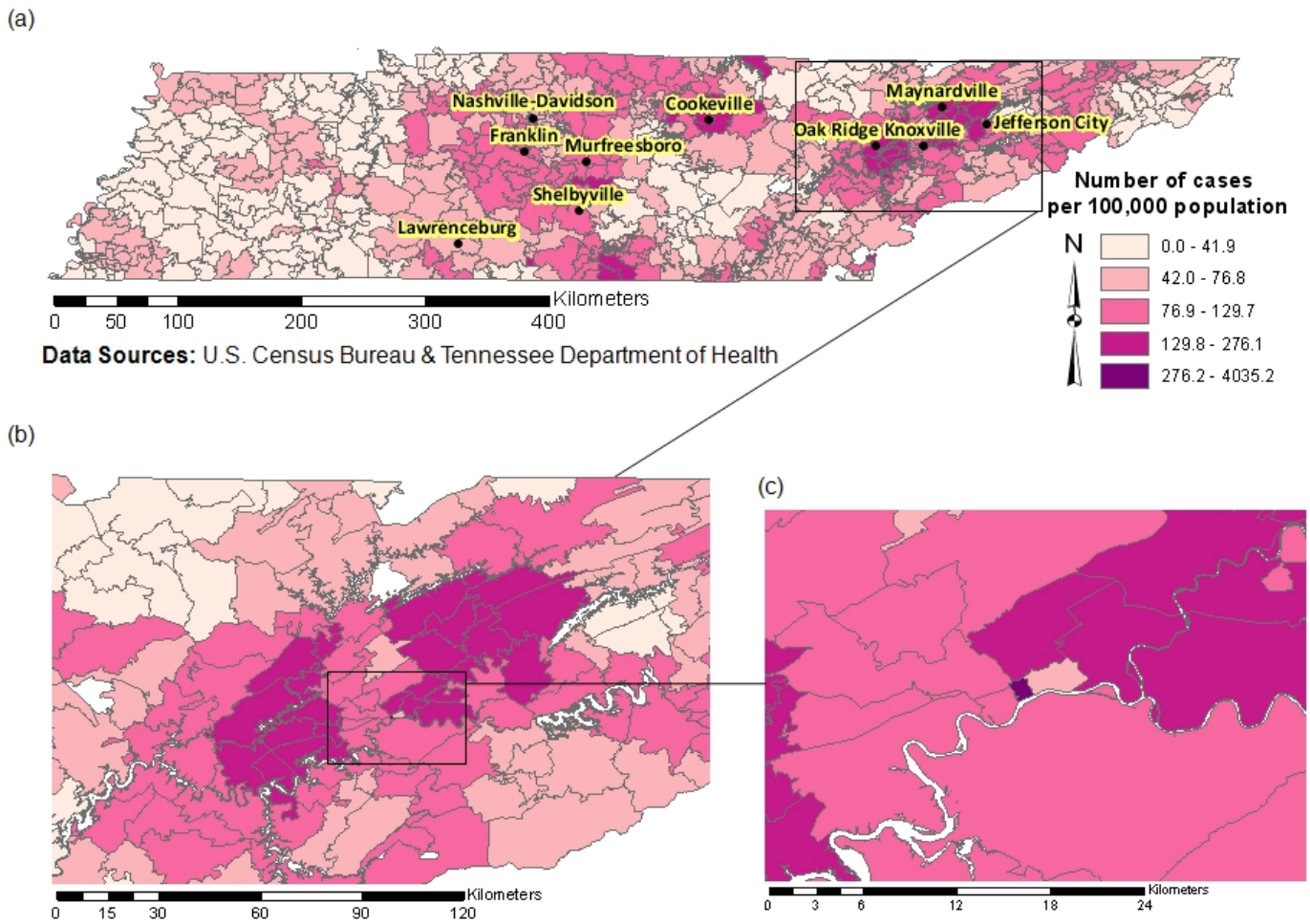


Figure 4.2.. Spatial empirical Bayesian smoothed risk of campylobacteriosis in Tennessee at the zip code spatial level. Primary insert incorporates the area surrounding Knoxville. Secondary insert highlights the highest risk region of central Knoxville (dark purple). The risks are standardized by age and gender.

