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Human *Campylobacter* and *Salmonella* infections in Michigan:
Environmental Drivers

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HUMAN *CAMPYLOBACTER* AND *SALMONELLA* INFECTIONS IN MICHIGAN: ENVIRONMENTAL DRIVERS

By

Tiffiani Joy Onifade

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ABSTRACT

HUMAN *CAMPYLOBACTER* AND *SALMONELLA* INFECTIONS IN MICHIGAN: ENVIRONMENTAL DRIVERS

By

Tiffiani Joy Onifade

BACKGROUND: Campylobacteriosis and Salmonellosis are common gastrointestinal infectious diseases primarily associated with foodborne routes of infection. Given the high incidence and health burdens of these diseases, much research has been done to reduce human transmission via that route. However, some of the national reported trends in the diseases (incidence peaks in the summer months and geographic variation) have not been fully explained and may suggest that other factors are driving these disease trends. OBJECTIVES: This study will explore these driving factors through three major objectives: 1) to analyze the incidence of historical human *Campylobacter* and *Salmonella* infections in Michigan with respect to demographic, geographic, and temporal trends (including evaluation of the seasonal high reporting period; obj 1b), 2) to evaluate the role of environmental and climatological factors in relation to changes in human incidence of *Campylobacter* and *Salmonella* infections in Michigan, and 3) to evaluate methods for analyzing *Campylobacter* and *Salmonella* environmental prevalence. METHODS: Objectives 1a & 1b: Retrospective study design along with linear modeling statistical techniques were used to evaluate the Michigan historical case data (1992-2005). Objective 2: Retrospective study design was used to evaluate historical case and environmental data creating Poisson Mixed regression models for statistically significant relationships between the incidence of disease and specific environmental factors. Objective 3: Published environmental sampling and laboratory culture methods were evaluated and hybridized to create cost effective, time efficient, reliable culture enumeration techniques. FINDINGS: 1a) Though exhibiting similar trends, incidences of these diseases in Michigan were substantially lower than national reports. 1b) Parameters of the seasonal high reporting period in Michigan varies by geography. 2) Environmental and weather related factors significantly explained some of the variation in incidence of the diseases. 3) The hybrid culture enumeration methods evaluated produced inconsistent results. CONCLUSIONS: This study aimed to add to the literature by explaining and filling a gap in the chain from animals to humans. By focusing on the environmental connections that may explain some of the variation in human rates of these diseases, the study was able to begin evaluating a missing link. The food route has been explained, and the water route has also been explained, but here the environmental contamination link between animals and the abundance of the bacteria through the environment was explored. By modeling the environmental effects on transport and prevalence of these bacteria in the farming and surrounding environments this has been a major step in understanding trends in prevalence and will potentially provide insight into how to lessen transmission between these animals and on to humans.

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INTRODUCTION

Purpose

In recent years, there has been a surge in research on the effects of environmental factors on, and their relations to, diseases given our changing climate and environment on macro (global warming) and micro (urbanization) levels, and some of this research has focused on the environmental effects on foodborne diseases. Campylobacteriosis and Salmonellosis are common infectious diseases caused by infection with *Campylobacter* spp. and *Salmonella* spp and are primarily associated with foodborne routes of infection. The growth and transport of the responsible bacteria can be influenced by the weather and several studies have evaluated the effect of ambient temperature on foodborne diseases that included campylobacteriosis and salmonellosis. Most causes of campylobacteriosis and salmonellosis are foodborne, however given previous research there is evidence that food may not fully explain the reported seasonal increases. The role of environment and climate in the transmission of these bacteria is complex, as there are many factors to consider (temperature, precipitation, landuse (agricultural, urban), water source, ect.). Given all this, there has not been a comprehensive analysis on the effects of a sum of environmental factors on the transport of *Campylobacter* and *Salmonella* and the association with human incidence of these infections. This study aims to add to the literature offering that analysis by retrospectively evaluating the associations of recorded environmental factors with reported human cases of *Campylobacter* and *Salmonella* infections and evaluating culturable environmental prevalence of these bacteria.

Specific Aims and Hypotheses

This research project, "Campylobacter and Salmonella Infections in Michigan: Environmental Drivers," consists of four smaller studies: two descriptive studies, (The Epidemiology of Campylobacteriosis and Salmonellosis in Michigan and *Campylobacter* and *Salmonella* Infections in Michigan: Evaluation of Seasonal and Geographic trends in Reporting (1992-2005), an ecological retrospective study, (Environmental Factors Influencing Rates of Human *Campylobacter* and *Salmonella* Infections in Michigan), and an environmental prevalence study (*Campylobacter* and *Salmonella* on Michigan Dairy Cattle Farms: Culture Isolation and Enumeration from Environmental Soil and Water). The long term objectives of this project were to identify and describe trends in rates of human *Campylobacter* and *Salmonella* infections and to evaluate the environmental and weather factors that statistically influence or explain them. The specific aims and hypotheses for each part of the study are given below.

<u>Descriptive Studies</u>

Descriptive Study 1: The Epidemiology of *Campylobacter* and *Salmonella* Infection Rates in Humans in Michigan (Chapter 2)

- Hypotheses
    - Incidences of *Campylobacter* and *Salmonella* infection in humans in Michigan will be comparable to those nationally with respect to temporal and demographic trends.
    - There will be geographic variation in the incidence of *Campylobacter* and *Salmonella* infections in Michigan.
- Specific Aims
    - To analyze historical trends in *Campylobacter* and *Salmonella* rates in Michigan with respect to temporal, demographic, and geographic trends
    - To identify counties with consistently high and consistently low incidence of *Campylobacter* and *Salmonella* infections

Descriptive Study 2: *Campylobacter* and *Salmonella* Infections in Michigan: Evaluation of Seasonal and Geographic Trends in Reporting (1992-2005) (Chapter 3)

- Hypotheses
    - There will be variation in the parameters of the seasonal reporting peak that will we explained by geographic variables.
- Specific Aims
    - To statistically evaluate the seasonal trend in case reporting in Michigan, identifying parameters peak-week, start and end-week, and duration of the high reporting period

- To evaluate the relation of geographic location and county incidence to high reporting period parameters

Ecological Retrospective Study

Environmental Factors Influencing Rates of Human *Campylobacter* and *Salmonella* Infection in Michigan (Chapter 4)

- Hypotheses
  - Environmental and weather related factors will be associated with changes in rates of *Salmonella* and *Campylobacter* infections in Michigan such that:
    - As temperature increases incidence of these diseases will increase.
    - As precipitation increases incidence of these diseases will increase.
    - Areas with more agricultural land use sources will be areas with higher incidences of these diseases.
    - As percentages of homes with non-municipal water and sewage disposal increase rates so these diseases will increase.
- Specific Aims
  - To evaluate the role of environmental and climatological factors in relation to changes in incidences of human *Campylobacter* and *Salmonella* infections in Michigan.

Field study

Campylobacter and *Salmonella* on Michigan Dairy Cattle Farms: Culture Isolation and Enumeration from Environmental Soil and Water (Chapter 5)

- Hypotheses
  - Culture methods can be used in conjunction with MPN to enumerate *Campylobacter* and *Salmonella* from environmental soil and water samples.
  - Recovery of *Campylobacter* and *Salmonella* will vary with temperature.
  - There will be variation in the amounts of *Campylobacter* and *Salmonella* present in the farm soils and surrounding waters that will relate to human incidence of these infections in county.
  - There will be a relationship between temperature and the amount of *Campylobacter* and *Salmonella* in cattle farm soils and surrounding waters such that higher prevalence of the bacteria in the environment corresponds to warmer temperatures.
- Specific Aims
  - To identify and validate methods for the enumeration of *Campylobacter* and *Salmonella* from environmental soil and water samples.
  - To evaluate the effect of temperature on *Campylobacter* and *Salmonella* recovery from soil and water.

- To evaluate Michigan dairy farms for the presence of *Campylobacter* and *Salmonella* in soil and water.

Overview

This research has been conducted with the goal of describing particular trends and geographical patterns with the hopes that this research can be used to predict variability in incidence of campylobacteriosis and salmonellosis in Michigan so that in future public health measures can be put into place to lessen the transmission. This dissertation is arranged into five major sections with a Literature Review: Drivers of *Campylobacter* and *Salmonella* Infections: Known and Suspected (Chapter 1), an analysis of the Epidemiology of Campylobacteriosis and Salmonellosis in Michigan (Chapter 2), the Evaluation of Seasonal and Geographic Trends in Reporting (Chapter 3), the modeling of Environmental Factors Influencing Incidence of Campylobacteriosis and Salmonellosis in Michigan (Chapter 4), and Culture Isolation and Enumeration of *Campylobacter* and *Salmonella* from Michigan Dairy Farm Environmental Soil and Water (Chapter 5).

The literature review details the background and historical reporting on campylobacteriosis and salmonellosis to better understand the known national trends and drivers influencing the burden of the disease. Chapter 2 progresses into the series of research studies by evaluating historical case data to describe these epidemiological trends for Michigan (a state that has not been included in the national extrapolations). The seasonal peak in reporting for both of these infections has also been noted in the literature but, Chapter 3 goes on further defining and describing the parameters associated with these reporting trends and evaluating possible geographic relationships. Chapter 4 evaluates environmental influences with the goals of explaining additional variability in incidence of campylobacteriosis and salmonellosis in Michigan that is not related to demographics. After the detailed evaluation of historical *Campylobacter* and *Salmonella* infection data and relating the trends to environmental factors (Chapters 2-4), Chapter 5 takes aim at the environmental prevalence. This chapter evaluates methods for the culture isolation and enumeration of these bacteria from environmental soil and water samples.

CHAPTER 1

Drivers of Campylobacter and Salmonella Infections: Known and Suspected A Review

1.1 Introduction

The notion that climate and health are linked was suggested as far back as Hippocrates, where he related the two around 400 BC (Rees, 1996). Through the years the idea lingered and in the Middle Ages herbalists would prescribe different remedies depending on the season. Today, we no longer believe that weather itself causes disease, but we are beginning to understand how it can create conditions for disease-causing organisms to thrive and migrate into areas where human exposure may occur, such as water sources. These types of relationships and the links to a changing global climate have been identified for diseases ranging from malaria and dengue fever to cholera (Lipp et al., 2002).

Campylobacter and *Salmonella* are commonly reported causes of bacterial enteritis in the United States, and throughout the world (Altekruse et al., 1999,Oberhelman and Taylor, 2000, Coker et al., 2002, USDA, 2003). They are generally considered food borne pathogens, but waterborne outbreaks are also known to occur (Blaser et al., 1979, Blaser et al., 1983, Palmer et al., 1983, Skirrow, 1991, Fahey et al., 1995, Ashbolt, 2004). It is estimated that 1% of the U.S. population is infected yearly by these disease, which primarily cause gastrointestinal illness including diarrhea, nausea, and bloody stool, but could lead to life threatening illnesses (WHO, 2003, Buzby and Roberts, 1997, Medema et al., 1996, Nachamkin, 2002, USDA, 2003, Mead et al., 2004). The health burden for these diseases is great, as there are sequales to infection for *Campylobacter* and *Salmonella*, Guillian-Barré syndrome and reactive arthritis, respectively, and death can occur from infection in vulnerable populations, immune compromised and the elderly.

Campylobacter and *Salmonella* transmission and trends for the diseases have been studied and noted in the literature for the United States and around other parts of the world. Poultry (Blaser et al., 1983, Skirrow, 1991), milk (Blaser et al., 1979, Fahey et al., 1995), and water have been implicated as major sources of infection (Blaser et al., 1983, Palmer et al., 1983, Ashbolt, 2004). This is of particular concern in Michigan, given the importance of the state's agriculture industry.

Many efforts have been made to curb transmission via the food borne route, and nationally a decline in *Campylobacter* cases has been seen (Van Gilder et al., 1999, Samuel et al., 2000, Samuel et al., 2004). This decline is significant 23% between 1996 and 2000 (Samuel et al., 2004, CDC, 2004) however, in recent years the national decline has slowed and *Salmonella* rates have only decreases slightly. A strong seasonal effect has been observed in the United States and elsewhere, where *Campylobacter* and *Salmonella* cases peak in the summer months and even with the overall decline in rates, the seasonal peaks remain (Padungton and Kaneene, 2003, Miller et al., 2004, Louis et al., 2005, Nylen, 2002, Lindback and Svensson, 2001, Potter et al., 2002). The relationship between infection rates and season of the year suggests a possible link to weather patterns. Determining the factors that influence transmission of the disease provides critical information for understanding the epidemiology of these diseases.

The aim of the literature review is to formulate the background and history of campylobacteriosis and salmonellosis to better understand the known trends and possible drivers influencing the burden of the disease.

1.2 Objectives

The objectives of this literature review were to:

1. Explore published literature on the epidemiology of human *Campylobacter* and *Salmonella* infections around the transmission of the diseases.
2. Evaluate studies on the environmental associations with reported cases of these infections.

1.3 Epidemiology of Campylobacteriosis and Salmonellosis

Campylobacter spp. and *Salmonella* spp., causative agents of Campylobacteriosis and Salmonellosis, are microbial pathogens known to be transmitted through eating foods that have not been properly prepared as these bacteria are commonly found on raw and undercooked meats (Blaser et al.1983, Skirrow, 1991). Currently, *Campylobacter* is the most commonly reported cause of acute bacterial gastroenteritis in developed countries (Mead et al. 1999) and *Salmonella* annually causes 1.3 billion cases world-wide. The health burden for these diseases is great, as there are sequels to infection for *Campylobacter* and *Salmonella*, Guillian-Barré syndrome and reactive arthritis, respectively, and death can occur from infection in vulnerable populations, immune compromised and the elderly. Given the high prevalence and health burdens for these diseases much research has been done examining the foodborne route to understand the trends in transmission and to minimize infection. However, there are other transmission routes for these bacteria that may be under appreciated in their contributions to the trends in disease.

Globally, microbiological contamination of water is the most common source of diarrhea-causing pathogens and may be the number one cause of childhood mortality. In the United States alone nine million cases of waterborne disease may occur annually (Mead et al. 1999). *Campylobacter,* frequently associated with poultry and other livestock, is often transmitted by the water route (Blaser et al.1983, Skirrow, 1991, Kapperud et al. 1992, Ashbolt, 2004). Likewise, *Salmonella*, a common zoonotic agent that can be transmitted by water, is one of the top three causes of waterborne disease (Ashbolt, 2004, Altekruse et al. 1999). However, this water route of infection has not been as extensively studied to evaluate environmental sources of contamination and environmental prevalence and transport of the bacteria that may lead to increased transmission to humans.

Many factors contribute to the human incidence of *Campylobacter* and *Salmonella* infection, and there is growing evidence that temporal and environmental factors are associated with incidence of these diseases (Rosef and Kapperrud 1983, Louis et al. 2006). Seasonality in human cases and environmental prevalence of both pathogens has been noted in the literature with peaks reported generally in the summer months (Louis et al. 2006). In the UK, *Campylobacter* detections in watersheds increased with or just prior to peaks in human cases in the late spring and early summer (Louis et al. 2006, Eyles et al. 2003). Highest frequency of *Salmonella* isolations from humans occurred in late summer months, and was also associated with increased rainfall. Dairy cattle infection with *Campylobacter* and *Salmonella* also show a seasonal trend in shedding rates (Jones, 2001). Given the associations of these bacteria with seasonal trends, temperature, and rainfall, there may be significant environmental factors driving these relationships.

Many epidemiological studies of foodborne disease in the United States have relied on FoodNet data (beginning in 1996) for analysis and description of the trends in the United States (Tauxe et al. 2004). Ten states participate in the FoodNet Program and although the states are diverse, all the data in some states come from only a few counties in that state. FoodNet provides the basis for a limited sample with cases only recorded from 1996 and the use of select regions of the country. This database has been used to track the effectiveness of foodborne pathogen control programs. In 1996, Hazard Analysis and Critical Control Points (HACCP) rules for poultry processing were implemented and FoodNet studies identified an annual decline in rates of *Campylobacter* infection from 1996 to the present. The reports attributed this decline to the new rule implementations, however, the seasonal trends (cases peaking in the summer months) remained (Buchanan and Whiting, 1998, Samuel et al. 2004).

Campylobacter spp.

Campylobacter has been reported as a cause of human enteric disease for over 100 years and it has probably existed for many centuries (Kist, 1985). The first mention of a *Campylobacter*-like bacterium occurs in 1886 when Theodor Escherich isolated a spiral bacterium from the intestinal mucus of people who died of diarrheal disease and from the stool of others with enteric diseases (Kist, 1985). Because of its similar comma-shaped appearance it was classified in the *Vibrio* genus (Sebald, 1963). In 1913, the same bacterium was isolated from bovine fetuses (Kist, 1985). It was not until 1957, that King described this "*Vibrio*" as the agent for the enteritis and later linked it to animals (Kist, 1985). With further investigation, Sebald and Veron (1963) found that the metabolism of this *Vibrio* was very different from others in the genus; this lead to the classification of a new genus, *Campylobacter* (from Greek meaning curved rod) (Sebald and Veron, 1963). In 1968, a technique was developed to isolate this microphile, and then improved in 1977, to isolate *Campylobacter* from feces. (Kist, 1985) This procedure allowed for further study of *Campylobacter* leading to more diagnoses and treatment. For more than thirty years, *Campylobacter* has been the leading cause of diarrheal illness in the United States, causing more disease than *Shigella* spp. and *Salmonella* spp. combined (USDA, 2003, Altekruse et al., 1999). It still persists as an important cause of enteritis.

Campylobacter spp. are motile, Gram-negative slender bacteria with a curved rod-shape. They range in size from 0.2-0.9 µm in width to 0.5-5 µm in length (Nachamkin, 2002). The flagellum can be monotrichous or amphitrichous and moves by corkscrew motion. Members of this genus are microaerophillic and thrive in an environment with 3-5% oxygen, 2-10% carbon dioxide, and 85% nitrogen. They are also thermopihlic, with optimum growth conditions at temperatures between 37 and 42° C, with better growth at the upper end of this range. *Campylobacter* are susceptible to environmental stresses such as freezing, drying, acidic conditions, and salinity (Altekruse, 1999).

The campylobacteria (which includes the genera *Campylobacter* and *Arcobacter*), also known as campylobacters can be divided into two classes based on a positive or negative catalase reaction. Catalase-negative campylobacters are sensitive to oxygen and require lower oxygen content (3% O_2) for growth. They are also able reduce nitrates and nitrites. Catalase-positive campylobacters can thrive in environments with higher oxygen content (5% O_2) and are able to reduce nitrates but not nitrites. *Campylobacter jejuni* and *C. coli,* the major causes of campylobacteriosis, are both catalase-positive campylobacters (Butzler, 1984).

<u>*Salmonella* spp.</u>

Salmonella was discovered by Theobald Smith in 1885 when it was isolated from pigs, and was named for Daniel Elmer Salmon. It was later discovered to be relevant as a human infectious agent. In 1920, Sir William Savage published the book “Food Poisoning and Food Infections” that detailed the previous 40 years of food poisoning outbreaks, many due to *Salmonella* (Savage 1920). Since then non-typhoidal *Salmonella* has been recognized as a leading cause of gastrointestinal illness world wide.

Salmonella are gram-negative, non spore forming rod shaped bacteria. They are usually motile with peritrichous flagella ranging in size from .7-1.5um diameter to 2-5um in length. They are also thermophilic with optimum growth conditions at temperatures between 35 and 37 C and pH between 7 and 7.5. *Salmonella* are susceptible to stresses of disinfectants and high temperatures. *Salmonella* are members of the family Enterobacteriaceae the genus Salmonella and one of the two species Enterica or Bongori. The over 2400 identified serotypes of *Salmonella* are further classified into two groups based on the O (somatic/cell wall) antigens or the H (flagellar) antigens. In the United States, serotypes *S.* Typhimurium, *S.* Enteritidis, and *S.* Newport make up over 50% of serotypes isolated from infected humans.