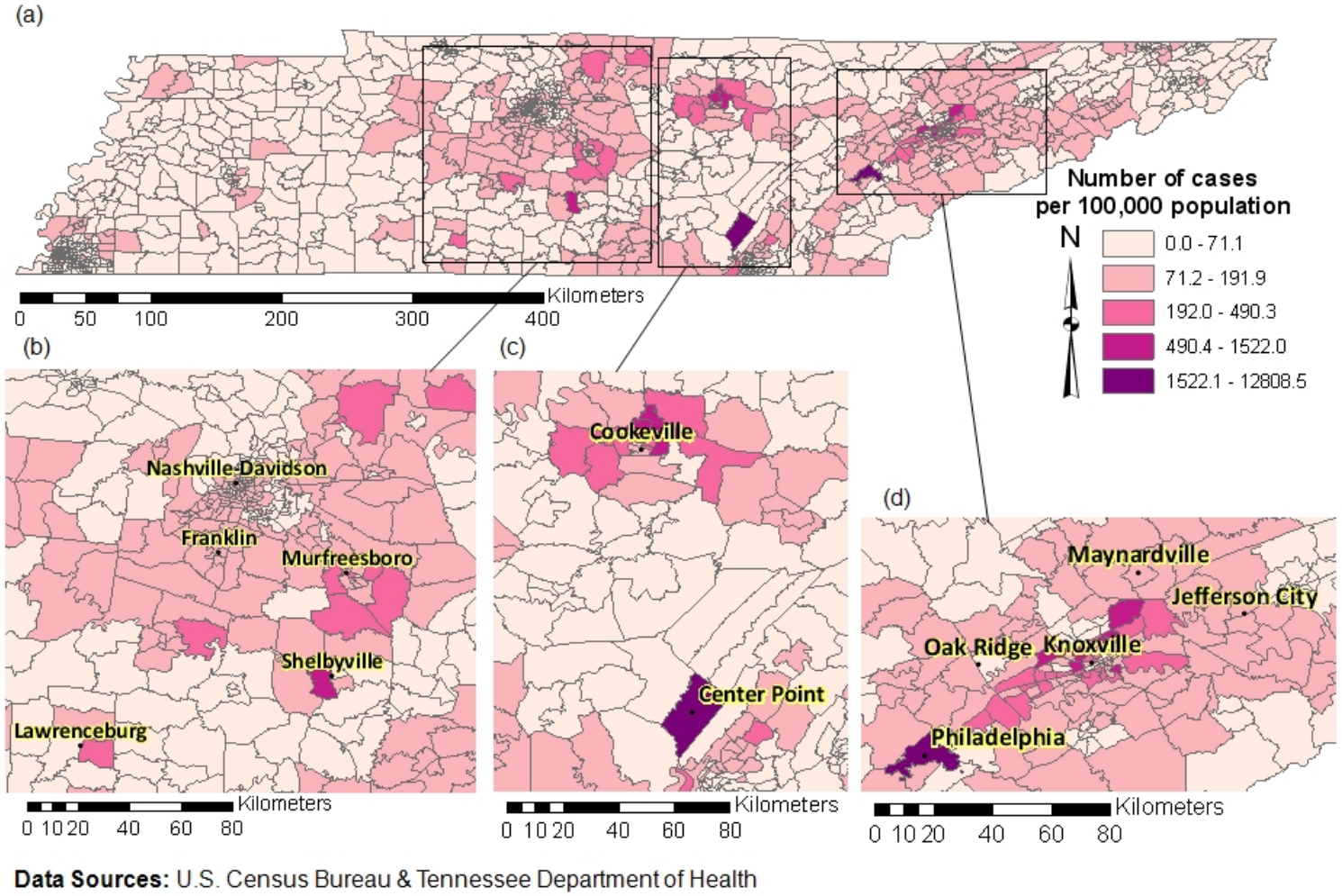


Figure 4.3. Spatial empirical Bayesian smoothed risk of campylobacteriosis in Tennessee at the census tract spatial level. The three regional inserts highlight important high risk areas at this scale. The risks are standardized by age and gender.

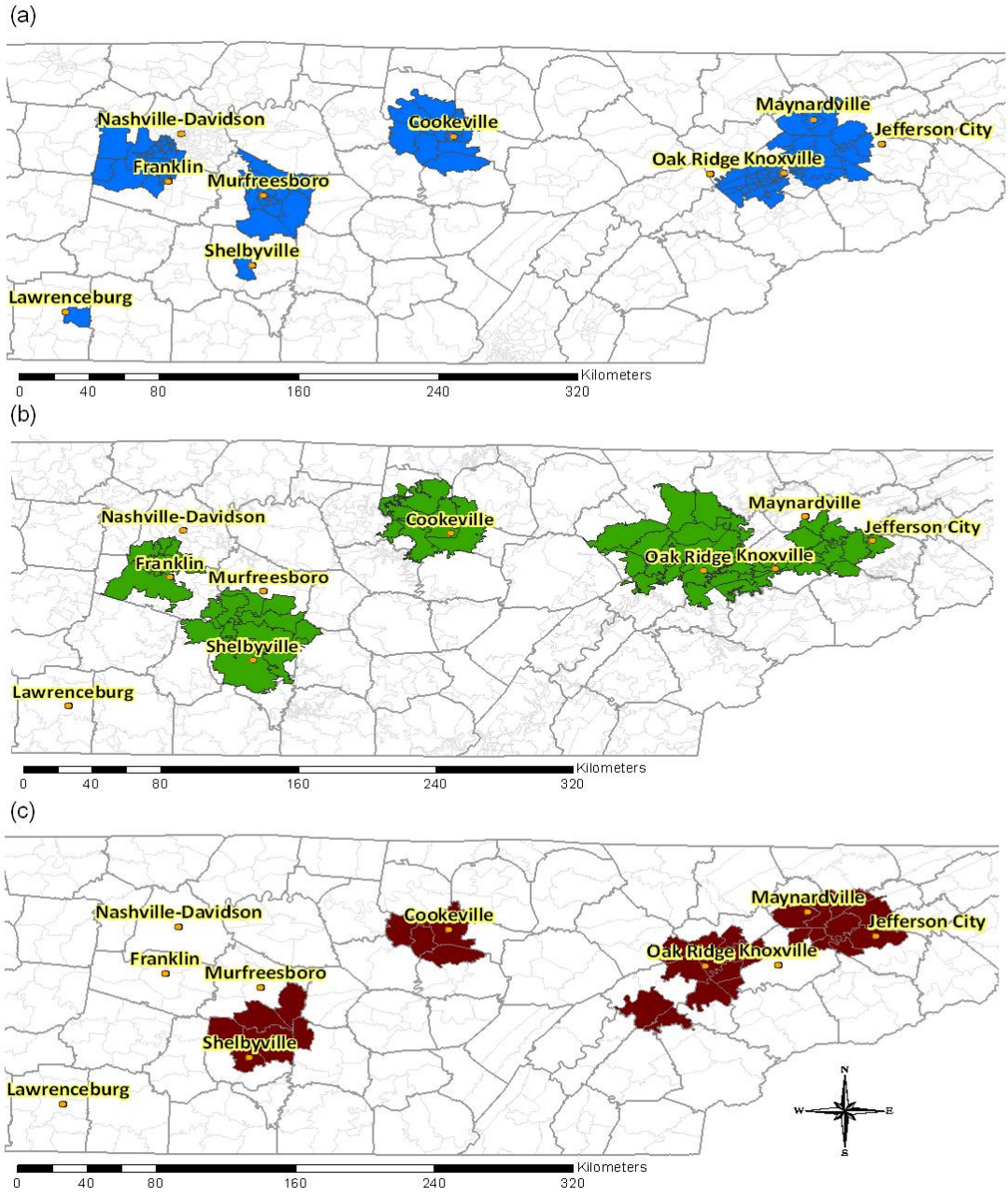