RESERVOIRS AND TRANSMISSION OF CAMPYLOBACTER AND SALMONELLA

Various warm-blooded animals serve as *Campylobacter* and *Salmonella* reservoirs including poultry, cattle, swine, sheep, dogs, cats, and rodents (Atwill, 1995, Cummings et al. 2009, Stanley and Jones, 2003, Farzan et al 2009). The USDA estimates that between 20 and 100% of retail chicken is contaminated with *Campylobacter* and a recent study found 22% contaminated with *Salmonella* (USDA, 2003, Lestari et al. 2009). Additionally, natural waters, sediment and sewage sludge have been found to contain these pathogens (Droppo et al. 2009, Ahmed et al. 2009, Lucey et al., 2000, Ashbolt, 2004, Sahlstrom et al., 2004, Jones, 2001).

Both campylobacteriosis and salmonellosis can occur as sporadic cases or as outbreaks. Outbreaks come from a single source such as in the town of Bennington, VT where 200 people were infected with *Campylobacter* by consumption of non-chlorinated drinking water (USDA, 2003) or the California *Salmonella* outbreaks associated with unregulated interactive water fountains (Kirian et al. 2008). Sporadic cases are often thought to have a foodborne or waterborne origin such as eating undercooked poultry or drinking unpasturized milk or untreated water (CDC, 2004). The infectious dose is low for both *Campylobacter* (400-500 bacteria) so one drop of juice from raw meat can cause infection (CDC, 2004) and for Salmonella where the infectious dose can vary based on the vector food source (Dunlop 1985).

Because the ideal growing environment for *Campylobacter* and *Salmonella* are in warm environments and they require hosts (Altekruse et al., 1999), it does not proliferate easily outside of the gut (Ketley, 1997). Therefore, reservoirs provide critical links to human disease. Livestock, domestic animals, and birds are some of the commonly known reservoirs for *Campylobacter* spp. (Atwill, 1995, Lefebvre et al. 2008, Stanley and Jones, 2003) and are shed in the feces of these animals in various concentrations throughout the year. *Campylobacter jejuni* can be isolated year round from slurry tanks around sheep farms (Stanley and Jones, 2003) and year round in varying amounts from environmental pig slurry (Mannion et al. 2007). Land application of fecal waste could lead to further contamination of the environment and possible runoff into nearby waterways.

DISEASE: CAMPYLOBACTERIOSIS AND SALMONELLOSIS

The disease caused by any member of the *Campylobacter* genus is termed campylobacteriosis or *Campylobacter* enteritis. *Campylobacter jejuni* causes over 99% of human cases (CDC, 2004). *Campylobacter* enteritis is a disease of interest to public health because of its high frequency in the population and potential chronic effects. The symptoms of the disease include mild or severe diarrhea often accompanied with fever and traces of blood in the stool. Symptoms often appear within two to five days of exposure and persists usually for one week. In immunocompromised persons, the bacteria can spread to the bloodstream and cause life-threatening infection. *Campylobacter* infection is also believed to be a precursor to Guillian-Barré Syndrome, an autoimmune disorder that can cause paralysis (Nachamkin, 2002, Takanhashi, 2005). One in 1,000 campylobacteriosis cases lead to Guillian-Barré syndrome (Allos, 1997).

Campylobacteriosis patients are treated with antibiotics and generally recover within one to two days. Without treatment, *Campylobacter* continues to be excreted even after a patient has recovered; cells may be shed in the feces for days to several weeks post-infection (Bulzer, 1984). Due to the amount of time that the organism is excreted there are potential environmental ramifications such that if sewage is not properly treated further transmission of disease is possible.

Campylobacteriosis is a global health concern. In developing countries, rates for *Campylobacter* infection are high, with 5% to 20% of the population infected annually, depending on the country (Oberhelman and Taylor, 2000). The incidence of campylobacteriosis for children under five years old in developing countries is 40,000 cases per 100,000 children under five (40% of the <5 population; Coker et al. 2002). In general, there is an increasing incidence of the disease in developing countries and an expanding spectrum of related diseases caused by *Campylobacter*. With the high incidence of HIV in developing countries there is consequently a greater potential for HIV-related deaths due to *Campylobacter* (Coker et al. 2002).

In developed countries, the rate of infection is lower, for example 1% of the United States population is infected each year (WHO, 2004). In the United States and other developed countries, *Campylobacter* remains the most frequently isolated bacterial enteric pathogen from clinical samples (WHO, 2004). In 1997, the reported incidence of campylobacteriosis in the United States was 25.2 people for every 100,000 people; however, it is estimated that about 1% of the US population are actually infected each year with *Campylobacter* (WHO, 2004). In the US, U.K., Canada, Denmark case rates are declining (Samuel et al., 2004, FDSCG, 2002, Samuelsson, 2004); however, in Australia cases have risen dramatically (CDA, 2005). The prevalence of the disease among children under 5 is also noted in developed nations but this peak is less dramatic and the disease is still common among other age groups (Coker et al. 2002, Padungton and Kaneene, 2003).

Salmonellosis is the disease caused by infection from *Salmonella*. Serotypes *S.* Typhimurium, *S.* Enteritidis, and *S.* Newport make up over 50% of serotypes isolated from infected humans in the United States. Symptoms of this disease include diarrhea, fever, vomiting, and abdominal cramps. Healthy individuals often recover within 5-7 days without treatment but occasionally severe dehydration will require hospitalization for intravenous hydration and antibiotics when the infection spreads from the intestines. Chronic infection can lead to Reiter's syndrome (pain in the joints or eyes) or chronic reactive arthritis. There is an estimated 400 deaths annually attributed to salmonellosis.

TRENDS IN DISEASE

Demographics

Gender. In general, *Campylobacter* prevalence is higher in males (Potter et al., 2002, Samuel et al. 2004, Hopkins and Olmsted, 1985). This trend is not well understood; however, it has been suggested that this is due to poor food handling practices more common among men or physiological differences between the genders (Altekruse et al, 1999, Louis et al., 2005). Recently, Younus et al. (2006) found higher rates of *Salmonella* infection for Michigan females.

Age. Prevalence of *Campylobacter* and *Salmonella* infections is distributed across age groups with the greatest number of cases reported for children under the age of five and with *Campyobacter* a second, smaller, peak in the 20-29 age group (Younus et al. 2006, Potter et al., 2002, Samuel et al., 2004). Several hypotheses have been proposed to explain this trend. First, parents are more likely to take their young children and infants to the doctor for symptoms of gastroenteritis (Friedman et al., 2000). Furthermore, children get sick more frequently due to an immature immune system. Subsequently, infections in childhood act to build immunity such that infection is less likely in later years (Perez-Perez and Blaser, 2005). The second peak in campylobacteriosis among the 20-29 year olds has not been explained.

Race. There has been little research in the area of race and campylobacteriosis. However, in one study in the U.S., Blacks were noted to have significantly lower rates than Whites, Hispanics, and Asians (Samuel et al. 2004). In the Younus et al. (2007) study there was no racial association for salmonellosis. Arshad et al. (2007) reported a higher average annual incidence of salmonellosis for Blacks.

Seasonality

In the U.S. and other parts of the world, there is a distinct peak in cases in the summer months (Miller et al., 2004, Louis et al., 2005, Nylen, 2002, Lindback and Svensson, 2001, Potter et al., 2002). The cause of this apparently universal seasonal trend is not fully understood. Some hypotheses have included increased risk of infection during peak summer travel times (Miller et al. 2004), increased consumption of poultry products in warmer weather and a higher likelihood of eating outdoors and outside of the home, in general (Friedman et al. 2000), and spread of *Campylobacter* via flies (Hald et al., 2004). In other systematic analyses, Louis et al. (2005) found a significant relationship between temperature change in England and Wales and seasonal campylobacteriosis rates and Naumova et al. (2007) found similar relationships with campylobacteriosis and salmonellosis and temperature in Massachusetts, USA.

Declining Cases in the United States

Data from states participating in the Centers for Disease Control and Prevention (CDC) FoodNet program show large declines in *Campylobacter* and from 1996-2000, with similar declines across all races, age groups, and genders (Van Gilder et al., 1999, Samuel et al., 2000, Samuel et al., 2004). This is noteworthy because the incidence rates are on the rise in other countries (Altekruse, 1999), particularly Australia and New Zealand. Between 1996 and 2005, the national (U.S) averages for *Campylobacter* and *Salmonella* were 16.9 and 14.5 cases per 100,000 people, respectively (CDC, 2000, 2001, 2002, 2003, 2004, 2005, 2006). The steepest decline in *Campylobacter* infections occurred prior to 2001, with rates declining 43% (an average of 8.7% decline annually from 1997 through 2001) then leveling off and only declining an additional 4% since (less than 1% annually). Since 1996, the decline in *Salmonella* incidence has been small (CDC, 2005). Possible explanations for these declines include improvements in the meat processing and poultry industries due to Hazard Analysis and Critical Control Points (HACCP) and Pathogen Reduction (PR) rule implementations (Buchanan and Whiting, 1998, Hariharan et al., 2004, Keener, 2004). These rules, which went into effect in 1997, require the use of more water when processing and disinfection of that water with trisodium phosphate.

1.4 Environmental Associations

One proposed environmental model for the transmission of campylobacteriosis to humans (Skelly and Weinstre, 2003) suggests that humans are exposed to the pathogen through feces, food, and aquatic environments. While *Campylobacter* and *Salmonella* have been found in all these environments, the modes of movement between them are not fully understood. Figure 1 illustrates the some of the transmission modes mentioned for *Campylobacter* and *Salmonella* that could be influenced by environmental factors. In this section we examine some environmental factors (Weather [precipitation and temperature], Landuse [agricultural], Sewage disposal, and Water source) and their possible associations with enteric diseases.

Precipitation Effects on Pathogen Loading in Watersheds

Changes in precipitation can affect the loading of enteric pathogens in waterways. Significant runoff and subsequent contamination of waterways after extreme rain events is a common occurrence (e.g., Lipp et al. 2001, Lipp et al. 2002, Leeming et al. 1998; Patz, 2001). The presence of waterborne disease agents, including *Giardia* cysts, *Cryptosporidium* oocysts, and enteric viruses, have been positively correlated with rainfall (Graczyk et al. 1999, Patz, 2001, Kristemann et al., 2002, Lipp et al., 2001). Microbial contamination in drinking water reservoirs in parts of Germany has been shown to increase by as much as 1- to 2-logs during extreme rainfall and runoff events (Kristemann et al., 2002). In areas, such as Florida, where wet winters are correlated with El Niño events, a direct relationship between the El Niño Soulthern Oscillation (ENSO) state and water quality (measured by fecal coliform bacteria) has been noted (Lipp et al. 2001). This is one of the only studies that has been able to relate ENSO events to the change in local weather patterns and then to discrete changes in water quality (Tampa Bay, FL).

Temperature and Environmental Survival

Despite the host requirement, *Campylobacter* and *Salmonella* are routinely found in environmental sources such as water, sediment, and sewage (Haley et al. 2009, Droppo et al. 2009, Buswell et al., 1998, Lucey et al., 2000, Ashbolt, 2004, Sahlstrom et al., 2004, Jones, 2001). There has been the discovery of a potentially environmentally adapted strain of *Campylobacter* jejuni that is prevalent in northwest England surface waters in late spring (Sopwith et al. 2008). Experimentally, for short periods of time, *Campylobacter* spp. can survive in sterile water but their survival increases when associated with a biofilm and at lower temperatures (Bruswell et al. 1998). In sterile water at 37° C *Campylobacter* survived an average of 21.8 hours while at lower temperatures the survival times went up with highest survival in sterile water at 4° C (201.6 hours). When autochthonous microflora were added to the microcosms to better represent the natural environment, survival rates increased significantly to ~ 200 hours at 30° C and ~ 550 hours at 4° C (Bruswell et al. 1998). Furthermore, by infecting protozoa (i.e., *Acanthamoeba polyphaga*) *C. jejuni* is able to prolong its survival in the environment and outside of a vertebrate host (Axelsson-Olsson et al., 2005). In a *Salmonella* almond soil microcosm, *Salmonella* recovery decreased more quickly with spiked samples stored at 35 C as compared to those at 20 and at 180 days could still be recovered from samples stored at 20°C but could not be detected in 35 ° C samples (Danyluk et al. 2008).

Landuse

The transport of pathogens via runoff can increase concentrations of waterborne pathogens in impacted watersheds (Ferguson el al., 2003); in turn the amount and quality of run off is directly related to land use. Runoff is affected by the amount and intensity of precipitation, surrounding land use, soil type, and topography (USGS, 2005, Tsubo, 2005, Sheresta, 2003). Sherestha (2003) suggested that urban land use resulted in the highest level of runoff followed by residential (village) areas, agricultural land, pasture land, and forests. These are related to land cover by impervious surfaces. Changes in land use have been associated with the emergence of pathogenic diseases in many regions of the world (Patz, 2001). Some of the land use changes include human settlement, commercial development, and road construction. Combinations of these types of changes have been linked with emergence of diseases such as malaria and schistosomiasis (Patz, 2001). Several studies have further implicated land use in the contamination of waterways (Interlandi & Crockett, 2002). Significant concentrations of fecal indicator microbes are found in waters that drain from confined livestock farming operations (Crowther et al., 2001). This information suggests that along with weather factors, the use of the land is an important factor in the amounts of pathogens in watersheds.

Sewage disposal

Proper disposal of wastewater is also an important consideration when investigating modes of disease transmission. Public means of sewage disposal is regulated by local, state, or federal agencies. The remainder of the State uses other means of disposal, usually a private on-site disposal system (OSDS; e.g., septic systems and cess pits), which do not include a mechanism for disinfection of waste. Septic systems include a tank which allows solid material to collect and scum to surface while the liquid portion is allowed to go into a leach field where the soil can assist in the filtration of microbes and organics from the waste water (American Ground Water Trust, 2005). Cesspools are less common and are simply pits where sewage is dumped. Local ordinances provide guidelines on how to properly locate these private systems but beyond that it is the homeowner's responsibility to ensure it is working properly. This is of particular importance because of the known links between sewage-contaminated water and human illness (Haflinger, 1999, Kambole, 2003, Exner, 2001). Public sewage treatment facilities have more stringent guidelines; however, all facilities are not required to perform tertiary levels of treatment which may be necessary to kill many microbial contaminants. Sahlstrom et al. (2004) found that 55% of sludge samples treated by common methods for secondary treatment (sedimentation, mesophilic or thermophilic aerobic digestion, composting, and storage) were positive for *Salmonella* and other potentially harmful microbes. Sludge, also known as biosolids, is often applied directly to land for use as fertilizer and may present a risk for infectious diseases (Sahlstrom et al., 2004).

Drinking Water Source

Waterborne disease agents have been identified as a major concern for human health (Patz, 2001). It has been estimated that in North America, 15-30% of gastrointestinal disease is a result of contaminated water (Ashbolt, 2004).

Once pathogens are in the watersheds, proper treatment of the water is required before consumption to prevent human infection and disease, including, campylobacteriosis and salmonellosis (Ashbolt, 2004).

Waterborne disease outbreaks have been a problem in the United States for many years. The US Environmental Protection Agency (EPA) regulates public drinking water systems that serve over 25 people; nationwide, these public systems serve 90% of the population (US Census, 1990). For those that are not served by public sources, individual wells are used. These wells are not regulated by the EPA but suggestions are given to prevent contamination of the water and each state determines the exact ordinances for that state. Some of the EPA's suggestions are for wells to be placed at least 50 feet from septic tanks and leach fields, silos, and livestock yards, 100 feet from petroleum tanks, liquid tight manure storage, and fertilizer storage and handling, and 250 feet from manure stocks (EPA, 2005). The regulation of these water sources are the responsibility of the homeowner who must carry out any testing to ensure water safety. The depth of private wells can also indicate likelihood of becoming contaminated. Drilled wells (deep wells of 100-1000 feet), are drilled below the bedrock and get water from confined ground water sources, while dug wells (10-30 feet deep) and bored or driven wells (30-100 feet deep), tap water from the saturated zone above the bedrock (an unconfined water source) which is more easily contaminated (EPA, 2005).

Review: *Campylobacter* and *Salmonella* seasonality temperature, precipitation, and environmental associations

A systematic review of the literature was conducted to examine how seasonal patterns of campylobacteriosis and salmonellosis, both primarily foodborne diarrheal illnesses (though the seasonal pattern is not fully explained through this route), relate to precipitation and temperature fluctuations and the environment (proxy measured by geographic distribution). Searches were conducted through the PubMed database and Google scholar and through a further snowball effort (researching the references of relevant articles to identify others) using key search terms precipitation, ambient temperature, seasonality, campylobacteriosis, salmonellosis, climate, environment, spatial distribution and any variation of those terms. The aim of this search was to identify studies where associations of temperature, precipitation, or geography were evaluated.

Temperature association

Incorporating a 4 week lag, Patrick et al. (2004), in the Denmark study found 68% of variation in human *Campylobacter* incidence could be explained by maximum temperature in a univariate model. England and Wales Tukey transformed data analyzed with autoregression techniques showed increased *Campylobacter* rates were correlated with temperature (Louis et al. 2006). In another English study a one degree rise in temperature corresponded to a 5% increase in the number of *Campylobacter* reports (Tam et al. 2006). Generalized linear models and additive models were used by Fleury et al. (2005) in the Canadian study to identify a non linear association between weekly

Campylobacter cases and temperature such that log relative risk increased by 2.2% for every degree increase in weekly mean temperature in Alberta and 4.5% in Newfoundland-Labrador. A similar temperature association was seen in Massachusetts, USA where *Campylobacter* daily incidence peaks 2-14 days following ambient temperature peaks (Naumova et al. 2007). Spanning Europe, Canada, Australia, and New Zealand, a slight 3 month lag association was reported (Kovats et al. 2005) and Australian study showed some unique findings with inverse associations reported in Adelaide and positive associations in Brisbane (Bi et al. 2008).

Several of the identified studies evaluated associations between *Salmonella* reported cases or incidence and temperature. Generalized linear models and additive models were used by Fleury et al. (2005) in the Canadian study to identify a non linear association between weekly *salmonella* cases and temperature such that log relative risk increased by 1.2% for every degree increase in weekly mean temperature. In Australia, D'souza et al. (2004) reported a positive association (1 month lag) between salmonellosis notifications and mean monthly temperatures through a log-linear model. In the Naumova et al. (2007) study, daily salmonellosis incidence peaked 2-14 days after the ambient temperature peak in Massachusetts, USA. Similarly, Zhang et al. (2008) found a 2 week lag associated with increase in cases in Adelaide, Australia. One study reported a 1 week time-series lag association consistent across six European countries, however, this was the only study to suggest that this a link to food handling practices. All of these studies found a positive relationship between ambient temperatures and cases of *Salmonella* infections however the specifics of the findings (strength of association and lag), methods of analysis, and location of the study vary. This may suggest that there is a universal temperature association but more research would need to be done to better define what that association is and what it means.

Precipitation association

Patrick et al. (2004), Denmark study also found precipitation to explain 6% of variation in human *Campylobacter* incidence with a 3 week lag in a univariate model. When seasonality of *Campylobacter* incidence was examined in England and Wales variations of measures for precipitation were used (continuous amount of rain vs. dichotomous rain yes or no) and only with the inclusion of temperature were small amounts (1%) of the variation in incidence explained and only for certain regions (Louis et al, 2006).

In the Zhang et al. (2008) study, when using a seasonal autoregressive integrated moving average (SARIMA) model rainfall was inversely related to the number of Salmonellosis cases in Adelaide, Australia.