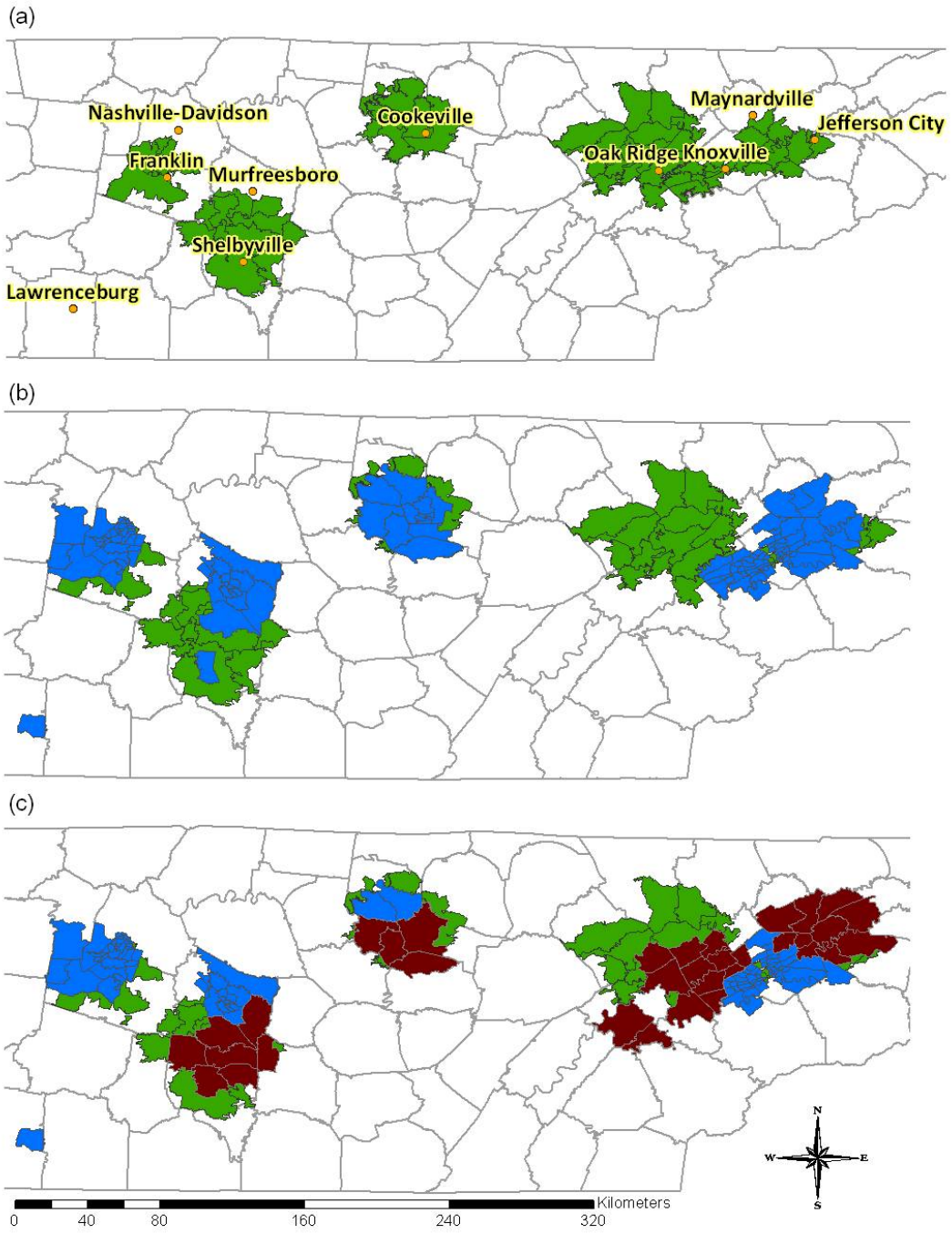