Environmental association

As noted in earlier sections (1.4) there are many environmental factors that could be associated with variation in transmission or incidence of *Salmonella* and *Campylobacter* infections. Under the assumption that environmentally related illness will show geographic clustering, studies assessing incidence distribution were evaluating (the spatial variation is used as proxy measure for environmental influence).

Many studies noted the spatial variation in *Campylobacter* and *Salmonella* infection incidences. Of particular note, the study by Jepsen et al. (2009) attempted to evaluate the clustering of *Campylobacter* incidences in a Danish county. This study clusters data based on space and time under the premise that data without environmental influence should be randomly distributed in space, finding that there was clustering around the northwestern portion of the study area. The researchers note that this may indicate an environmental "cause" in that area and further research should be done to identify it (Jepsen et al. 2009).

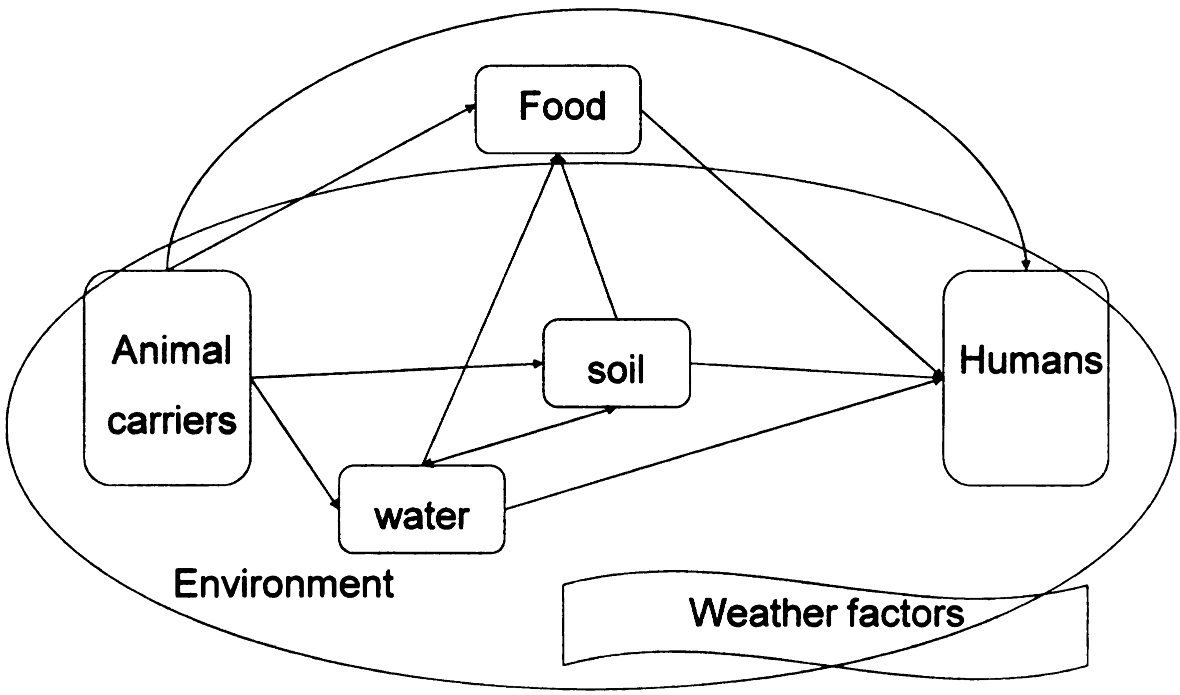
The Louis et al. (2006) study not only reports on the geographic variation in *Campylobacter* rates, but attempts to explain some of that variation by examining the high rate areas (rural and agricultural). The study was not able to link surface water with incidence.

Studies were not identified that specifically examined the environmental associations with human incidence of *Salmonella* infection.

1.5 CONCLUSIONS

Published literature has shown the relationship of climate to health and disease. Large climatic events affect global and local weather patterns resulting in increased precipitation and runoff. Based on the type of land use this runoff can be great and can contain pathogens such as *Campylobacter* or *Salmonella*. These pathogens are able to persist in natural waters, where humans may be exposed. The source of the drinking water may also be a key factor in the transmission of the disease. The goal of the research, presented in this dissertation, is to evaluate significant weather and other environmental factors for their association with and potential driving force influencing changes in Michigan *Campylobacter* and *Salmonella* case rates.

Figure 1.1: Model of Bacterial transport with Environmental Influence



This figure shows the various known infection pathways for human infection with Campylobacter and Salmonella Infections. The larger circle suggests the variables that could be influenced by environment and weather factors.

CHAPTER 2

The Epidemiology of Campylobacteriosis and Salmonellosis in Michigan

2.0 STRUCTURED ABSTRACT

Background - *Campylobacter* spp. and *Salmonella* spp. are some of the most commonly reported causes of bacterial enteritis in the United States; however, relatively little is known about regional and local scale variability of these diseases.

Specific Aims - To describe demographic, temporal, and geographic trends of campylobacteriosis and salmonellosis in Michigan, US 1) analyzing historical trends in *Campylobacter* and *Salmonella* rates in Michigan with respect to temporal, demographic, and geographic trends and 2) identifying counties with consistently high and consistently low incidence of *Campylobacter* and *Salmonella* infections.

Design – Retrospective descriptive study

Methods and Results - Data were analyzed on culture-confirmed cases of campylobacteriosis and salmonellosis from 1992-2005. The average annual incidence of these diseases in Michigan were 4.3 cases per 100,000 people (ranging from a high of 6.3 cases per 100,000 in 2004 to a low of 3.1 cases per 100,000 in 1997) for campylobacteriosis and 4.5 cases per 100,000 people (ranging from a high of 5.8 cases per 100,000 in 1998 and 2004 to a low of 3.5 cases per 100,000 in 1997) for salmonellosis. Incidence among the 0-4 age group for both diseases (7.7 *Campylobacter* spp. and 13.7 *Salmonella* spp. cases per 100,000) were significantly higher than all other age groups. There

were no significant differences for males and females. A marked seasonal trend, for both diseases, was also evident with rates peaking in the summer months. Geographically, incidence varied across the state among counties with no campylobacteriosis cases reported in Baraga and Lake to a mean annual high of 22.1 Campylobacter cases per 100,000 in Menominee and from 0.28 *Salmonella* cases per 100,000 in Cheboygan to 17.3 in Wexford. Case rates state-wide for both diseases were significantly higher in counties with intermediate population densities, 55-144 people/mi^2. Counties most frequently noted as the highest case rate counties in the state were identified and evaluated to find no distinguishable geographic trend, however, demographically these counties are all >90% white, have a large elderly population, and have a low population density. Overall, Michigan rates are lower than the national rates (12.7 Campylobacter cases per 100,000 and 14.6 salmonella nationally) and have no annual trend while nationally annual rates have been on the decline (for the period of this study).

Conclusions - There is geographic and seasonal variation in reporting of campylobacteriosis and salmonellosis and demographics of the high reporting areas do not follow expected trends. These results may suggest that non-demographic factors, including environmental influences, may affect the rates of campylobacteriosis and salmonellosis in these areas.

Significance - This study adds to the growing body of information on the epidemiology of *Campylobacter* and *Salmonella* in the United States. Through this retrospective descriptive study, data from Michigan available beginning in 1992 (four years prior to FoodNet) will provide information comparable to

FoodNet, as Michigan is not a FoodNet state and has not previously been represented in the national reported data.

2.1 INTRODUCTION

Campylobacter and *Salmonella* are commonly reported causes of bacterial enteritis in the United States, and throughout the world (Altekruse et al., 1999,Oberhelman and Taylor, 2000, Coker et al., 2002, USDA, 2003). They are generally considered a foodborne pathogen, but waterborne outbreaks are also known to occur (Blaser et al., 1979, Blaser et al., 1983, Palmer et al., 1983, Skirrow, 1991, Fahey et al., 1995, Ashbolt, 2004). *Campylobacter* and *Salmonella* transmission and trends for the diseases have been studied and noted in the literature for the United States and around other parts of the world. These trends include temporal (declining incidences beginning in 1996 and seasonal peaks in the summer months), demographic (men having higher incidences than women and children under five years old having the highest incidences for age groups), and geographic (rates vary from rural to urban areas) aspects (Buchanan and Whiting, 1998, Van Gilder et al., 1999, Samuel et al., 2000, Lindback and Svensson, 2001, Allos et al., 2004, Hariharan et al., 2004, Keener, 2004, Samuel et al., 2004, CDC, 2004, USDA, 2006). However in the United States, national rates and the trend in rates are determined based on the Food Net Program, which only began in 1996 and may not be generalizable to the entire country (Hardnett et al., 2004). Michigan is a non-Food Net state and has complete data archived from 1992. Michigan also has a diverse population to compare to the nation demographically, distinct seasons to examine seasonal trends, and geographic variation with urban centers and large agricultural areas. Here we evaluate the trends in reported cases in Michigan with respect to national findings.

This study is descriptive and includes a retrospective analysis of *Campylobacter* and *Salmonella* infection incidences in Michigan from 1992-2004. This study aims to examine historical data on reported cases of *Campylobacter* and *Salmonella* infection with respect to geographic (state and county), temporal (year, season, and month), and demographic trends (gender and age). This study also aims to compare these trends to reported national data and identify Michigan counties with consistently high and low incidence of disease for future study.

2.2 HYPOTHESES

The specific hypotheses tested in the study were:

1) incidences of human *Campylobacter* and *Salmonella* infection in Michigan will be comparable to those nationally with respect to temporal and demographic trends; and that
2) there will be geographic variation in the human incidence of *Campylobacter* and *Salmonella* infections in Michigan.

2.3 METHODS

a. Study Design

A retrospective study design was used to evaluate the aforementioned hypotheses. Historical data were collected from public data sources and linked for further analysis based on associated data identifiers.

b. Sources of Data

Case Data: Michigan local health departments are responsible for supervising the collection and reporting of notifiable disease data from health boards, practitioners, and laboratories in their jurisdiction. The Michigan Department of Community Health (MDCH) receives all these reports of culture confirmed laboratory human cases of *Campylobacter* and *Salmonella* infections. Information from these records was abstracted from the period of 1991-2006. These abstracted de-identified (per Human Subjects Exempt Research Protocols) data included race, county, organism (*Campylobacter* or *Salmonella*), gender, age, and year, month, and day of disease onset. Annual and monthly state and county incidence and population densities were calculated using these case data, population data, and county area data (Incidence = (# cases/ population)*100,000).

Population Data: To evaluate the relationship between county-level case rates and demographic characteristics of Michigan counties, state and county level population data were collected from the US Census Bureau. This data were collected from the annual estimates for Michigan for the demographic subsets by race, county, gender, age group, and state from the years of 1992-2005.

County land area data were collected from the US Geological Survey and assessed with the population data to calculate annual state and county population densities.

c. Data Analysis

Descriptive analyses of annual case rates (per 100,000 people) among categories of gender, age group, race, month, and season were performed for the entire state of Michigan and for each of 83 counties. Monthly case rates for age group, gender, and race were also calculated for each county. In instances where there was a missing identifier, the case was excluded from calculation based on that identifier. For seasonal analyses, months were collapsed into seasons defined as spring (March, April, and May), summer (June, July, and August), fall (September, October, and November), and winter (December, January, and February). Data were analyzed for differences in mean rates between counties and for temporal trends.

The distributions of cases among all demographic variables were calculated for each county and compared to that county's case rates in the general population. Counties were grouped according to the distribution of demographic variables (deciles) by age, gender, race, and geographic location. Population density was calculated as the number of people per square mile in a given county. Quartiles for population density were determined by evaluating the distribution of total number of data points (all counties for all years). Percent distributions of county populations were calculated for each age group, gender, and race. Differences in county case rates were analyzed among deciles for population demographic distribution and quartiles for population density analysis.

To determine statistically significant differences in case rates among study variables, an analysis of variance (PROC ANOVA) was performed using SAS software (v.9.1, ESRI, Redlands, CA) and post hoc Least Significant Difference (LSD) or Student-Newman-Keuls (SNK) tests were used to determine the pair-wise differences. In all measures, statistical significance was declared when $p<0.05$.

High and Low incidence counties: To avoid outliers a method was developed to identify counties that consistently have the highest and lowest incidence of *Campylobacter* and *Salmonella* infections over the period of study. This method takes into account consistency and overall rates for evaluation. Consistently high or low incidence counties are defined as counties with incidence of disease that shows up with high frequency in the upper 25% of counties for highest or lowest monthly incidence over the period of study. The overall rates were evaluated by identifying counties with the highest overall annual mean and monthly mean. Counties that showed up on all these lists were determined to be the "High and Low incidence counties".

2.4 RESULTS

Campylobacter: Geographic. The mean annual incidence of *Campylobacter* infections in Michigan was 4.28 cases per 100,000 people. There were nine counties with significantly higher incidences of *Campylobacter* infections. These counties are Menominee (22.1 annual cases / 100,000 people), Marquette (16.7), Missaukee (14.2), Leelanau (13.6), Wexford (13.4), Isabella (13.0), Hillsdale (12.8), Berrien (12.1), and Alcona (12.0). The counties of Mackinac (0.65 cases / 100,000 people), Cheboygan (0.63), Presque Isle (0.55), and Gogebic (0.45) had the lowest incidences with the exceptions of Baraga and Lake that reported no cases over the period of record (Figure 2.1a).

Temporal. Incidence of campylobacteriosis in Michigan was on a marked decline from a high of 6.16 cases / 100,000 in 1992 to a low of 3.1 per 100,000 in 1997. Thereafter, the rates rose to 6.29 in 2004 (Figure 2.2). Evaluation of the monthly incidence shows the cyclical pattern of reported disease incidence where cases were significantly highest in the summer months peaking in July with a mean monthly incidence of 0.72 cases per 100,000 people and the cases were significantly lowest in the winter and spring from November through April (monthly incidences of 0.26, 0.20, 0.19, 0.17, 0.21, and 0.22, consecutively) (Figure 2.3 and 2.4). Seasonally, the summer was statistically higher than all other seasons (Figure 2.5).

Demographic. The 0-4 age group had the highest incidence of disease (7.7 cases / 100,000 people) while the 5-19 age groups had the lowest (5-9 age group: 2.9, 10-14 age group: 2.3, and 15-19 age group: 2.5 cases / 100,000 people) (Figure 2.6). There was no significant difference in incidence between males and females (4.5 and 4.0 cases / 100,000 people, respectively) (Figure 2.7a). African Americans had significantly lower incidence than all other groups (Figure 2.8).

<u>*Salmonella:*</u> *Geographic.* The mean annual incidence of *Salmonella* infections in Michigan was 4.50 cases per 100,000 people. All counties reported cases of *Salmonella* infections over the period of record with Wexford (17.3 annual cases / 100,000 people), Benzie (14.1), Missaukee (13.6), Marquette

(13.2), Lenawee (13.2), and Menominee (12.6) having the highest mean incidences over the period. Mackinac (.79 cases / 100,000 people), Midland (.68), and Cheboygan (.28) had the lowest mean incidences (Figure 2.1b).

Temporal. Incidence of salmonellosis in Michigan shows no significant overall trends. The incidence in 1992 was 4.5 and remained between 4.5 and the overall minimum reached in 1997, 3.5 cases / 100,000 people until 1998, when it increased to 5.8 cases / 100,000 people. From then rates declined through 2003 to a local minimum of 3.9 and increased again in 2004 to 5.8 once again (Figure 2.2). Evaluation of the monthly incidence shows the cyclical pattern of reported disease incidence where cases were significantly the highest in the summer months peaking in July with a mean monthly incidence of .67 cases / 100,000 people and the cases were lowest in the winter from November through February (monthly incidences of 0.29 cases / 100,000 people, 0.23, 0.23, and 0.21, consecutively) (Figure 2.3 and 2.4). Seasonally, incidence of disease was statistically higher in the summer months (Figure 2.5).

Demographic. The incidence of disease for the 0-4 age group was significantly higher than all other groups (13.7 cases / 100,000 people). The lowest incidences were seen in the 50-59 age group (3.2 cases / 100,000 people) and the 10-14 age group (3.0) (Figure 2.6). There was no significant difference in incidence between males and females (4.2 and 4.7 cases / 100,000 people, respectively) (Figure 2.7b). African Americans had statistically significantly lower incidence of disease than other groups (Figure 2.8).

County Demographic Distributions: There were significant differences in incidence for counties based on population density. Counties with population densities in the 3rd quartile (population density of 55 – 144 people per square mile) had significantly higher rates than other population density county categories (Table 2.1). County racial distribution did not show significant differences between county groups (Table 2.2a, 2.2b). County age distribution did not show counties with significantly skewed populations having disproportionate incidence (Table 2.3a, 2.3b).

High and Low Incidence Counties: The high incidence counties were determined to be counties that consistently were in the upper or lower quartiles for high or low annual incidence. The counties that appeared the most frequently in the upper quartile for *Campylobacter* are Menominee, Marquette, Isabella, Wexford, Leelanau, Emmet, Alcona; and for *Salmonella are* Wexford, Benzie, Branch, Kenweenaw, Oscoda (Figure 2.1). Counties most frequently appearing in the lower quartile for *Campylobacter* are Arenac, Baraga, Cheboygan, Genesee, Gogebic, Lake, Lapeer, Mecosta, Presque Isle, Saginaw, Shiawassee, St Clair, Wayne; and for *Salmonella* are Arenac, Bay, Cheboygan, Eaton, Gladwin, Grand Traverse, Macomb, Mecosta, Midland, Montclam, Ogemaw, Sanilac, Shiawassee, St Clair, Tuscola, Wayne (Figure 2.1).

2.5 DISCUSSION

In this study historical incidences of *Campylobacter* and *Salmonella* cases in Michigan were analyzed with respect to temporal variation, geographic area, and demographic variation. It was expected that the incidences of

Campylobacter and *Salmonella* infections in Michigan would be comparable to national data in terms of averages and with respect to temporal and demographic trends and that there would be geographic variation in rates across the state.

Temporal

During the 14 year period of analysis (1992-2005), 6,111 culture-confirmed cases of *Campylobacter* infection and 6,483 *Salmonella* cases were reported in Michigan, at means of 437 and 463 cases per year and mean incidences of 4.3 and 4.5 *Campylobacter* and *Salmonella* cases per 100,000 people, respectively. Between 1996 and 2005, the national (U.S) averages for *Campylobacter* and *Salmonella* were 16.9 and 14.5 cases per 100,000 people, respectively (CDC, 2000, 2001, 2002, 2003, 2004, 2005, 2006). Nationally, there was a steep decline in *Campylobacter* infections prior to 2001, with rates declining 43% (an average of 8.7% decline annually from 1997 through 2001) then leveling off and only declining an additional 4% since (less than 1% annually). In Michigan during the same time period, there is no distinguishable corresponding overall trend (Figure 2.2). However, there was a steep decline that ended in 1997 and began at the beginning of the recorded period for an overall decline of 49% (9.9% annually 1992 through 1997). *Campylobacter* incidence then increased dramatically throughout the remainder of the record period. Since 1996, the decline in *Salmonella* incidence has been small (CDC, 2005). In Michigan, over the reporting period, there have been no significant trends in *Salmonella* incidence.

Nationally, the decline in *Campylobacter* and *Salmonella* rates has been observed since 1996 with similar declines across all races, age groups, and genders (Samuel, 2004). This is significant due to the fact that around the world in both developed and developing countries the incidence of campylobacteriosis has risen substantially over the past 20 years (Coker et al., 2002). Several possible explanations for this disparity have been suggested, including improvements in the meat processing and poultry industries due to Hazard Analysis and Critical Control Points/ Pathogen Reduction (HACCP/PR) rule implementations (Buchanan and Whiting, 1998, Allos et al., 2004, USDA, 2006). These rules require the use of more water when processing and disinfection of that water with trisodium phosphate; however, these were implemented in 1997 and does not explain the significant decline noted in Michigan that begins at the beginning of the recorded period (1992). Another possible reason for the decline is better education of the public on food safety (Samuel, 2004).

Another finding of this study is that the mean rates of both *Campylobacter* and *Salmonella* infections in Michigan (4.3 and 4.5 cases / 100,000 people, respectively) over the period of study were far below the national averages (12.7 and 14.6) (CDC, 2006). Further study will be needed to fully explain this difference, however it is possible that climate factors may explain some of this difference as the expected seasonal temporal trends are seen in Michigan with high incidence in the summer months and low incidence in the winter months, but due to Michigan's high latitude the winter low lasts for six months while the summer high is only present for one month.

Campylobacteriosis and Salmonellosis cases peak in the summer months (from May to July in the northern hemisphere) across the US and around the world (Miller et al., 2004, Louis et al., 2005, Nylen, 2002, Lindback and Svensson, 2001, Potter et al., 2002). The cause of this apparently universal seasonal trend is not fully understood. Some hypotheses have suggested an increased risk of infection during peak summer travel times (Miller et al., 2004, Coker et al., 2002; Louis, 2005), increased consumption of poultry products in warmer weather and a higher likelihood of eating outdoors and outside of the home, in general (Friedman et al., 2000). Jones (2001) suggests that the seasonal trends are due to variations in *Salmonella* and *Campylobacter* infections of livestock and poultry flocks, which could be associated with increased *Campylobacter* transmission by flies in the summer months along with variations in environmental loading (Rosef and Kapperud, 1983; Hald et al., 2004; Nichols, 2005). In this study, highest rates in Michigan were also reported in the summer, particularly in July, followed by June and August. In a systematic analysis, Louis et al. (2005) found a significant relationship between temperature change in England and Wales and seasonal campylobacteriosis rates suggesting that environmental factors such as climate affect case rates.

Demographic

There was a high degree of variability in campylobacteriosis rates within the state over the period of record, which was not related to the demographic makeup of the county as Michigan counties followed all expected reported demographic trends; high rates in the 0-4 age group, men higher rates than women, and Blacks lower rates than other racial groups.

The distribution of *Campylobacter* and *Salmonella* infection rates peaked in the 4 year and under group and is similar to previous reports (Friedman et al., 2000, Louis et al., 2005, Perez-Perez and Blaser, 2005). *Campylobacter* and *Salmonella*, as well as other pathogens that cause gastroenteritis are vastly underreported in the general population (Gillespie et al., 2002). It has been speculated that high rates noted in the under five age group may be a reporting bias, with parents being more likely to take their young children to the doctor for symptoms of gastroenteritis (Friedman et al., 2000). Because of this Louis et al. (2005) suggested that this group may better represent the actual case load. In addition to a reporting bias, children get sick more frequently due to an immature immune system (Perez-Perez and Blaser, 2005). Subsequently, infections in childhood act to build immunity such that infection is less likely in later years (Perez-Perez and Blaser, 2005). It is unclear what factors may have lead to the second peak in campylobacteriosis among the 20-29 year olds.

In this study we also found a slight difference between the rates of salmonellosis and campylobacteriosis in males and females in Michigan, with males having higher rates. This is consistent with previous studies although this trend is not well understood (Potter et al., 2002, Samuel et al., 2004). It has been suggested that this is due to poor food handling practices more common among men; however this seems unlikely given that the trend was evident among all age groups including those <5 (Samuel et al., 2004). Therefore, physiological differences between the sexes may explain this trend (Altekruse et al., 1999).

There was a significant difference in incidence of campylobacteriosis and salmonellosis based on race, with rates for blacks significantly lower than for whites and other races. This is consistent with previous findings; however it is unclear what may drive this trend. One explanation may be cultural differences that result in different consumption and food preparation patterns. Samuel et al. (2004) speculate that blacks may be less likely to be seen by a physician for mild gastrointestinal illnesses like *Campylobacter* or *Salmonella* infections. This is suspected because blacks have the highest rates of hospitalizations due to *Campylobacter* infection (Samuel et al., 2004).

High case rates were found in the counties with disproportionate populations of these high rate groups. Noting the state-wide demographic trends, the counties with the highest mean case rates for the total population have no apparent similarities. This suggests that other factors not identified here may be affecting the case rates in these areas. Case rates in these consistently high counties averaged 15.8 *campylobacter* and 11.8 *salmonella* cases per 100,000 people, which is well above the Michigan average but is still lower than the national average. One avenue for further exploration would involve the evaluation of environmental factors known to be associated with the presence of these bacteria. One such environmental factor is farming/agriculture where animal shedding and slurry spreading has lead to known environmental bacterial contamination (Jones, 2001). This factor along with water source (many Michigan homes acquire their drinking water from private (often untested) wells (EPA, 2005)) and the spike in Michigan summer recreational activity (increasing likelihood of environmental exposure) it is likely that some of these influences may affect the incidence of these diseases in Michigan.

Geographic

There was variation in rates across the state as expected. Many of the counties with high rates of infection show up as the top high infection rate counties for both *Campylobacter* and *Salmonella* (Wexford, Missaukee, Marquette, and Menominee Counties). These counties are grouped in two areas of the state, which may suggest some geographic factors influencing the variations in rates.

2.6 CONCLUSIONS

Many studies to date have relied on Food Net data to describe and identify trends in campylobacteriosis and salmonellosis (CDC, 2005). However, these data only represent a very small portion of the US population (5.4% in 1996 and 9.5% in 1999), the state samples are not representative of the entire state, and the data are only available starting in 1996. Although the surveyed population is similar it may not be generalizable to the entire US population (Hardnett et al., 2004). With these data, extrapolations have been made to describe many trends, particularly the decline in *Campylobacter* incidence (Samuel et al., 2004). This decline in case rates from Food Net states has been noted from 1996-2003 and has been attributed to improvements in the poultry industry (Buchanan and Whiting, 1998, Allos et al., 2004, Samuel et al., 2004). This expected declining temporal trend was not seen in Michigan, as there was no trend in annual rates. There was also no change in rates after the HACCP rule implementations in 1996. This would suggest that the rates in Michigan may be less strongly influenced by food routes. Michigan's rates are also much lower than national rates. This makes Michigan an important site to evaluate as all other demographic factor trends are consistent with the national data.

Further studies would need to be done to evaluate the relation of geographic and environmental factors in order to understand the geographic variability in rates from county to county. These findings also suggest that there may be drivers other than food that may explain the variation in rates of these diseases. The following studies will further define the seasonal variation and evaluate possible environmental drivers.

Table 2.1. Incidence (per 100,000 people) within counties by quartiles of population density.

Quartile	County Population Density people/mi^2	*Campylobacter* Incidence	*Salmonella* Incidence
>75%-ile	>144	4.92	5.03
51-75%-ile	55 -144	6.93*	6.82*
26-50%-ile	34-55	5.18	5.59
≤25%-ile	<34	5.37	4.97

** This incidence was significantly higher than all other quartiles for the aforementioned disease.*

Population density quartiles were based on intervals of all data points. Density is reported as the number of people per square mile for all counties and all years. This table reports the mean human *Campylobacter* and *Salmonella* incidences of counties within a certain a certain population density range.

Table 2.2.a. *Campylobacter* Incidence (per 100,000 people) by percent distribution of county by race.

Percentage of County Population	County Racial groups: *Campylobacter* rates		
	White	Black	Other
>90%	6.04*	-	-
81-90%	4.76	-	-
71-80%	1.82	-	-
61-70%	-	-	-
51-60%	0.73	-	-
41-50%	-	0.73	-
31-40%	-	-	-
21-30%	-	1.14	-
11-20%	-	5.23	1.51
≤10%	-	5.77	5.76*

**This incidence was significantly higher than all others in that racial group.*

-There were no counties with populations with populations for this racial group within this percentage range.

This table reports the mean human *Campylobacter* incidence of counties with a certain percentage of its population composed of a particular race.

Table 2.2.b. *Salmonella* Incidence (per 100,000 people) by percent distribution of county by race.

Percentage of County Population	County Racial groups: *Salmonella* rates		
	White	Black	Other
>90%	5.72	-	-
81-90%	5.20	-	-
71-80%	4.86	-	-
61-70%	-	-	-
51-60%	0.81*	-	-
41-50%	-	0.81*	-
31-40%	-	-	-
21-30%	-	5.56	-
11-20%	-	5.86	4.61
≤10%	-	5.58	5.58

** This incidence was significantly lower than all others in that racial group.*

-There were no counties with populations for this racial group within this percentage range.

This table reports the mean human *Salmonella* incidence of counties with a certain percentage of its population composed of a particular race.

Table 2.3.a. *Campylobacter* Incidence (per 100,000 people) by percent distribution of county by age group.

Percentage of County Population	County Age Groups: *Campylobacter* Incidence								
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	≥60
>30%	-	-	-	-	-	-	-	-	4.80
21-30%	-	-	-	-	5.75	-	-	-	4.23
10-20%	-	-	-	5.24	5.82	5.55	5.55	5.38	6.38
<10%	5.55	5.55	5.55	5.55	4.66	5.24	-	-	6.14

-There were no counties with populations for this age group within this percentage range.

This table reports the mean human *Campylobacter* incidence of counties with a certain percentage of its population composed of a particular age group.

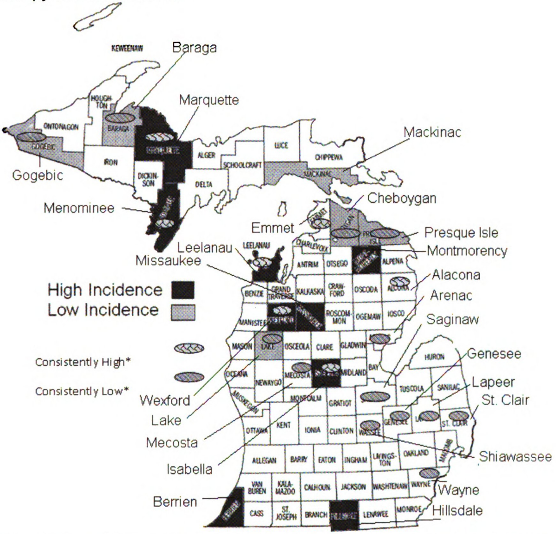
Table 2.3.b *Salmonella* Incidence (per 100,000 people) by percent distribution of county by age group.

Percentage of County Population	County Age Groups: *Salmonella* Incidence								
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	≥60
>30%	-	-	-	-	-	-	-	-	4.80
21-30%	-	-	-	-	5.75	-	-	-	4.23
10-20%	-	-	-	5.24	5.82	5.55	5.55	5.38	6.38
<10%	5.55	5.55	5.55	5.55	4.66	5.24	-	-	6.14

-There were no counties with populations for this age group within this percentage range.

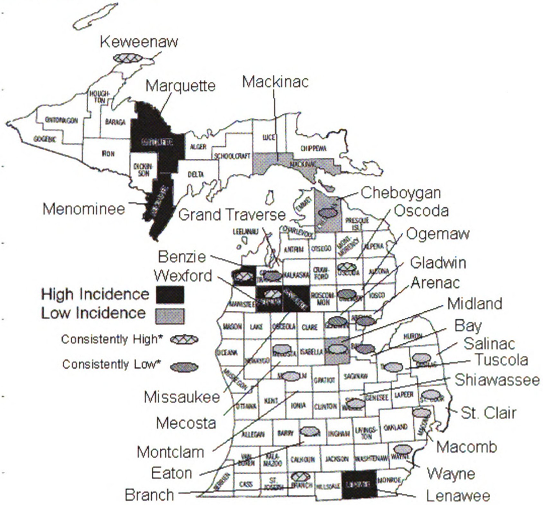
This table reports the mean human *Salmonella* incidence of counties with a certain percentage of its population composed of a particular age group.

Figure 2.1.a. Michigan Counties with high and low incidences of human *Campylobacter* infections.



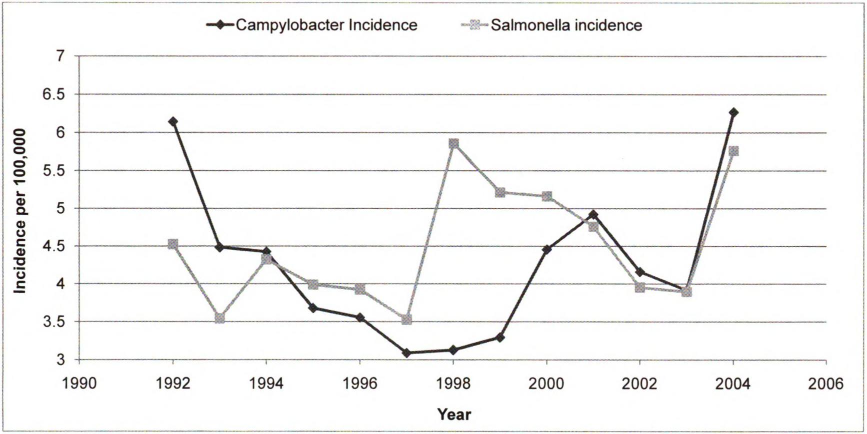
**Consistently High or Low counties are counties that frequently showed up in the top or bottom quartile for annual mean county Campylobacter incidence.*
High and low incidence counties had significantly higher or lower mean overall Campylobacter incidences.
This figure illustrates the geographic relationships of high and low incidence counties and the overlap between those and counties with consistently high and low human *Campylobacter* incidence.

Figure 2.1b. Michigan Counties with high and low incidences of human *Salmonella* infections.



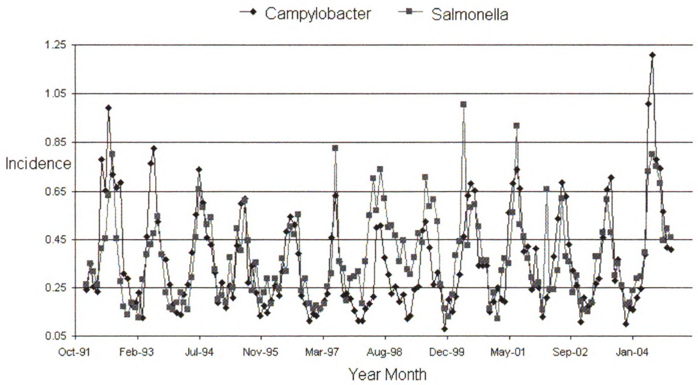
**Consistently High or Low counties are counties that frequently showed up in the top or bottom quartile for annual mean county Salmonella incidence.*
High and low incidence counties had significantly higher or lower mean overall Salmonella incidences.
This figure illustrates the geographic relationships of high and low incidence counties and the overlap between those and counties with consistently high and low human *Salmonella* incidence.

Figure 2.2 Annual incidences of Campylobacter and Salmonella infections in Michigan 1992-2005.



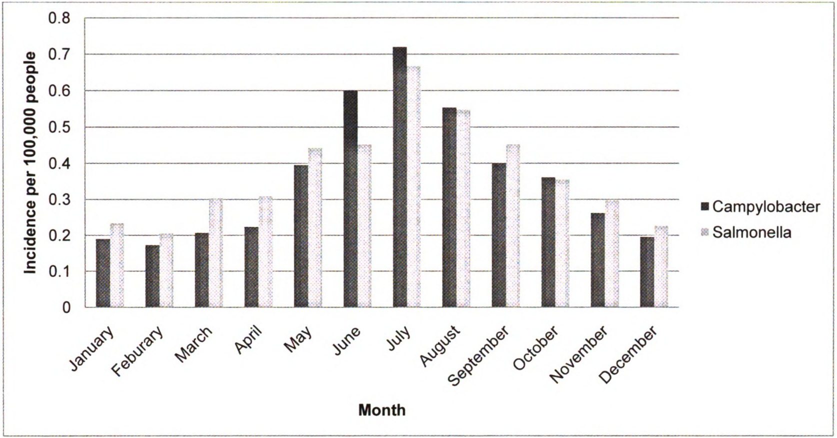
This figure shows the annual incidence of reported *Campylobacter* and *Salmonella* cases in Michigan per 100,000 people, over the period of study from 1992-2004.

Figure 2.3 Monthly incidences of Campylobacter and Salmonella in Michigan 1992-2005



This figure shows the time series of Michigan monthly mean human *Campylobacter* and *Salmonella* incidence over the period of the study.

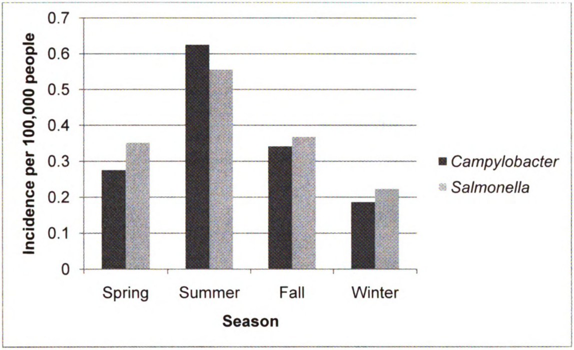
Figure 2.4 *Campylobacter* and *Salmonella* Incidence by Monthly average



The month of July is significantly higher for Campylobacter and Salmonella incidence. May and June follow, as being significantly higher than all others for Campylobacter incidence: then April, August, and September.

This figure graphs the mean monthly incidence of Michigan human *Campylobacter* and *Salmonella* infections.

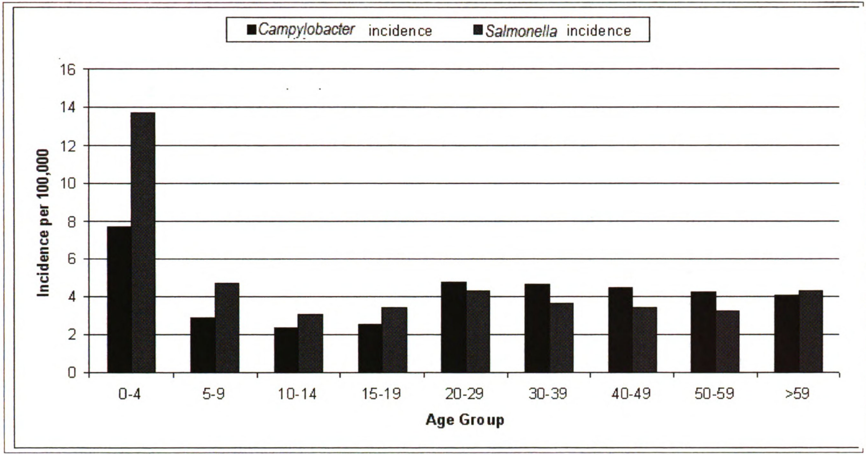
Figure 2.5. Average *Campylobacter* and *Salmonella* Incidence by Season



The summer is significantly higher for both Campylobacter and Salmonella seasonal mean incidence.