Figure 4.4. Significant high risk clusters using 3% scanning window at three levels: (a) county subdivision, (b) zip code, (c) census tract.



Data Sources: U.S. Census Bureau & Tennessee Department of Health

Figure 4.5. Magnified view of Tennessee demonstrates simultaneous overlay of clusters using 3% scanning window: (a) includes only the zip code (green), (b) contains the zip code and census tract (blue) and (c) shows all three (from bottom to top): zip code, census tract and county subdivision (brown).
Note: Order of overlay was chosen for improved visualization.

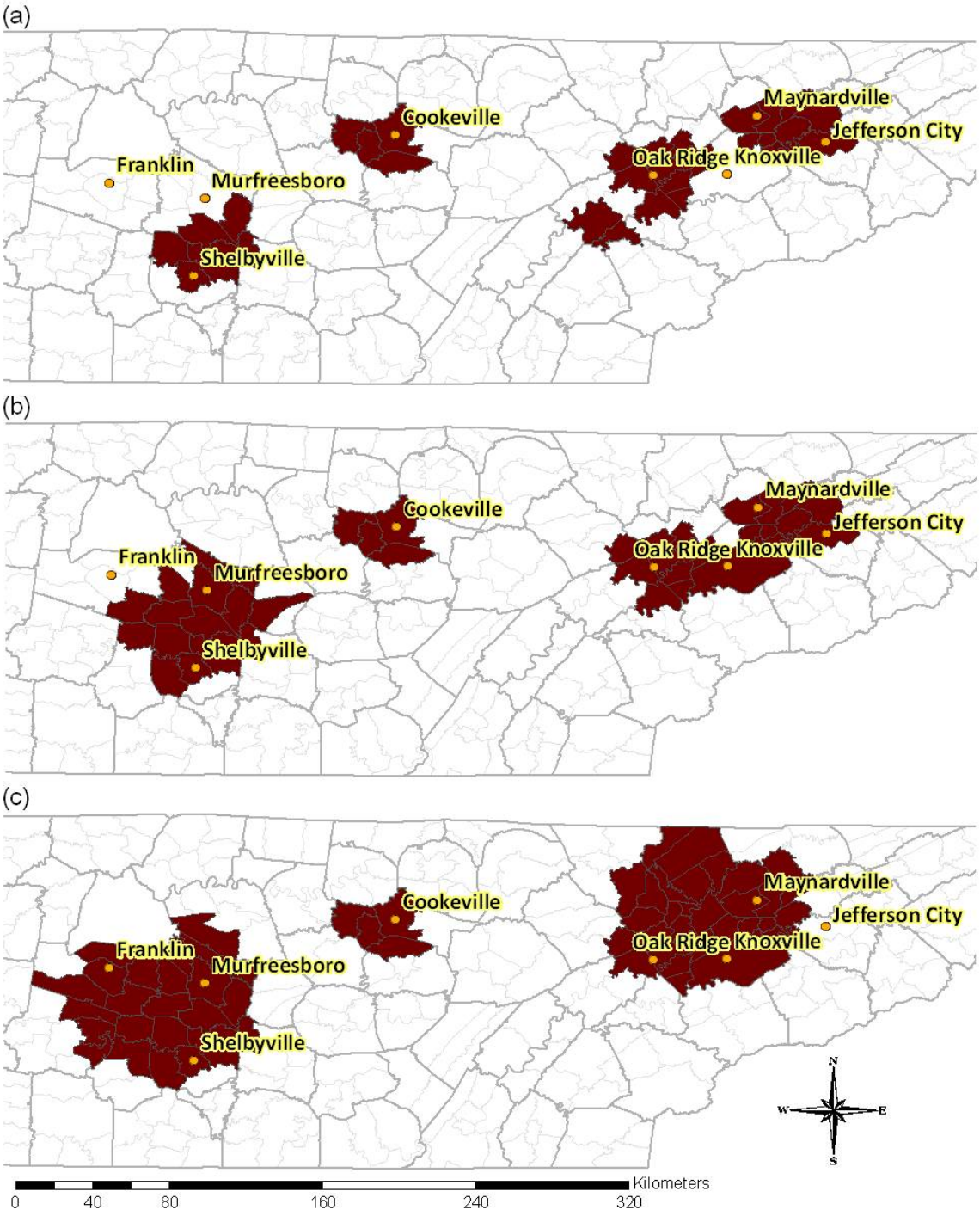


Figure 4.6. Significant ($p<0.05$) high risk cluster results for county subdivision spatial level using (a) 3% scanning window, (b) 5% scanning window, (c) 10% scanning window.