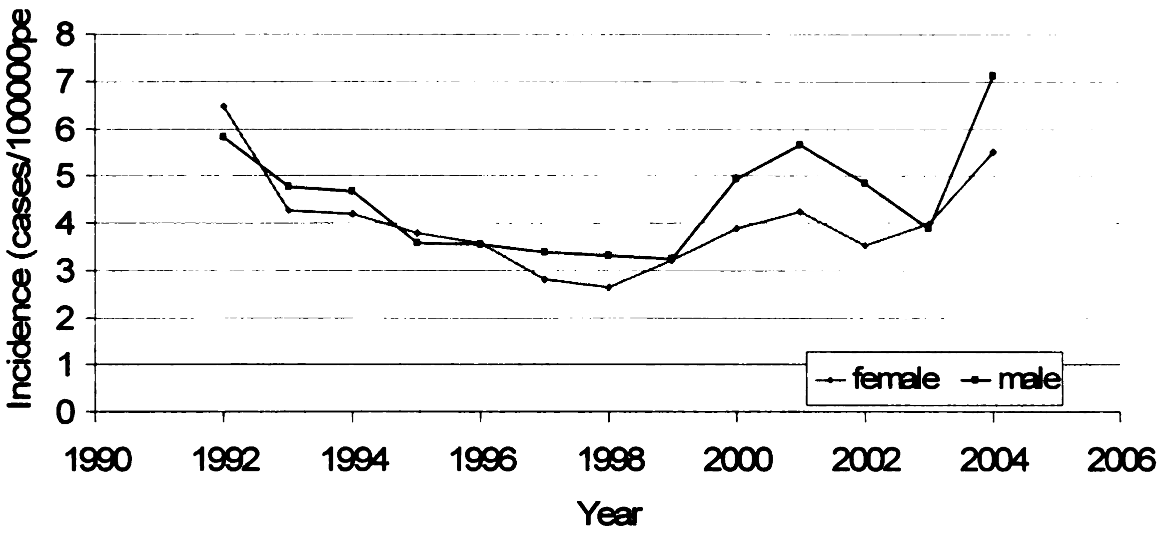
This figure graphs the mean seasonal incidence of Michigan human *Campylobacter* and *Salmonella* infections.

Figure 2.6. *Campylobacter and Salmonella* Incidence by Age group



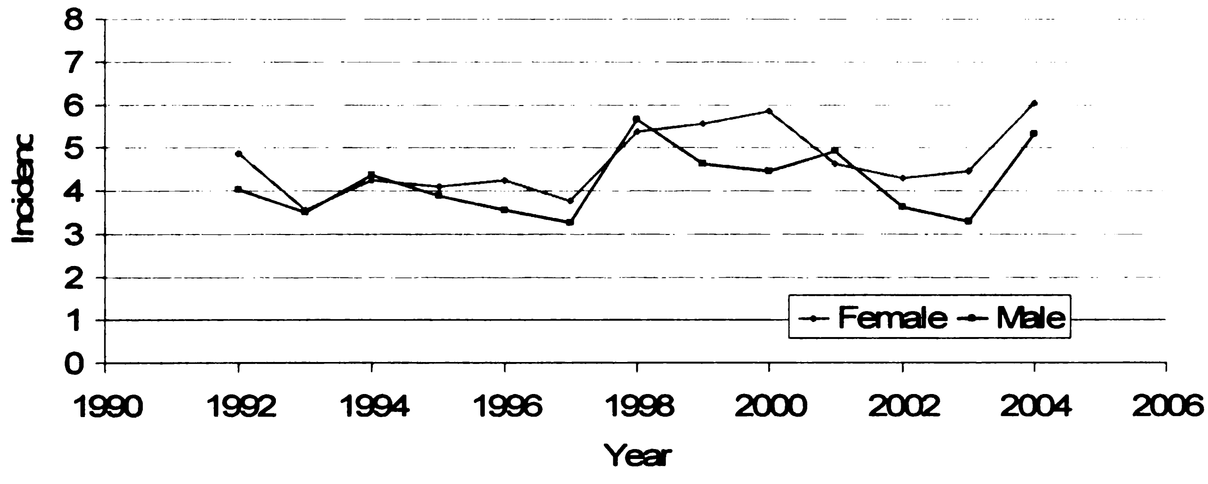
The 0-4 year age group is significantly higher for both Michigan Campylobacter and Salmonella mean incidence. For Campylobacter incidence the 5-9, 10-14, and 15-19 groups have the significantly lowest incidences. This figure graphs the mean annual incidence of Michigan human *Campylobacter* and *Salmonella* infections by age group.

Figure 2.7a *Campylobacter* Incidence by Gender



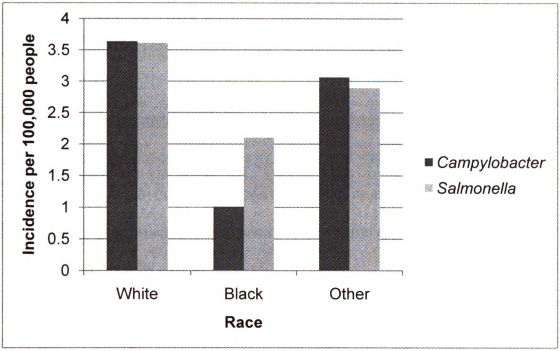
This figure graphs the mean annual incidence of Michigan human *Campylobacter* infections by gender.

Figure 2.7b *Campylobacter* and *Salmonella* Incidence by Race



This figure graphs the mean annual incidence of Michigan human *Salmonella* infections by gender.

Figure 2.8 *Campylobacter* and *Salmonella* Incidence by race.



Blacks were significantly lower than Whites and Other races for both Campylobacter and Salmonella infections.

Data collected from the Michigan Department of Community Health reported the race as Black or African American, White, Asian, American Indian/Alaskan Native, or Multiracial. These groups were collapsed into three groups Black which included Black/African American reports, White which included White reports, and other which included Asian, American Indian/Alaskan Native, and Multiracial.

This figure graphs the mean annual incidence of Michigan human *Campylobacter* and *Salmonella* infections by race.

CHAPTER 3

Campylobacter and *Salmonella* Infections in Michigan:

Evaluation of Seasonal and Geographic Trends Reporting (1992-2005)

3.0 STRUCTURED ABSTRACT:

Background - Campylobacteriosis and Salmonellosis are common gastrointestinal infectious diseases in the US and world-wide. Given the high incidence and health burdens of these diseases, much research has been done and trends in incidence of these diseases (seasonal and geographic) have been reported, but not fully explained.

Specific Aims - To statistically evaluate the seasonal trend in case reporting in Michigan, identifying parameters peak-week, start and end-week, and duration of the high reporting period. This study also evaluates the relation of geographic location and county incidence to high reporting period parameters.

Design - Retrospective analysis

Methods - Historical case data on *Campylobacter* and *Salmonella* infections in Michigan (1992-2005) were evaluated, time series techniques applied to identify the parameters, and linear modeling techniques were used to evaluate relationships of geographic location and county incidence to disease reporting trends.

Results - Time series shows regular behavior with high reporting for both campylobacteriosis and salmonellosis in the summer and early fall. This period varies between counties with respect to all parameters. Duration of the high reporting period was the only variable consistently associated with geographic

location and incidence.

Significance – Given the previously reported associations of these diseases with temperature and the changing global climate (global warming) there is increasing urgency to fully understand the seasonal trend of these diseases. By geographically defining the parameters of seasonality, more detailed models can be created incorporating variables for which geography may be serving as a proxy, and uncover the drivers of campylobacteriosis and salmonellosis seasonality.

3.1 INTRODUCTION

Rationale:

Disease seasonality is the systematic recurrence of a compact cluster of cases followed by a long interval of low incidence forming a typical pattern for a specific pathogen in a given population in a given locality (Naumova, 2006). Seasonality is characterized by 1) a point in time when the seasonal curve reaches its maximum, 2) the amplitude from peak to nadir, and 3) the duration of the increase defined by a shape of a curve (Naumova, 2006). Campylobacteriosis and Salmonellosis are known to exhibit seasonality with cases peaking in the summer months. This seasonality has been evaluated but the causes remain unclear. Further investigation into the shapes and parameters of the seasonal patterns and possible associates of those parameters could inform future research into drivers of the diseases and whether there seasonal variations in host susceptibility, pathogen survival and transmissibility, or environmental load.

This study evaluates the high reporting period (seasonality) for these diseases, identifying the critical weeks (start and end week, peak weak, and duration) and their associations with incidence level and geographic location. This study highlights time frames and patterns for use in further studies identifying environmental factors that could be driving these trends.

Background:

Campylobacteriosis and Salmonellosis are common infectious diseases caused by infection with *Campylobacter* spp. and *Salmonella* spp. and are primarily associated with foodborne routes of infection. Currently, *Campylobacter* is the most commonly reported cause of acute bacterial gastroenteritis in developed countries (Mead et al., 1999) and non-typhoid *Salmonella* annually causes 1.4 million cases annually in the United States (CDC 2004). The health burden for these diseases is great, as there are sequales to infection for *Campylobacter,* Guillian-Barré syndrome (Kalra et al., 2009, Vucic et al., 2009), and for *Salmonella*, reactive arthritis (Girschick et al., 2008). In vulnerable populations such as the immune compromised and the elderly, death can occur from infection. There are commonly reported demographic, geographic, and temporal trends and given the high prevalence and health burdens for these diseases much research has been done examining the foodborne route to understand the trends in transmission and to minimize infection. The reported temporal trend consists of two parts, the overall declining trend that has been studied and attributed to improvements in the meat processing industry (Samuel et al., 2004), and the seasonal trend which is not fully explained.

Many factors contribute to the human incidence of *Campylobacter* and *Salmonella* infections, and there is growing evidence that temporal and climate factors are associated with incidence of these diseases (Louis et al., 2006, Jepson et al., 2009, Naumova 2006). In efforts to understand seasonality, studies conducted, using various methods and data from developed countries all around the world, have assessed the relationships with ambient temperature and changing incidence relationships. These studies consistently report seasonal increases in incidence of these diseases exhibit a lag relationship with increases in temperature (Zhang et al., 2006, Patrick et al., 2004). The lag in these studies ranged from 2 days to 5 weeks. The authors reporting longer lag times suggest that factors (food prep and handling) close to the time of the reported infection may not be the most important step in transmission (D'Souza et al., 2004) and other routes should be explored. Authors report shorter lags suggest that food handling could be the cause (Kovats et al., 2004). Given the varying results, methods, and locations of these studies, further investigation is needed to tease out the consistencies and differences in the seasonal trends.

The study by Lindback and Svensson on *Campylobacter* infections in Sweden attempted to define seasonality for reported cases (2001). This study found, there was variation in the high reporting period with respect to the start-week and peak-week between counties and that there was a relationship between the high reporting period parameters and geography (north/south position) such that more southern counties had earlier start and an earlier peak than in northern counties. This study highlighted the regularities and variations in reporting patterns between geographic areas that make unique comparisons possible.

Michigan Department of Community Health (MDCH) receives reports on culture confirmed human cases of *Campylobacter* and *Salmonella* infection in Michigan and has data archived from 1991 through the present. Previous studies in Michigan have reported a seasonal peak and geographic variation that have not been explained (Arshad et al., 2007, Younus et al., 2007). Using some of the Lindback and Svensson methods, our study evaluated the seasonal peak in reporting of *Campylobacter* and *Salmonella* infections in Michigan with respect to peak-week, start and end-weeks, and duration. Further, relationships between the high reporting period parameters, geographic location, and county incidence level were evaluated. It is expected that results of such analysis will be used in modeling the high reporting period and identifying critical time points for future analysis in conjunction with environmental factors that possibly drive these disease rates.

3.2 HYPOTHESIS

The specific hypotheses tested in the study were:

1) there will be variation in the seasonal peak parameters (start week, end week, peak week, and duration) with respect to geographic location; and that
2) there will be variation in the seasonal peak parameters with respect to incidence.

3.3 METHODS:

a. Case Data

Reported cases of *Campylobacter* and *Salmonella* infections in Michigan were collected from the Michigan Department of Community Health (MDCH). These data were collected as a part of a mandatory reporting system for communicable diseases of interest in Michigan. Cases are reported to local health departments by doctors, hospitals, and laboratories once the bacteria have been culture confirmed. To protect the identity of individuals the data were requested with limited identifiers. These data were collected with the identifiers of state, county, onset date, race, gender, and age, but for this study the relevant identifiers are state, county, and onset date. Cases that did 1) not report the state as Michigan, 2) not report a county in Michigan, or 3) did not report a date in the range of 1/1/1992 to 12/31/2005 were excluded. Of the 6148 *Campylobacter* and 6508 *Salmonella* infections reported, 6113 cases (99%) and 6041 cases (93%) respectively, were used in this study. The cases were sorted and counted by year, month, and week based on the onset date provided for the aggregated state and county data.

b. Evaluation of Specific Aims

Aim 1: Evaluate high reporting period

Data Management: SAS 9.1 software was used to manage the data for calculations. All calculations were made for the *Campylobacter* and *Salmonella* data sets. The weekly average over the 1992-2005 period for each week (1-52) was calculated. The sum of reported cases in the i^{th} week (i is the designation for one week in the set of 1-52 weeks) of each year is averaged yielding the i^{th} week average. The overall weekly average was calculated; all reported cases are summed and averaged over the weeks (reported cases/728 weeks). A 9-week moving average was also calculated for the state and counties (centered on the mid-week).

Identify Dependent Variables: All variables were identified for *Campylobacter* and *Salmonella* data sets on the county and state levels. Peak-week (continuous variable) was determined based on the week with the highest associated 9-week moving average. The high reporting period is defined as the period when reporting, defined by 9-week moving average, is higher than the overall weekly average, and that period contains the peak-week. The start-week (continuous) of the high reporting period is the first week (in a period containing the peak-week) where the 9-week moving average is above the overall weekly average value. The end-week (continuous) is the last week in that period where the 9-week moving average is above the overall weekly average value. The duration (continuous) is the range of weeks between the start and end-week (difference, start-week from end-week).

Aim 2: Evaluate geographic relation to seasonal high reporting period

Data Collection and Management of Independent Variables: Data were collected on county latitude and longitude measures (continuous). Counties were ranked and ordered 1-83 based on the latitude and longitude, for the variables lat rank (ordinal) and long rank (ordinal). Counties were also grouped based on latitude and longitude quartile. The ranges of latitude and longitude values were 41.89-47.42 and 82.59-89.80. These ranges were divided into fourths to group the counties into four geographic lat regions (ordinal) and long regions (ordinal). Data were also collected on Michigan climate zones and counties were grouped based on the climate zone (categorical).

Aim 3: Evaluate incidence in relation to seasonal high reporting period

Data collection and Management of Independent Variables: Population data estimates were collected from the US Census Bureau on Michigan and Michigan counties from 1992-2005. This data along with MDCH case data for *Campylobacter* and *Salmonella* infections in Michigan was used to calculate the state and county annual incidence for each disease, for variable incidence (continuous). The counties were divided based into quartiles (defined by 25% groups of the highest annual incidence), for the variable incidence rank (ordinal).

c. Statistical Analysis

General linear modeling (proc Glm code in SAS) procedures were used to evaluate the dependent variables peak-week, start-week, end-week, and duration with respect to the independent variables latitude, longitude, lat rank, long rank, lat region, long region, climate zone, and incidence rank. Models were constructed for each dependent variable with each individual independent variable and with combinations of non-correlated independent variables. Statistical significance was determined at the $p<0.05$ level.

3.4 RESULTS

Weekly reporting of *Campylobacter* and *Salmonella* Infections in Michigan

The time trends of reported cases of *Campylobacter* and *Salmonella* infections in Michigan by week are shown in Figures 3.1a and 3.1b. The seasonal pattern seen here is also present in the county level data (Table 3.1). Peaks and durations of these seasonal fluctuations vary between counties.

Temporal Distribution

The weekly average (grey), the overall weekly average (blue horizontal line), and the 9 week moving average (black) for *Campylobacter* and *Salmonella* infections in Michigan are shown in Figures 3.2a and 3.2b. The area above the overall weekly average and below the 9 week moving average represents the model (definition) of the high reporting period. These calculations were made for Michigan and its counties (Table 3.1).

High Reporting Period Parameters

The parameters for the high reporting period, peak-week, start-week, end-week, and duration for the aggregate Michigan case report data were calculated and are reported in Table 3.2. The parameters are similar for the *Campylobacter* and *Salmonella* high reporting periods with the *Salmonella* period starting three weeks earlier in the year than for *Campylobacter* and lasting four weeks longer. These measures were calculated for Michigan and its 83 counties and the values varied by county (Table 3.1). Of counties with annual incidence over 1 per 100,000 people, the *Campylobacter* peak-week ranged from week 8 (Luce county) to week 47 (Alger), start-week 8-43 (Luce, Alger), end-week 11-52 (Luce, Alger), and duration 0-28 (Mecosta, Wexford) for *Salmonella* the peak-week 8-48 (Ogemaw, Marquette), start-week 8-43 (Ogemaw, Arenac), end-week 14-52 (Ogemaw, Allegan), duration 6-28 (Ogemaw, Marquette).

Relation of Geography and Incidence to High Reporting Period Parameters

The duration of the period consistently significantly ($p<0.05$) correlated with all evaluation variables for *Campylobacter* incidence rank and all except longitude derived variables for *Salmonella* infections. The parameter end-week was associated with climate zone and incidence for *Campylobacter* infections and lat region and incidence for *Salmonella*. Parameter peak-week was associated with lat region and incidence for *Salmonella*. Parameter start-week was associated with lat region*long region.

3.5 DISCUSSION

Time series shows regular behavior with high reporting for both campylobacteriosis and salmonellosis in the summer and early fall. This period varies between counties with respect to all parameters. Duration of the high reporting period for campylobacteriosis and for salmonellosis was the only variable consistently associated with geographic location and incidence. For *Campylobacter* reporting, the longer periods of high reporting were associated with more southern and more eastern geographic locations and *Salmonella*'s longer reporting period (duration) showed associations with more southern locations. The Lindback and Svensson (2001) *Campylobacter* study found geographic variation (north south) related to in the start and peak weeks such that the more southern counties had earlier start and an earlier peak than northern counties. Our study showed similar results. This north to south association could be an artifact of temperature zones and the known temperature disease relationship following the assumption that southern regions of Michigan would be warmer longer; however, the climate zones did not show any relation to the high reporting period parameters. Also, the eastern trend seen with Campylobacteriosis reporting may be related to the higher population density in the eastern portion of the state.

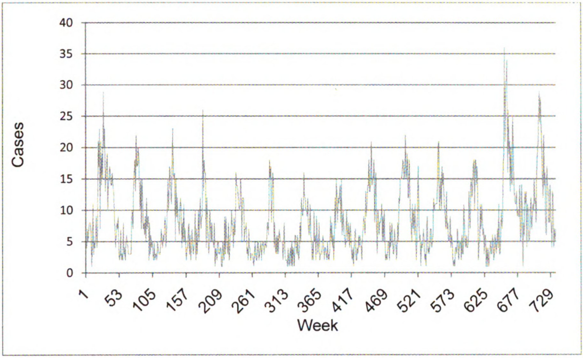
Our study went further to evaluate the relationships between the high reporting period parameters and county incidence level. We found that the incidence variable was associated with all of the high reporting period parameters at the county level (start and end week, duration, and peak week). As incidence increased the reporting parameters were affected as such: 1) start week was earlier, 2) end week was later, 3) duration was longer, and 4) peak week was earlier. These findings have not been seen in the literature and require further investigation.

This evaluation is an essential step toward modeling the high reporting period and identifying critical time points for future analysis in conjunction with environmental factors and seasonal changes in human behavior that possibly drive these disease rates. This study adds to the literature giving more information on the previously reported, but not fully explained, seasonal peaks in reported cases of these diseases, and moves toward offering a greater understanding of these peaks. The larger aim of this study is to eventually lead to the reduction of incidence and health burdens through understanding the mechanisms of transmission that can prevent future *Campylobacter* and *Salmonella* infections in humans.

3.6 CONCLUSIONS

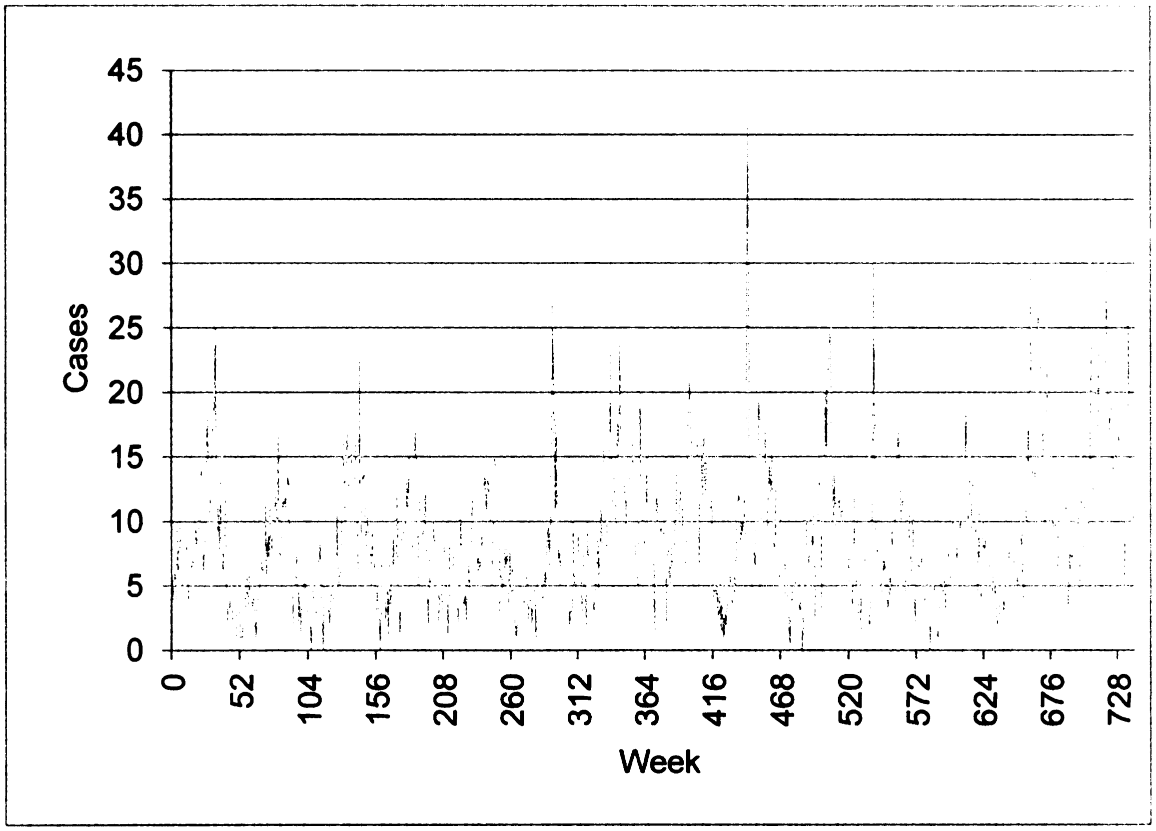
This study takes a step toward understanding the seasonal trends of campylobacteriosis and salmonellosis through defining the parameters of seasonality by geographic location. This is significant due to the relationship of geography to environmental factors (climate and weather, land use, water source). By understanding how the parameters of seasonality vary we can begin to explain and relate that variability to other seasonal variations. These may have discernable relationships with a specific start or end time, peak week, or duration that may suggest a link with host susceptibility, pathogen survival and transmissibility, or environmental load as drivers for that parameter of seasonality. Future research is needed to evaluate the differences that these geographic locations represent and modeling those differences to identify the drivers of seasonality.

Figure 3.1.a. Weekly reported cases of Human *Campylobacter* cases in Michigan



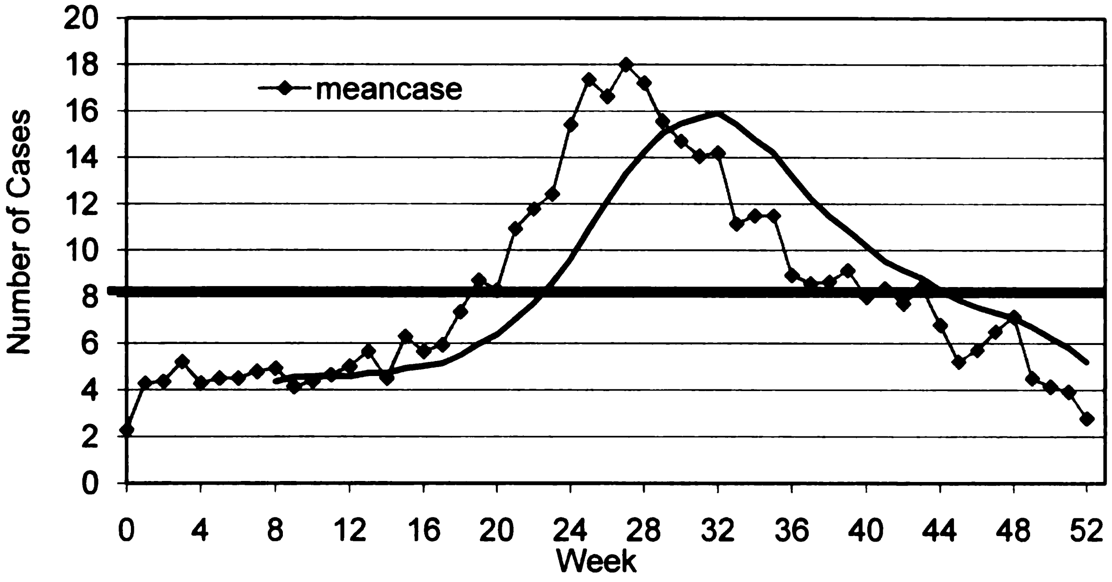
This figure shows the weekly number of *Campylobacter* cases reported in Michigan, over the period of study from 1992-2005.

Figure 3.1.b. Weekly reported cases of Human *Salmonella* cases in Michigan



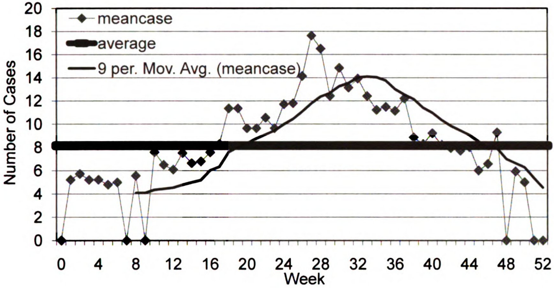
This figure shows the weekly number of *Salmonella* cases reported in Michigan, over the period of study from 1992-2005.

Figure 3.2.a. Weekly averages for *Campylobacter* cases in Michigan.



This figure shows the average number of weekly *Campylobacter* cases reported in Michigan, over the period of study from 1992-2005. The horizontal line represents the overall average and the smoothed line represents a nine week moving average trend line.

Figure 3.2.b. Weekly averages for *Salmonella* cases in Michigan.



This figure shows the average number of weekly *Salmonella* cases reported in Michigan, over the period of study from 1992-2005. The horizontal line represents the overall average and the smoothed line represents a nine week moving average trend line.

Table 3.1. Michigan Counties High Reporting Period Parameters and Geographic Data

County	*Salmonella*					*Campylobacter*					Geographic Data		
	peak week	start week	end week	duration	Incidence	peak week	start week	end week	duration	Incidence	Latitude	Longitude	Climate Zone
Alcona County	30	24	45	21	6.16	26	22	31	9	12.02	44.68	83.56	15
Alger County	30	30	38	8	0.79	47	43	52	9	2.35	46.39	86.63	16
Allegan County	46	38	52	14	3.73	31	24	40	16	5.36	42.59	85.90	14
Alpena County	36	26	52	26	2.24	26	22	40	18	2.73	45.03	83.54	15
Antrim County	24	19	44	25	3.29	29	23	37	14	8.60	45.01	85.18	15
Arenac County	47	43	51	8	2.79	26	18	37	19	1.81	44.06	83.87	15
Baraga County	40	36	48	12	5.28	48	48	52	4	0.00	46.71	88.40	17
Barry County	30	23	38	15	3.74	33	26	50	24	6.42	42.61	85.32	14
Bay County	28	23	41	18	2.77	33	25	41	16	3.14	43.64	83.92	15
Benzie County	35	27	40	13	14.08	31	25	38	13	9.41	44.64	86.04	15
Berrien County	34	18	45	27	8.33	34	24	41	17	12.12	41.97	86.43	14
Branch County	39	36	44	8	11.84	35	25	43	18	7.70	41.93	85.05	14
Calhoun County	35	20	46	26	5.59	29	23	38	15	7.11	42.28	85.08	14
Cass County	34	23	40	17	7.35	33	26	47	21	7.09	41.92	86.02	14
Charlevoix County	37	32	40	8	9.77	32	29	44	15	8.08	45.25	85.10	15
Cheboygan County	19	19	27	8	0.28	43	35	51	16	0.63	45.48	84.51	15
Chippewa County	36	30	50	20	7.77	41	24	49	25	3.49	46.32	84.50	16
Clare County	33	27	38	11	5.84	32	19	39	20	7.43	43.97	84.86	15
Clinton County	35	27	45	18	7.78	33	20	47	27	4.80	42.93	84.57	14
Crawford County	30	26	37	11	6.71	24	24	29	5	6.85	44.68	84.64	15
Delta County	32	24	40	16	11.19	29	23	37	14	10.40	45.88	86.95	16
Dickinson County	15	10	23	13	4.77	40	33	46	13	6.74	45.87	87.97	16
Eaton County	31	26	37	11	4.72	33	24	43	19	4.80	45.87	87.97	14
Emmet County	28	21	42	21	9.97	32	26	43	17	9.21	45.48	84.91	15
Genesee County	35	24	43	19	5.46	34	23	45	22	1.33	43.01	83.70	14
Gladwin County	15	11	19	8	3.35	10	9	13	4	3.53	43.98	84.42	15
Gogebic County	22	22	30	8	0.90	32	32	40	8	0.45	46.40	89.80	17
Grand Traverse County	19	15	24	9	1.39	34	27	41	14	5.00	44.70	85.57	15
Gratiot County	32	27	41	14	5.34	31	27	47	20	7.46	43.30	84.62	14

Table 3.1 Continued

County	*Salmonella*					*Campylobacter*					Geographic Data		
	peak week	start week	end week	duration	Incidence	peak week	start week	end week	duration	Incidence	Latitude	Longitude	Climate Zone
Hillsdale County	28	20	42	22	8.88	31	23	50	27	12.76	41.90	84.60	14
Houghton County	36	28	50	22	8.37	30	23	36	13	3.64	47.03	88.61	17
Huron County	21	13	22	9	2.37	37	34	45	11	3.05	43.84	83.07	14
Ingham County	32	25	44	19	5.79	31	24	48	24	4.08	42.67	84.47	14
Ionia County	39	34	46	12	4.84	32	28	39	11	4.73	42.96	85.08	14
Iosco County	37	25	43	18	6.19	27	8	35	27	3.87	44.36	83.57	15
Iron County	17	13	25	12	1.21	32	25	33	8	2.98	46.20	88.56	17
Isabella County	33	27	46	19	7.85	31	25	51	26	13.01	43.63	84.84	15
Jackson County	22	18	29	11	5.98	32	19	47	28	4.04	42.24	84.40	14
Kalamazoo County	29	21	45	24	8.99	32	24	41	17	7.85	42.26	85.56	14
Kalkaska County	35	29	43	14	8.07	43	31	51	20	11.20	44.71	85.12	15
Kent County	30	22	45	23	3.41	28	22	45	23	3.45	42.99	85.61	14
Keweenaw County	34	34	42	8	7.28	26	25	34	9	7.20	47.43	88.24	17
Lake County	33	33	41	8	3.03	0	0	0	0	0.00	43.95	85.83	15
Lapeer County	33	28	49	21	4.82	43	25	50	25	3.83	43.07	83.23	14
Leelanau County	27	22	42	20	5.55	38	27	45	18	13.64	44.95	85.77	15
Lenawee County	30	21	41	20	13.20	35	25	43	18	10.87	41.92	84.07	14
Livingston County	34	20	46	26	8.27	32	23	39	16	7.76	42.57	83.87	14
Luce County	31	31	39	8	1.12	8	8	11	3	1.09	46.46	85.59	16
Mackinac County	14	14	22	8	0.68	21	21	29	8	0.65	46.04	84.99	16
Macomb County	34	23	42	19	7.17	32	22	40	18	6.90	42.59	82.95	14
Manistee County	37	31	52	21	1.22	25	24	32	8	1.90	44.32	86.14	15
Marquette County	48	24	52	28	13.24	29	22	42	20	16.72	46.46	87.61	16
Mason County	39	31	48	17	5.51	31	27	38	11	1.62	43.98	86.31	15
Mecosta County	41	35	49	14	1.76	25	25	25	0	1.33	43.65	85.35	15
Menominee County	30	23	40	17	12.57	31	23	44	21	22.07	45.46	87.57	16
Midland County	30	27	39	12	0.55	35	27	47	20	1.27	43.64	84.34	15
Missaukee County	28	25	34	9	13.64	38	26	45	19	14.23	44.33	85.15	15
Monroe County	31	21	41	20	6.59	31	24	43	19	6.39	41.92	83.48	13
Montcalm County	17	16	24	8	2.10	27	20	47	27	4.47	43.31	85.18	14

Table 3.1 Continued

County	*Salmonella*					*Campylobacter*					Geographic Data		
	peak week	start week	end week	duration	Incidence	peak week	start week	end week	duration	Incidence	Latitude	Longitude	Climate Zone
Montmorency County	33	24	48	24	4.04	23	23	31	8	0.73	45.02	84.15	15
Muskegon County	29	26	42	16	7.82	31	23	44	21	5.32	43.25	86.22	14
Newaygo County	31	25	33	8	4.09	26	20	36	16	4.91	43.53	85.81	15
Oakland County	32	20	48	28	3.15	32	22	43	21	3.43	42.59	83.31	14
Oceana County	41	18	45	27	4.80	26	18	38	20	2.45	43.64	86.29	15
Ogemaw County	8	8	14	6	1.08	44	40	52	12	4.41	44.33	84.13	15
Ontonagon County	30	30	38	8	1.98	34	27	51	24	2.97	46.68	89.28	17
Osceola County	31	28	37	9	3.67	22	22	30	8	2.01	43.98	85.35	15
Oscoda County	26	24	32	8	7.39	40	32	47	15	6.77	44.68	84.91	15
Otsego County	21	13	41	28	6.82	27	21	32	11	2.17	45.01	84.63	15
Ottawa County	31	17	40	23	10.16	29	22	42	20	11.23	42.93	86.05	14
Presque Isle County	30	30	38	8	1.08	24	13	32	19	0.55	45.37	83.93	15
Roscommon County	36	23	41	18	6.02	30	26	46	20	2.56	44.36	84.65	15
Saginaw County	34	26	35	9	4.16	35	21	48	27	2.74	43.38	84.00	14
Sanilac County	40	31	48	17	4.13	31	24	39	15	5.33	43.38	82.76	14
Schoolcraft County	18	18	26	8	0.86	15	15	23	8	3.46	46.14	86.24	16
Shiawassee County	47	39	51	12	3.33	43	34	52	18	3.00	42.94	84.13	14
St. Clair County	40	32	49	17	0.95	30	25	45	20	1.16	42.92	82.60	14
St. Joseph County	28	21	42	21	7.23	32	24	43	19	8.62	41.90	85.53	14
Tuscola County	22	17	35	18	3.98	41	33	49	16	4.65	43.46	83.43	14
Van Buren County	33	25	38	13	8.53	31	28	39	11	6.54	42.26	86.04	14
Washtenaw County	37	24	49	25	3.56	32	24	46	22	3.58	42.26	83.76	13
Wayne County	33	25	44	19	0.81	30	23	41	18	0.73	42.34	83.20	13
Wexford County	32	26	48	22	17.32	27	21	49	28	13.38	44.33	85.55	15

Table 3.2. Michigan High Reporting Period Parameters

	Start Week	Peak Week	End Week	Duration
Campylobacter	23	32	44	21
Salmonella	20	33	45	25

This table reports the high reporting period parameters for the state of Michigan over the course of the study for *Campylobacter* and *Salmonella* reported human infections.

CHAPTER 4

Environmental Factors Influencing Rates of *Campylobacter* and *Salmonella* Infections in Michigan

4.0 STRUCTURED ABSTRACT

Objective- To evaluate *Campylobacter* and *Salmonella* infection data collected in Michigan from 1992-2005 in conjunction with environmental factors across various scales from county level, to watershed, to climate division.

Specific Aims- To evaluate the role of environmental and climatological factors in changes in incidences of human *Campylobacter* and *Salmonella* infections in Michigan.

Design- Retrospective Analysis

Methods- Data were analyzed on multiple scales including county for localized effects due to land-use and water resources, watersheds, and by climate division for climate related variables. Statistical analyses included mixed methods to account for temporal relationships in the data.

Results- Counties with large percentages (greater than 40%) of their land in agricultural production were significantly associated with higher rates of campylobacteriosis. Areas with mid ranges 21-40% in agricultural production were associated with the lowest levels of campylobacteriosis and salmonellosis. While source of potable water and sewage disposal method both were significant factors in predicting rates in counties, they explained small amounts of the observed variability in both *Campylobacter* and *Salmonella* infection rates.

Significant differences in rates were seen between the climate divisions with the highest rates in division 3 (north west lower peninsula).

Conclusion- Time (year and month) and daily maximum temperature were the best predictors of human *Campylobacter* and *Salmonella* incidence by county across the climate divisions. These types of models may help to explain excess case rates in high rate counties which do not fit demographic trends, as described earlier.

4.1 INTRODUCTION

The gram-negative bacteria, *Campylobacter* and *Salmonella* have been recognized as leading causes of diarrheal illness in the United States and worldwide (USDA, 2003, Altekruse et al., 1999, CDC, 2005). These bacteria are commonly associated with poultry, and other livestock, and consumption of poultry is a major risk factor for both campylobacteriosis and salmonellosis (Kapperud et al., 1992, Wysok and Uradziński, 2009). *Campylobacter* and *Salmonella* have also been transmitted by the water route (Kussin et al., 2005, Kirian et al., 2008), which may be related to contamination from animal reservoirs (including poultry and cattle) especially in rural areas and human behavior.

In US an estimated 1% of the population is infected annually with campylobacteriosis, with an average 12.7 reported cases per 100,000 people per year due to underreporting (CDC, 2006). For salmonellosis, an average of 14.6 cases per 100,000 people is reported annually (CDC, 2006). In many regions of the world, cases for both these diseases are on the rise (Coker et al., 2002). In the US, case rates of campylobacteriosis have declined since the beginning of coordinated surveillance in 1996 (Samuel et al., 2004); however, distinct seasonal trends continue to cause a high burden of disease in summer months. Rates for salmonellosis have only decreased slightly (CDC 2005). Given the high prevalence of campylobacteriosis (Oberhelman and Taylor, 2000, Lindback and Svensson, 2001,Coker et al., 2002, Samuel et al., 2002) and the potential for the infection to lead to more serious illnesses (Guillian-Barré Syndrome (1 in 1,000 cases) (CDC 2004), which costs the United States up to 1.8 billion dollars annually (Buzby et al., 1997), it is important to identify and understand factors that influence the incidence of the disease both spatially and temporally (between years and seasons). This is also important for salmonellosis as it too has great impact on health with the possibility of leading to reactive arthritis or Reiter's syndrome and an economic impact. A recent study in Spain calculated the economic burden of salmonellosis related hospitalization to find an average annual cost of 12.4 million Euros (Gil Prieto et al., 2009).

In analyses of campylobacteriosis and salmonellosis in developed nations (e.g., US and U.K.) consistent demographic trends in disease incidence have been observed, most notably a peak in cases among young children (<5 years old) and males (CDC, 2005, Samuel et al., 2004, Louis et al., 2006). Additionally, a distinct seasonal pattern with cases peaking in the summer months has been noted world-wide. While this seasonal trend is often attributed to food preparation issues related to picnics and eating outside of the home (Coker et al., 2002, Miller et al., 2004, Louis et al., 2005, Fullerton et al., 2008), this does not explain why the same pattern exists among cultures with different summertime customs.