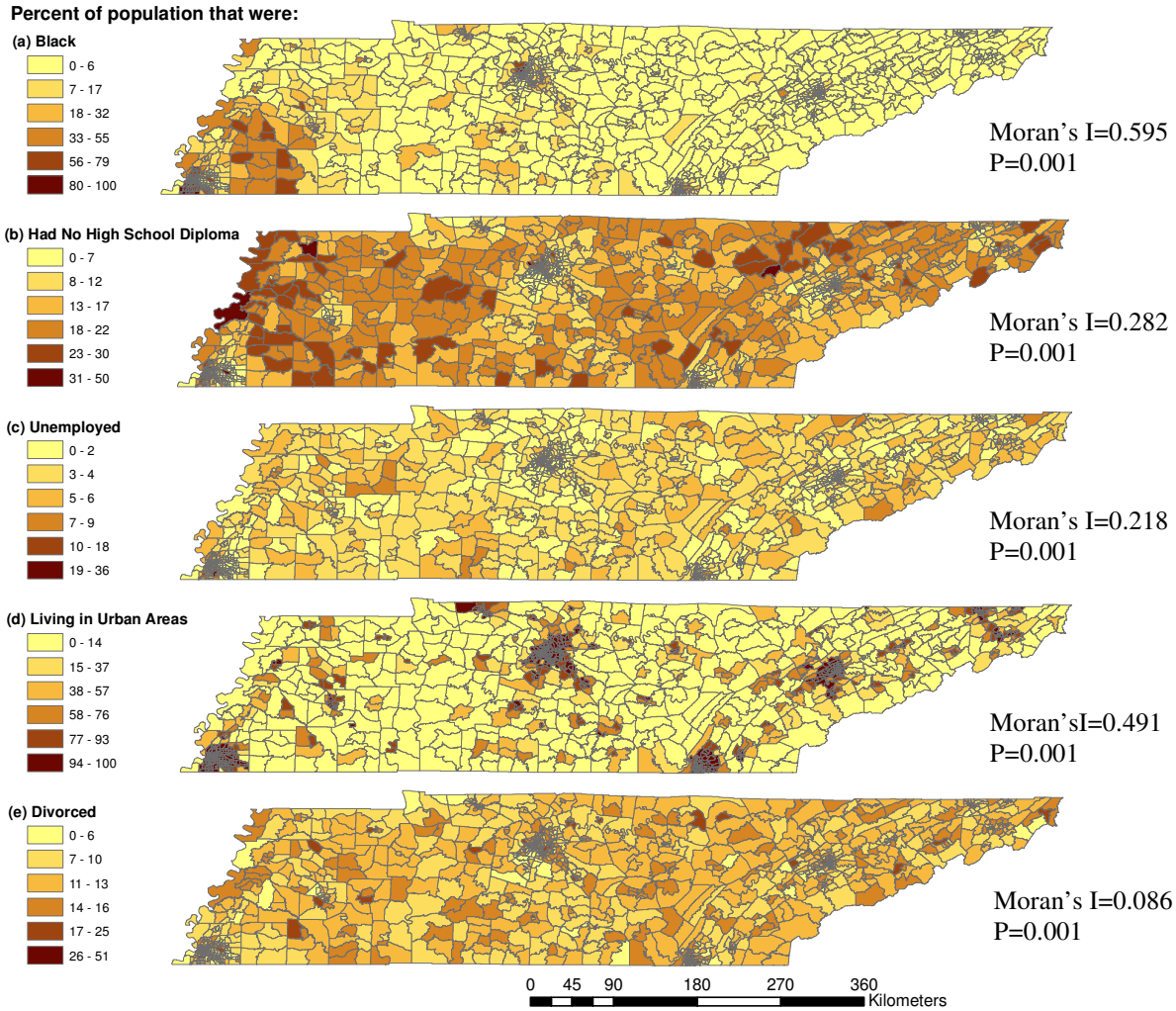


Figure 5.1. Geographic distribution of selected socioeconomic variables investigated for potential association with campylobacteriosis risk in Tennessee.

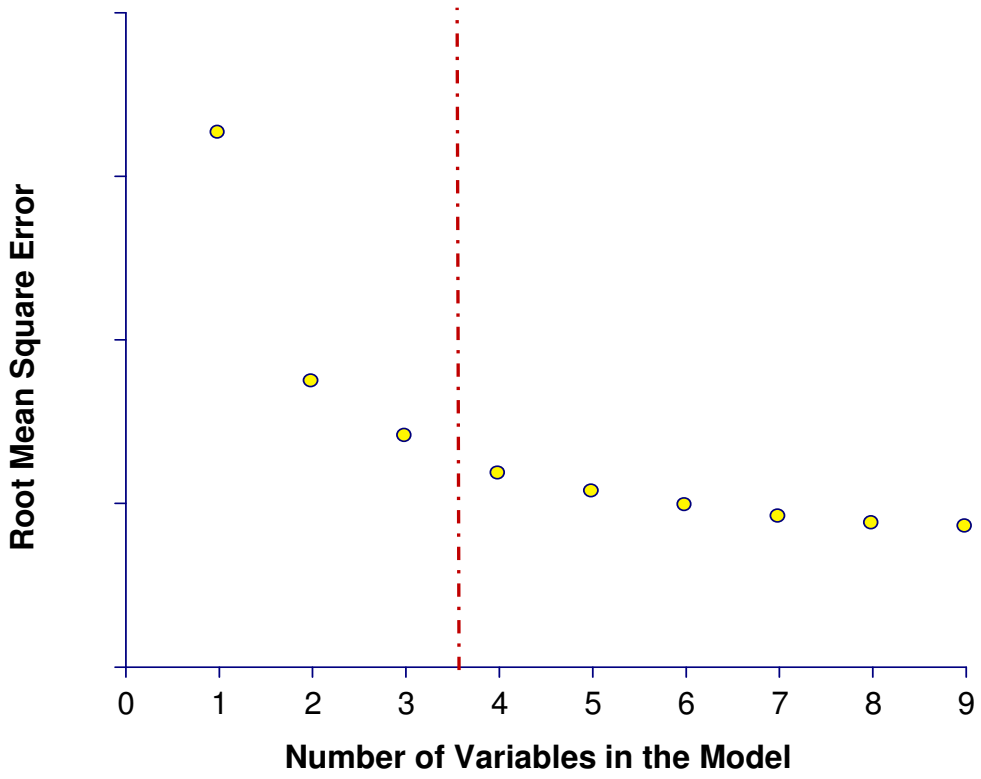


Figure 5.2. McHenry's All Possible variable selection procedure scree plot demonstrating root mean square error improvement in the top model combinations. Improvement is optimized with four variables (dashed red line).