Others have suggested environmental factors may drive this seasonal pattern, including higher loading of the bacteria in livestock and increased *Campylobacter* transmission among poultry flocks by flies (Rosef and Kapperud, 1983, Hald et al., 2004, Nichols, 2005) that could lead to an increase in food animal carriage and potential for greater human exposure and infection.

As evaluated and reported in Chapter 2, demographic analyses in Michigan for case data collected between 1992 and 2005 revealed that counties with a high population density, young populations (<5 year age group), and largely white populations were positively correlated with case rates; however, in this study several counties reporting the highest case rates historically (annual county averages up to 22.1 *Campylobacter* cases and 17.3 *Salmonella* cases per 100,000) did not follow these trends. These high incidence counties were often located in rural areas of Michigan. This disparity in population density is particularly interesting given that in a nation-wide study of campylobacteriosis in the U.K., Louis et al. (2006) found that case rates were negatively correlated with population density and positively correlated with agricultural land use. Louis et al. (2006), and others, have demonstrated that both environmental and weather related factors influence contamination of surface waters with enteric pathogens and human campylobacteriosis and salmonellosis disease patterns (Patz, 2001, Lipp et al., 2002, Kambole, 2003, Ashbolt, 2004, D' Souza et al. 2004, Fleury et al. 2005, Zhang et al. 2008). Given the rural and suspected agrarian nature of the 'anomalous' high case rate counties in Michigan and the potential for environmental transmission to explain these rates, we hypothesized that non-demographic factors including environmental and weather-related variables may be important influences in disease incidence. Here we evaluate the role of agricultural land-use, water and sewage disposal resources, and weather variability on a campylobacteriosis and salmonellosis patterns in Michigan over a 13 year period (1992 – 2005).

4.2 HYPOTHESES

Environmental and weather related factors are associated with changes in rates of *Salmonella* and *Campylobacter* infections in Michigan such that:

- As temperature increases incidence of these diseases will increase.
- As precipitation increases incidence of these diseases will increase.
- Areas with more agricultural land use sources will be prone to higher incidences of these diseases.
- As percentages of homes with non-municipal water and sewage disposal increase rates of these diseases will increase.

4.3 METHODS

In order to capture multiple scales of possible influence on rates of campylobacteriosis and salmonellosis, data were analyzed at the county, watershed, and climate division levels. Data were obtained for each of the 83 counties and aggregated into ten climate divisions (defined by NOAA) (Figure 4.1) and into 62 watersheds (defined by USGS eight digit HUC codes) for analysis (MI DEQ 2009). Geographic analyses were conducted at the county level to examine local-level land use factors that may contribute to campylobacteriosis and salmonellosis rates, and at the watershed and climate

division level to evaluate the role of regional impacts that may be associated with regional trends in reported campylobacteriosis. All data variables are described in Table 4.2 and all model outcomes are described in Tables 4.3 and 4.4.

a. Campylobacteriosis and Salmonellosis Rates

Over the thirteen year period of study, records of all culture confirmed *Campylobacter* and *Salmonella* cases in Michigan from 1992 to 2005 (from onset date) were provided by the Michigan Department of Community Health (MDCH). All culture confirmed cases of campylobacteriosis and salmonellosis in Michigan must be reported to public health officials when diagnosed. Variables that were extracted from the State database include the reportable condition, case status, state and county of residence, onset, confirmation, diagnosis, and referral dates, age, race, and gender. In instances where there was a missing identifier, the case was excluded from the calculation of incidence for that variable. (For example, if a case did not report the county of residence for the individual but did give the state the case would be included in the state rate calculation but would not be included with any county level calculations.) Population estimates were obtained from the US Census Bureau (http://www.census.gov/popest/datasets.html.). Monthly county incidence rates were calculated by dividing the number of cases reported in a county in the study month by the number of people in that demographic group in the county and multiplying by 100,000 to give the number of individuals infected per 100,000 people per month. *Campylobacter* and *Salmonella* rates were also evaluated using the Freeman Tukey Square Root Transformation (transformed rate = $(100,000)^{1/2}$ $\{[C/N]^{1/2}$

$+[(C+1)/N]^{1/2}\}$ where C is the number of *Campylobacter* or *Salmonella* cases and N is the population) to accommodate model assumptions (Cressie, 1993).

b. <u>County-level Geographic Analyses</u>

Agricultural land-use data values were approximated from Michigan Agricultural Statistics land in agricultural production report (MASS, 2005). The land in agricultural production was reported in acres and this value was divided by the county's total land area to get the percent of agricultural land. No data were reported for the counties of Luce and Keweenaw. For counties reporting land in agriculture production, percentages ranged from 79% to less than 1%. This range of percents was broken into the following category clusters: 0-20%, 21-40%, 41-60%, and <60%.

Information on the number of occupied homes, the source of drinking water (public or non-public), and type of waste water disposal (centralized sewer or on-site disposal, i.e., septic system or cess pit) for households in each county was obtained from the US Census Bureau. The percent of homes using public water sources and percent using public sewage disposal within each county were calculated and these variables are described in Table 4.2. Percents ranged from 2.5% to 99.5% of homes in the counties on public water and 2.6% to 98.1% of homes on public sewage. These were broken into 10% incremental categories (deciles) to create 10 groups for public water source and 10 for public sewage disposal.

Statistical associations between monthly county case rates and land-use classification were assessed using the Generalized Estimating Equation, GEE, (PROC GENMOD) analyses (SAS v.8, Cary, NC) with a repeated (county) option (Table 4.2). All associations were considered significant at $p \leq 0.10$. The GENMOD model (SAS v.8, Cary, NC) was also used to model homes using non-public water sources and using septic systems in relation to disease incidence rates.

c. Seasonal and Monthly Analysis

For the state, case rates were analyzed with respect to month of the year. At the climate division level of analysis, an autocorrelation procedure using month and incidence were performed for each of the divisions to determine the seasonal patterns.

d. Watershed and Climate Division Geographic Analyses

County data were assigned to watersheds (defined by USGS eight digit hydrologic unit codes [HUC]) based on the location of the county center using geographic information software (ARCGIS v.9.1, ESRI, Redlands, CA). County data were assigned to climate divisions per the National Oceanic and Atmospheric Administration (NOAA) division boundaries.

e. Meteorological Factors

Daily average precipitation, average maximum daily temperature (F), average minimum daily temperature, average mean daily temperature, high temperature, and low temperature monthly data were obtained from the National Climate Data Center (NCDC) for all weather stations in Michigan for 1992 – 2005. These variables are further described in table 4.1. Data were available from stations state-wide. The counties Wayne, Washtenaw, St. Clair, Oakland, Newaygo, Monroe, Macomb, Livingston, Lenawee, Lapeer, Genesee, Clare, and Antrim did not have any weather station data reported from NCDC over the study period. As these counties were missing these variables, they were excluded from county level analysis for the missing variables. Data from all stations within an individual county, watershed, or climate division were compiled taking the average values from all stations with in the geographic area. Precipitation data were analyzed as the amount of precipitation or the amount of snow (in inches) for the month. Monthly average daily maximum and minimum temperatures were evaluated for all stations within the counties. These values were used in conjunction with the precipitation data in a GEE model (SAS v.8, Cary, NC) to predict county case rates (Table 4.2).

The GEE analysis required evaluation of the variables in a two step process. The first step was to perform a univariate analysis evaluating climate, and environmental/geographical factors individually with respect to human cases of *Campylobacter* and *Salmonella* infections. Variables were considered significant at the 0.10 level ($p<0.10$). If variables were significant in this univariate analysis, they were then combined with other significant variables in a multivariate analysis. The multivariate analysis was run with a variable removal criterion of $p>0.05$. The final multivariate model contained all variables with significance at the 0.05 level. Given that cases were count data and zero counts were frequently recorded for study counties, the *Campylobacter* and *Salmonella* cases were evaluated using the GEE model with a Poisson distribution with and offset (log of population) function.

4.4 RESULTS

a. Campylobacteriosis and Salmonellosis Rates

Between 1992 and 2005, the average annual incidence of culture confirmed *Campylobacter* infections in Michigan (statewide) was 4.28 cases per 100,000 people. There were 5,864 reported *Campylobacter* cases and an average of 419 cases each year. The annual incidence of *Salmonella* infections were 4.50 cases per 100,000 people. There were 6,263 reported *Salmonella* cases and an average of 447 cases each year.

There were significant differences between climate divisions. For the rates of Campylobacteriosis, division 3 (northwest lower peninsula) was significantly higher than all other divisions (9.28 cases per 100,000 people) and divisions 7 (mideast lower peninsula) and 5 (midwest lower peninsula) the lowest (3.6 and 2.8 cases per 100,000 respectively). For Salmonellosis, the highest rates were in climate division 3 (8.0 per 100,000) and the lowest in divisions 6 (middle lower peninsula), and 7 (3.8 and 3.5 per 100,000) (Figure 4.1) (http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/regional_monitoring/CLIM_DIVS/michigan.gif). Throughout Michigan, annual incidences for both *Campylobacter* and *Salmonella* infections showed no trend. For campylobacteriosis, climate divisions 3, 6, 7, 8 (southwest lower peninsula), and 9 (lower mid lower peninsula) showed significant relationships with temporal measures month and year. Divisions 10 (southwest lower peninsula) and 3 showed significant temporal relationships with salmonellosis rates.

There were significant differences in rates of human disease between 62 watersheds evaluated. Watershed 42 (Cedar) had the highest rates of campylobacteriosis followed by 46 (Escanaba) and 28 (Platte) (http://www.michigan.gov/documents/deq/lwm-mi-watersheds_202767_7.pdf). Lowest rates were seen in 25 (Pere Marquette) and 56 (Presque Isle). Highest salmonellosis rates were seen in 46, 42, and 4 (Betsie) and the lowest rates in 56 and 39 (Au Train).

b. County-Level Geographic Analyses

At the county level, land use, potable water source and method of waste water disposal were evaluated to determine their relationship to incidence of campylobacteriosis and salmonellosis. There were significant differences in land in agriculture categories with respect to incidence rates. Counties with greater than 40% of land area in agriculture had significantly higher rates of campylobacteriosis than other counties. Counties with 21 to 40% of land area in agriculture had the lowest rates for both campylobacteriosis and salmonellosis. For salmonellosis, the 21 to 40% group was the only group significantly different from the others. Lower rates of campylobacteriosis were associated with counties with greater than 70% of homes on public sewage and counties with 20-30% on public sewage. The counties with the highest percentages of homes using municipal sewage had the lowest rates of salmonellosis. Counties with greater than 80%, 50-60%, and 20-30% of homes using public water sources have the lowest campylobacteriosis and salmonellosis rates statistically.

c. Seasonality

State-wide between 1992 and 2005, case rates peaked in the summer months for both campylobacteriosis and salmonellosis. Mean rates in June, July and August were significantly greater than those reported in other months (Chapter 2, Fig. 2.4). For all the climate districts campylobacteriosis case rates peaked in June and July and exhibited a second, lower peak in the late fall, but this was not statistically significant. Salmonellosis rates peaked in July and August. When regression techniques were use to evaluate the amount of variation in *Campylobacter* and *Salmonella* explained by time (year and month), the amount of *Campylobacter* variation explained ranged from 1.5% in division 8 to 9.5% in division 10 with division 5 having no significant temporal relationship, and the amount of *Salmonella* variation explained ranged from 2.1% in division 1 to 10.4% in division 8. The results of analysis for significant seasonal autocorrelation of case rates varied among the different climate divisions. Using Tukey transformed case rates, division 4 was the only climate division to show no monthly autocorrelations with campylobacteriosis rates, while all other divisions showed autocorrelations for various monthly cycles with transformed *Campylobacter* and *Salmonella* infection rates. Most climate divisions showed autocorrelations at cycles 10 or greater suggesting an interannual trend, while a many showed autocorrelations of the first or second order. This short period suggests relationships between cases reported within one month of each other.

d. Meteorological Analysis

Case rates in all climate divisions were evaluated for temperature (5 measures) and mean precipitation over the 14 year period of record. There was no climate data reported for climate division 10. In the univariate analysis, average temperature variables consistently significantly explained a small percentage of the variation in *Campylobacter* and *Salmonella* infection rates (*Campylobacter*: up to 10.5% of the variation in rates were explained by the mean daily minimum temperature for that month in climate division 8 (southwest lower peninsula) *Salmonella:* up to 5.8% explained by the same factor in the same division (8)). Precipitation significantly explained some of the *Campylobacter* rate variability in 4 of the 9 divisions with available climate data (divisions 9, 8, 6, and 3). Precipitation explained *Salmonella* rate variability in divisions 9 and 3. In all climate zones, average maximum daily temperature and time were the most significant predictors of incidence using the Tukey transformed data and produced the best fitting models predicting incidence.

4.5 DISCUSSION

During the 13 year period of analysis (1992-2005), rates of *Campylobacter* and *Salmonella* infection in Michigan averaged annually 4.28 and 4.5 cases per 100,000 people respectively and varied across the state from 3.5 cases per 100,000 people in division 7 to 8.1 cases in climate division 3 for campylobacteriosis and for salmonellosis cases ranged from 2.8 cases per 100,000 people in division 5 to 9.3 cases in division 3. During this same period the national (U.S) averages for *Campylobacter* and *Salmonella* infections were

16.9 and 15.4 cases per 100,000 people, respectively. To explain the variations in rates that are not explained by demographic factors, this study examined case data by county and by climate district to evaluate the possible environmental influences on rates of campylobacteriosis and salmonellosis.

Counties in Michigan with consistently high *Campylobacter* rates have been identified (Chapter 2) including Menominee, Marquette, Isabella, Wexford, Leelanau, Emmet, Alcona; and for *Salmonella* Wexford, Benzie, Branch, Keweenaw, and Oscoda. These counties are dispersed throughout the state with the largest grouping of these consistently high rate counties in climate division 3. Most of these counties followed demographic trends of low population density and tended to trend toward the older age groups. In contrast, national reported trends suggest that high density counties with young populations should have higher rates. These observations combined with recent studies associating *Campylobacter* infection rates with climate and agricultural land use (Patrick, 2004; Kovats, 2005; Louis et al., 2005) make it evident that previous research using only demographic variables have not adequately explained the variation in *Campylobacter* infection rates in the US. This study examined possible environmental factors to explain the variations in the Michigan *Campylobacter* and *Salmonella* infection rates.

Environmental variables of interest include: land-use, potable water source, method of sewage disposal, and meteorological factors (temperature variables and average precipitation) as these factors are closely tied to water quality and can affect large geographic areas. In order to examine these factors, the scale of effect must be considered. The data were first examined on the county level to ascertain the relationship between land-use, potable water source, and method of sewage disposal to rates of *Campylobacter* infection. As weather patterns often affect large geographic areas, the variables daily maximum and minimum temperatures and daily precipitation were evaluated at both the county and climate divisions level.

County. It has previously been shown that land use has a great effect on the local environment and human health (urban areas allowing for more runoff; forest lands allowing the least) (Interlandi and Crockett, 2003, Sherestha, 2003). The source of run-off can in turn affect the types and amounts of pathogens found in the waterways with agricultural and farmlands often associated with fecal pathogens (Atwill, 1995, Mallin et al., 2000, Graczyk et al., 2000, Crowther et al., 2002, Stanley and Jones, 2003, Ferguson et al., 2003, Kelsey et al., 2004). Indeed, Potter et al. (2002) has found an association between high concentrations poultry/farmland and high rates of campylobacteriosis (Potter et al., 2002). In this study we found that counties with greater percentages of land in agriculture had the highest incidence of campylobacteriosis while an intermediate group, 21-40% of land in agriculture, had the lowest incidence of campylobacteriosis and salmonellosis, suggesting that significantly more rural areas may be more prone to high rates of *Campylobacter* infection. However, this presents a disparity between these findings and the demographic analyses that revealed that counties with intermediately higher population densities (presumably more urban) had higher rates (Chapter 2). Despite these differences, the trends noted here for agricultural land-use may suggest that areas with more agrarian lands had higher disease burden, which is consistent with other reports (e.g., Louis et al. 2006).

Statistically significant relationships were noted between the percentage of homes using non-municipal potable water source and on-site sewage disposal and rates, which indicated that these factors were predictors of lower case rates. However, only small amounts of the variability in the case data could be explained by either of these variables, suggesting that they are of low value in studying the epidemiology of this disease.

Climate Division. The climate division level analysis allowed for examination of large scale factors, such as climate, on disease rates. It has been shown that variation in precipitation affects the local environment and human health. Extreme changes in precipitation are known to be associated with decreased water quality (Leeming et al., 1998, Lipp et al. 2001, Interlandi and Crockett, 2003) and increased gastrointestinal disease (Curreno et al., 2001, Lipp et al, 2002), including campylobacteriosis and salmonellosis (Louis et al., 2006, Zhang et al. 2008). Temperature has also been shown to have a great effect on *Campylobacter* and *Salmonella* survivability such that lower temperatures are favorable (Buswell, 1998, Danyluk et al. 2008); however, published studies have found that higher temperatures and hours of sunshine are significantly associated with the campylobacteriosis and salmonellosis incidences (D'Souza et al. 2004, Patrick, 2004, Fleury et al. 2005, Kovats, 2005; Louis, 2006). Both high temperature and the number of hours of sunlight in the summer help to explain the consistent seasonality of this disease; but still do not provide a mechanism for the trend. These associations are consistent with our findings. Models that included precipitation or either maximum daily temperature or minimum daily temperature explained a significant percentages of the variability in incidence of campylobacteriosis and of salmonellosis by climate division. Maximum daily temperature alone was best able model most climate divisions.

4.6 CONCLUSIONS

In Chapter 2 we presented results that demonstrated demographic patterns associated with high case rates including high population density, young populations (<5 age group), and largely white populations; however, in the counties with the most frequent high case rates over time this trend was not observed. These high incidence counties were scattered throughout the state but were primarily rural areas. We hypothesized that other factors were influencing the distribution of rates in Michigan, particularly in these regions.

Here the environmental variables land use, source of potable water, sewage disposal method, and climatological factors were evaluated for their contribution to case rates in Michigan. As the major trend in rates across the state is seasonality, as expected, time was often among the best predictors of Campylobacter and *Salmonella* case rates in time series analyses. While precipitation had some influence on certain climate divisions, temperature (maximum daily temperature) was the most important environmental predictor of climate division-wide variability in case rates.

The counties of interest identified in chapter 2, which had high rates despite a low population density, included several counties in climate division 3 (north west lower peninsula). This division had the highest rates over all others and showed significant associations with precipitation and all temperature measures for both *Campylobacter* and *Salmonella* incidences. While differing from the demographics of the state, the trends in case rates in this division better correspond to previous research that suggest that rural and agricultural areas are more prone to high incidence of campylobacteriosis (e.g., Patrick, 2004; Kovats, 2005; Louis et al., 2006). Furthermore, the primary role of temperature in explaining the case rate variability is also consistent with reports from other areas (D'Souza et al. 2004, Patrick, 2004, Kovats, 2005; Louis et al., 2006; Zhang et al. 2008). Therefore, it seems that in Michigan, environmental factors should be further explored as a driver of disease in some rural areas whereas demographics may play a greater role in highly metropolitan areas. These observations are difficult to interpret, however, because of the incompatibilities between the various units of analyses and the lack of understanding of the natural history of campylobacteriosis and salmonellosis among these different demographic and geographic units of analysis. This study highlights the poorly understood environmental ecologies of these diseases and suggests that there are multiple risk factors of disease at the individual level that are modified by large scale and regional environmental impacts on pathogen presence. This information on the difference between urban and rural centers is important in attempts to prevent and understand this disease and suggests that different strategies may be needed.

Figure 4.1. Michigan Climate Divisions and Counties.