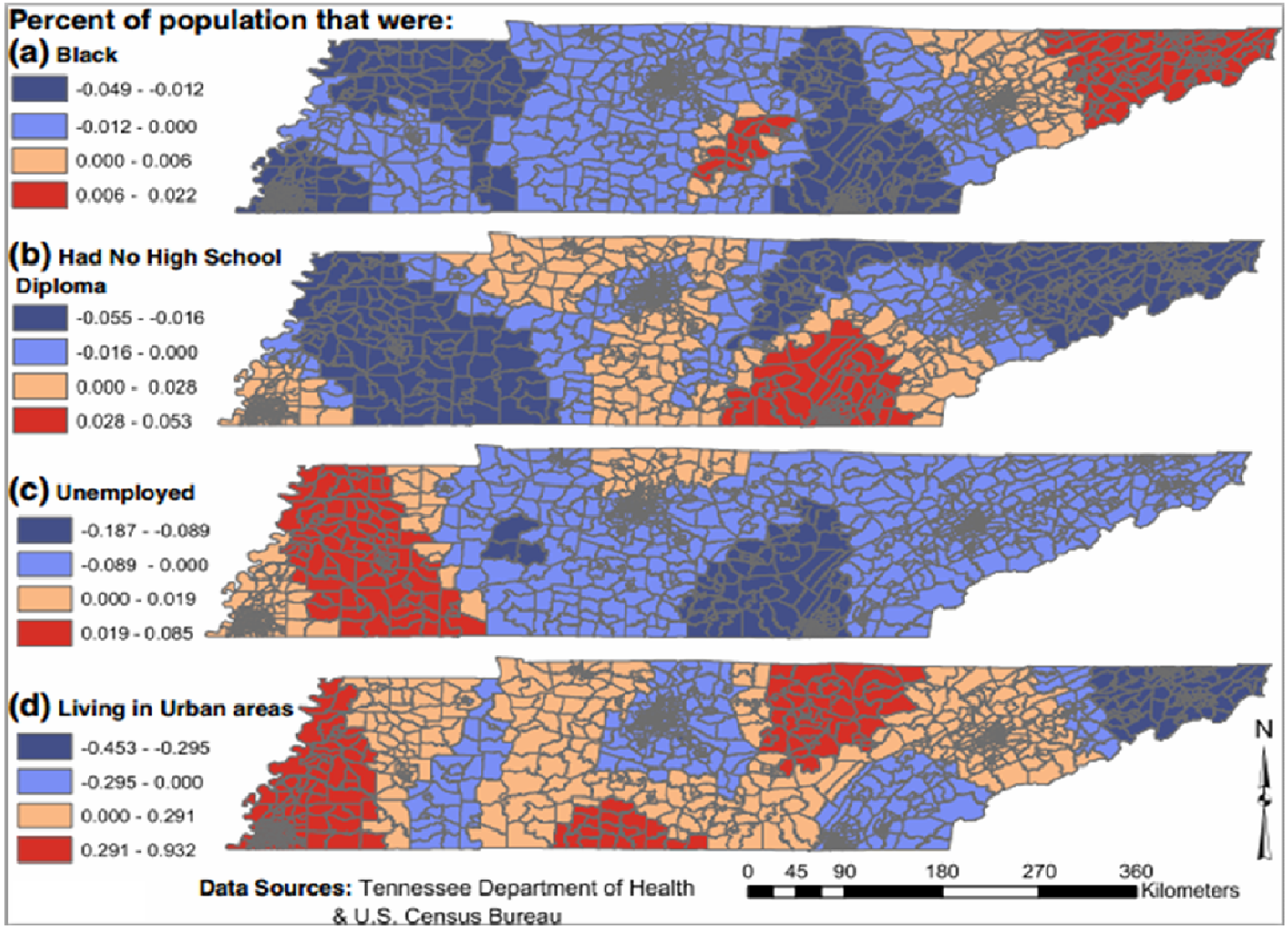


Figure 5.3. Model 1 geographically weighted parameter estimates of the significant socioeconomic determinants of campylobacteriosis risk in Tennessee.

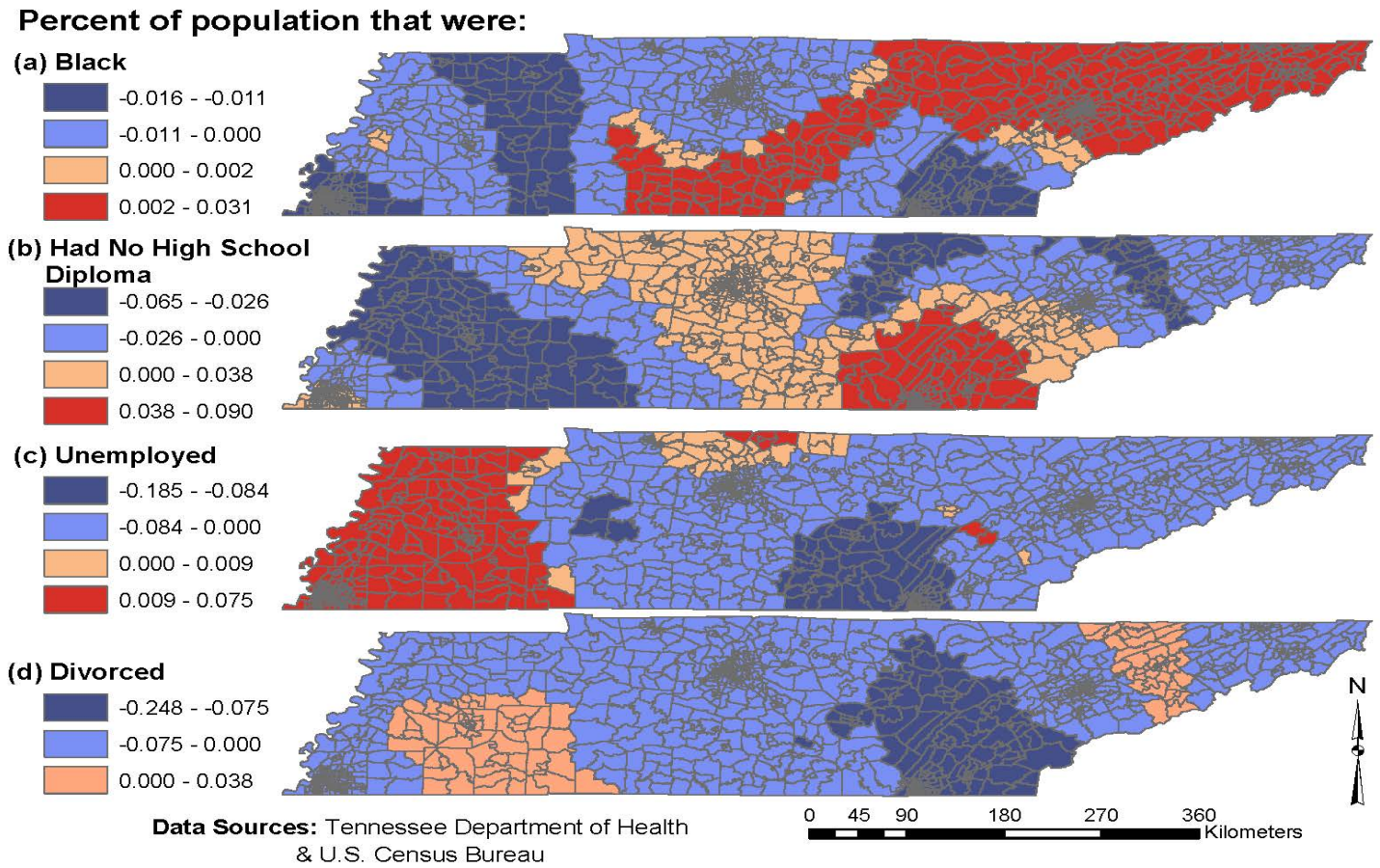


Figure 5.4. Model 2 geographically weighted parameter estimates of the significant socioeconomic determinants of campylobacteriosis risk in Tennessee.

Vita

Jennifer Weisent was born in Queens, New York on March 6, 1973. She graduated from Mattituck High School in 1991 and attended Cornell University where she received a Bachelor of Science degree in Animal Science in 1995. She served as a Peace Corps volunteer for two years in Ecuador, designing and implementing sustainable animal and agriculture farming systems with the indigenous Quechua. She then attended Ross University School of Veterinary Medicine, on the island of St. Kitts, West Indies and received her DVM in 2003. She worked in small animal private practice and shelter medicine in New York for three years, specializing in pediatric critical care and spay/neuter surgery. In 2007 she began her doctoral studies in the Department of Comparative and Experimental Medicine at the University of Tennessee in Knoxville, TN where she received her PhD in Epidemiology with a minor in statistics. In the coming year, Jennifer intends to continue self-directed epidemiological research, complete her first full length non-fiction book, as well as continue to publish her poetry and personal essays.