1 West Upper	
2 East Upper	
3 Northwest Lower	
4 Northeast Lower	
5 West Central Lower	
6 Central Lower	
7 East Central Lower	
8 Southwest Lower	
9 South Central Lower	
10 Southeast Lower	

This figure illustrates a map of Michigan with the counties and climate division boundaries.

Table 4.1 Michigan Counties with Watershed and Climate Division classification

COUNTY	Watershed	CLIMATE DIVISION
ALCONA	2	4
ALGER	39	2
ALLEGAN	17	8
ALPENA	36	4
ANTRIM	13	3
ARENAC	30	7
BARAGA	48	1
BARRY	14	9
BAY	18	7
BENZIE	4	3
BERRIEN	34	8
BRANCH	34	9
CALHOUN	17	9
CASS	34	8
CHARLEVOIX	10	3
CHEBOYGAN	11	4
CHIPPEWA	62	2
CLARE	32	
CLINTON	14	9
CRAWFORD	2	4
DELTA	58	2
DICKINSON	50	1
EATON	14	9
EMMET	11	3
GENESEE	32	10
GLADWIN	32	6
GOGEBIC	56	1
GRAND TRAVERSE	9	3

Table 4.1 Continued

COUNTY	Watershed	CLIMATE DIVISION
GRATIOT	32	6
HILLSDALE	21	9
HOUGHTON	55	1
HURON	26	7
INGHAM	14	9
IONIA	14	9
IOSCO	1	4
IRON	50	1
ISABELLA	32	6
JACKSON	14	9
KALAMAZOO	17	8
KALKASKA	20	3
KENT	14	8
KEWEENAW	55	1
LAKE	25	5
LAPEER	32	10
LEELANAU	28	3
LENAWEE	29	10
LIVINGSTON	32	10
LUCE	60	2
MACKINAC	41	2
MACOMB	12	10
MANISTEE	20	3
MARQUETTE	46	1
MASON	5	5
MECOSTA	22	6
MENOMINEE	42	1
MIDLAND	32	6

Table 4.1 Continued

COUNTY	Watershed	CLIMATE DIVISION
MISSAUKEE	22	3
MONROE	29	10
MONTCALM	14	6
MONTMORENCY	36	4
MUSKEGON	22	5
NEWAYGO	37	5
OAKLAND	12	10
OCEANA	37	5
OGEMAW	30	4
ONTONAGON	53	1
OSCEOLA	22	6
OSCODA	2	4
OTSEGO	11	4
OTTAWA	14	8
PRESQUE ISLE	23	4
ROSCOMMON	22	4
SAGINAW	32	7
SANILAC	6	7
SCHOOLCRAFT	49	2
SHIAWASSEE	32	9
ST. CLAIR	27	10

Table 4.1 Continued

COUNTY	Watershed	CLIMATE DIVISION
ST. JOSEPH	34	9
TUSCOLA	32	7
VAN BUREN	34	8
WASHTENAW	15	10
WAYNE	31	10
WEXFORD	20	3

Case data were collected at the county level and to evaluate watershed and climate division levels the county case data had to be assigned to the respective units. This table shows which counties were assigned to each watershed and to each Climate division.

Table 4.2 Description of Model Variables

Variable Type	Variables		Reporting Level	Level	Interval	Time Range	Source
Climate	Precipitation	Daily Ave Precipitation	Weather Stations	Continuous	Daily	1992-2005 Note: some stations report incomplete time periods	National Climate Data Center (NCDC)
	Temperature	Ave Daily Mean Temp					
		Ave Max Daily Temp					
		Ave Min Daily Temp					
		Daily High Temp					
		Daily Low Temp					
Environmental Data	Sewage Disposal: *Percent of occupied homes using public disposal*		County	Continuous interval	Every 10 years	1990, 2000	US Census Bureau
	Water Source: *percent of occupied homes using public water*						
	Land use: *Percent of Land in Agricultural Production*		County	Continuous interval	Annual	1992-2005	Michigan Agricultural Statistics Service MASS
Case Data	Campylobacteriosis Cases		County	discrete	Daily	January 1, 1992 - December 31, 2005	Michigan Department of Community Health
	Salmonellosis Cases						
Population Data	Population		County, State	discrete	Annual Estimates	1992 -2005	US Census Bureau

Table shows the variables evaluated in the study along with the parameters of those variables.

Table 4.3 Univariate analysis results for climate variables with respect to disease cases.

Hypothesis	Dependent Variables	Independent Variables	GEE model Results	
			p	B
As temperature (°F) *increases incidence will increase.*	*Campylobacter* disease cases	Mean daily	<0.0001	0.0277
		Mean Maximum Daily	<0.0001	0.0253
		Mean Minimum Daily	<0.0001	0.0292
		Month Maximum	<0.0001	0.0279
		Month Minimum	<0.0001	0.0252
	Salmonella disease cases	Mean daily	<0.0001	0.0213
		Mean Maximum Daily	<0.0001	0.0196
		Mean Minimum Daily	<0.0001	0.0228
		Month Maximum	<0.0001	0.0213
		Month Minimum	<0.0001	0.0191
As precipitation (inches) increases incidence will increase	*Campylobacter* disease cases	Monthly Snow	<0.0001	-0.0425
		Monthly precipitation	<0.0001	0.0861
	Salmonella disease cases	Monthly Snow	<0.0001	-0.0291
		Monthly precipitation	0.0070	0.0758

This table describes the models created in the study to evaluate each specific hypothesis, details the variables included in each, and presents the findings.

Table 4.4 Univariate analysis results for environment/geographic variables with respect to disease cases.

Hypothesis	Dependent Variables	Independent Variables	GEE model Results	
			p	B
As % of land in agricultural production increases incidence will increase.	*Campylobacter* disease cases	Percentage of land in Ag production	>0.10	--
	Salmonella disease cases	Percentage of land in Ag production	0.0934	0.2797
As % of homes with private water and sewage disposal methods increase incidence will increase.	*Campylobacter* disease cases	Water source	>0.10	--
		Sewage Disposal	>0.10	--
	Salmonella disease cases	Water source	>0.10	--
		Sewage Disposal	>0.10	--
There will be a relationship between geographic parameters and incidence of disease.	*Campylobacter* disease cases	Climate Division	0.0930	-0.0931
		Health District	>0.10	--
		Watershed	>0.10	--
	Salmonella disease cases	Climate Division	>0.10	--
		Health District	>0.10	--
		Watershed	>0.10	--

This table describes the models created in the study to evaluate each specific hypothesis, details the variables included in each, and presents the findings.

CHAPTER 5

Campylobacter and *Salmonella* on Michigan Dairy Cattle Farms: Culture Isolation and Enumeration from Environmental Soil and Water

5.0 STRUCTURED ABSTRACT:

Objective: To isolate and enumerate, via culture methods, *Campylobacter* and *Salmonella* from Michigan dairy cattle farm environments in counties with varying rates of human disease and relate recoverability to temperature.

Specific Aims: 1) To evaluate culture methods for the enumeration of *Campylobacter* and *Salmonella* in environmental soil and water samples, 2) To experimentally evaluate the effect of temperature on *Campylobacter* and *Salmonella* recovery from soil and water, and 3) To evaluate Michigan dairy farms in counties, with varying rates of human disease, for the presence of *Campylobacter* and *Salmonella* in soil and water.

Methodology: 1) Methods were derived from available literature on Most Probable Number (MPN) enumeration techniques and environmental soil and water isolation methods for *Campylobacter* and *Salmonella*. These methods were straightforward for water but required modification for soil as there was a gap in the literature on isolation of *Campylobacter* and *Salmonella* from soil. For water, standard methods were used. For soil, published methods for enumeration of these bacteria in food and detection in soil were evaluated for: a) the necessity of shaking the sample during incubation, b) volume of media, c) number of replicates for MPN, d) number of days for *Campylobacter* incubation, e) effects of background organisms, f) range of detection, and g) time untill evaluation. 2)

Microcosm experiments were carried out with sampling and processing of soil and water from varied temperate environments (Freezing 3.5°C, Cool 32°C, and Room temp 70.8°C) every 24 hours for 72 hours. 3) Soil and Water samples were collected from Michigan dairy cattle farms. The water samples were filtered at 2 volumes (10ml and 25ml) and soil samples processed at 2 measures (5g and 1g), pre-enriched in *Campylobacter* and *Salmonella* selective media (Preston, BPW), replicated and diluted in enrichment media (Preston, Tetra), Plated (CCDA, XLD), and biochemical tests were performed on presumed positive samples.

Results: 1) Results from soil and water methods validation procedures did not follow expected patterns. Methods used to evaluate autoclaved spiked soil samples showed sensitivity to *Salmonella* from as low as 5 cfu/g and *Campylobacter* 50 cfu/g, while non-autoclaved spiked samples showed inconsistent results. Methods used to evaluate autoclaved spiked water samples showed sensitivity to *Salmonella* as low as 0.5 cfu/ml and only 500 cfu/ml for *Campylobacter*. Non-autoclaved spiked water samples showed inconsistent results. 2) Autoclaved spiked soil and water samples stored in the cool environment had the greatest recovery rates for both *Campylobacter* and *Salmonella* (all positive). Lowest recovery rates for both *Campylobacter* and *Salmonella* came from those stored at room temperature. 3) Fourteen farms were sampled over the course of the study (sampled for soil and water once or twice during the study sampling period October 2008-June 2009). One water

sample (sampled during the October sampling cycle) was positive for *Salmonella*. All other samples were negative for *Campylobacter* and *Salmonella*.

Conclusions: MPN techniques in conjunction with culture isolation methods for *Campylobacter* and *Salmonella* from environmental soil and water samples were insufficient to allow for enumeration in raw samples. It is suspected that background organisms play a major role in competition for nutrients during the culture processes as in very dilute spiked non-autoclaved samples there was some recovery of *Campylobacter*. While in more concentrated samples there was growth of other organisms on the plates. This interference is also suspected to have influenced the *Salmonella* results, as recovery of *Salmonella* did not always decrease with dilutions. As expected, per the literature, the lowest recovery of *Campylobacter* and *Salmonella* was associated with the warmer temperature. Further research would have to be done to determine if there is a relationship between the 1) prevalence (enumeration) of *Campylobacter* and *Salmonella* on Michigan dairy cattle farms and temperature and 2) prevalence and human incidence of disease in that county due to the low recovery of *Salmonella* (1 positive sample) and no recovery of *Campylobacter*.

Significance: *Campylobacter* and *Salmonella* are common infectious bacteria often associated with the foodborne route of infection in humans. Environmentally, these bacteria are closely linked with poultry and cattle environments. This study aimed to evaluate the amounts of these bacteria in Michigan dairy cattle environments in counties with varying rates of human disease and the relation to environmental temperature. By modeling the environmental effects on transport and prevalence of these bacteria in the farming and surrounding environments this study aimed to provide a major step towards understanding trends in prevalence and gain insight into how to lessen transmission between these food animals and on to humans. The actual findings of this study were inconclusive in this regard; however strides were made in the field of laboratory methods. In the literature, there were many methods published for isolation of *Campylobacter* and *Salmonella* from water and food, however there was a lack of adequate instruction for MPN techniques and culture isolation methods of these bacteria from soil. This study was able to identify the barriers to this process and to discover that culture techniques for isolation and enumeration of these bacteria from environmental soil samples is not likely to yield reliable results given the competition of other organisms in the samples.

5.1 INTRODUCTION:

Background: Campylobacteriosis and Salmonellosis are common infectious diseases often associated with food routes of infection and cattle serve as a major reservoir for *Campylobacter* and *Salmonella* with high carrying rates in these animals (Stanley et. al, 1998, Madden et. al, 2007). Through these and other food animals, *Campylobacter* spp. and *Salmonella* spp. go on to infect humans causing high rates of gastrointestinal disease, world-wide. Given the high incidences and health burdens for these diseases, much research has been done to better understand the routes of transmission and minimize infection; however this has mainly been done through the food route (food to mouth). As a result, safeguards were put into place which leads to a decrease in incidence of these diseases, however the seasonal trends (human cases peaking in the summer months) remained (Buchanan and Whiting, 1998, Allos et al., 2004, USDA, 2006, CDC, 2006). Numerous factors likely contribute to the disease burden and there is growing evidence that environmental factors such as climatic variability, including changes in temperature and precipitation, are associated with outbreaks of intestinal diseases.

Seasonality in human outbreaks and in environmental prevalence of both pathogens has been noted in the literature with peaks reported generally in the summer months. In the United Kingdom and other areas, *Campylobacter* spp. detections in watersheds increase with or just prior to peaks in human cases in the late spring and early summer (Louis ., 2005, Eyles et al., 2003, Arvanitidou et al., 2005). The highest frequency of *Salmonella* isolations from humans occurs in the late summer months also associated with increased rainfall (Haley et al., 2009, Geather et al., 2009). These links should be further studied to fully understand and prevent future cases of *Campylobacter* and *Salmonella* infections.

With this study, we aim to add to the literature by exploring and reporting on the environmental presence of these bacteria in the dairy farm environment of counties with varying human rates of *Campylobacter* and *Salmonella* infections.

Rationale: Research has been done to show the trends in human infection with *Campylobacter* and *Salmonella* showing an overall declining rate noted since 1996 (attributed to improvements in the poultry and food processing industries) and a distinct seasonal trend that remains. Research has also been done to show the trends in food animal infection with *Campylobacter* and *Salmonella* showing a seasonal trend in shedding rates. There is also research showing seasonal trends in water prevalence of these bacteria. However, there has not been much research done to model this potential pathogen transport route (Skelly Weinstein, 2003). This study aims serve as a preliminary step in bridging the gap in the literature. These studies explores through laboratory evaluation, experimentation, and field sampling the culturable recoverability of these bacteria in the dairy farm environments along with possible influences (temperature) that could affect the environmental survival and recovery of these pathogens. This study should lead to larger studies where tracers or molecular typing will be used to confirm the path of transport for these bacteria from farm animals, to the environment and water, and then on to humans (Wilson et al 2008).

5.2 HYPOTHESES

- Culture methods can be used in conjunction with MPN to enumerate *Campylobacter* and *Salmonella* from environmental soil and water samples.
- Recovery of *Campylobacter* and *Salmonella* will vary with temperature.
- There will be variation in the amounts of *Campylobacter* and *Salmonella* present in the farm soils and surrounding waters that will relate to human incidence of these infections in county.
- There will be a relationship between temperature and the amount of

Campylobacter and *Salmonella* in cattle farm soils and surrounding waters such that higher prevalence of the bacteria in the environment corresponds to warmer temperatures.

5.3 SPECIFIC AIMS

- To identify and validate methods for the enumeration of *Campylobacter* and *Salmonella* from environmental soil and water samples.
- To evaluate the effect of temperature on *Campylobacter* and *Salmonella* recovery from soil and water.
- To evaluate Michigan dairy farms for the presence of *Campylobacter* and *Salmonella* in soil and water.

5.4 METHODS

a. Study Design

This study will employed a three tiered design that included 1) a validation of laboratory procedures, 2) a microcosm experiment in varied temperate simulated environments, and 3) field sampling from Michigan dairy cattle farms.

b. Sampling and Laboratory Procedures

Collection of Soil and Water samples:

Soil. Samples from areas of exposed soil (near lagoon/manure storage area) were collected; about 20g of surface soil will be collected and stored in the Whirl-Pak bags using methods described by Johnson et al.,(1997).

Water. Water samples were collected from waters in the direct draining area (as determined by the drainage commission) of the sampled farm (2 samples for each bacteria). At each site, samples were collected in sterile bottles from standing water on the farm. The sample was taken from the top 20cm of water using methods described by Sayah et al.,(2005).

Campylobacter enrichment and culture:

For processing the soil, 1g and 5g samples were measured and pre-enriched in 45ml of Preston Enrichment (PE) broth, incubated at 42°C for 48 hours under microaerophilic conditions. Samples of 10ml and 25mls of water were filtered through 0.45um membrane filters. These filters were then pre-enriched in 45ml PE broth and incubated at 42°C for 48 hours under microaerophilic conditions. From both the water and soil pre-enrichment broths, secondary enrichments were performed by adding aliquots of the broth to 9ml of PE and further diluting by adding aliquots from that tube to 9ml of PE. Both of these broths were done in triplicate and incubated at 42°C for 48 hours under microaerophilic conditions. All samples were then streaked onto CCDA-Preston agar plates, then incubating them at 42°C for another 48 hours under microareophilic conditions. Gram-stain, oxidase test, and motility testing were performed to further confirm the presence of suspected *Campylobacter* growth on those plates.

Salmonella enrichment and culture:

For processing the soil, 1g and 5g samples were measured and pre-enriched in 45ml of Buffered Peptone Water (BPW) and incubated at 37°C for 24 hours. Samples of 10ml and 25mls of water were filtered through 0.45um membrane filters. These filters were then pre-enriched in 45ml BPW broth and incubated at 37°C for 24 hours. From both the water and soil pre-enrichment broths, secondary enrichments were performed by adding aliquots of the broth to 9ml of Tetra and further diluting by adding aliquots from that tube to 9ml of Tetra. Both of these broths were done in triplicate and incubated 37°C for 24 hours. All samples were then streaked onto Xylose Lysine Desoxycholate (XLD) agar to be incubated at 37°C for 24 hours. TSI, Urea, Citrate and LIA testing were performed to further confirm the presence of suspected *Salmonella* growth on those plates.

c. Validation Study

This portion of the study initially required a review of the literature around laboratory methods for the 1) culture isolation and 2) enumeration of *Campylobacter* and *Salmonella* from environmental soil and water and the 3) range of detection for these methods with respect to recorded environmental concentrations. From these findings laboratory procedures were optimized through experimentation that evaluated the necessity of shaking soil samples, media volume, time of dilution, order of replication, number of days for campylobacter incubation, time till evaluation, effects of background organisms, and the range of detection.

For all optimization experiments soil and water samples were collected from a dairy farm using methods described above and treated appropriately for each experiment. To evaluate the necessity of shaking soil samples, autoclaved soil samples were spiked; one set of samples with 50cfu/g of *Salmonella* and another set with 50cfu/g of *Campylobacter.* These samples were evaluated using laboratory procedures described above with one set of both *Salmonella* and *Campylobacter* shaken during the pre-enrichment incubation and one set not shaken. To evaluate the optimum pre-enrichment media volume, spiked autoclaved soil and water samples were pre-enriched in varying concentrations of media. Spiked autoclaved samples were evaluated for the effect of diluting the sample at the pre-enrichment as compared to the enrichment phase of isolation. To evaluate the effect of replicating the samples at prior to or post dilution, spiked sample sets were replicated (triplicate) at the pre-enrichment phase and compared to the set replicated at the enrichment phase. *Campylobacter* spiked samples were processed with varying incubation times (24 vs. 48hrs) to determine the optimum incubation time. *Campylobacter* and *Salmonella* spiked autoclaved soil and water samples were stored in the refrigerator and processed every 12hrs for 48hrs to assess the maximum time till evaluation. To assess the effect of background organisms, both autoclaved and not autoclaved spiked samples were processed and compared. Autoclaved samples were spiked with varying concentrations of *Campylobacter* and *Salmonella* then processed and compared.

d. <u>Microcosm Temperature Study</u>

Microcosm experiments were carried out with sampling and processing of soil and water from varied temperate environments (Freezing 3.5°C, Cool 32°C, and Room temp 70.8°C). Autoclaved *Campylobacter* and *Salmonella* spiked soil

and water samples were stored in a freezer, refrigerator, and desk top with temperature monitors. A set of the samples were removed from the environment and processed every 24 hours for 72 hours. Results were recorded.

e. Environmental Dairy Farm Sampling Study

Study Area

Michigan is a state in the upper mid-western area of the United States, bordering Canada, and has both rural and urban areas. The state has a population of 10,120,860 people, a land area of 56,804 square miles, and 40,001 square miles of water (US Census, 2006). Michigan has both urban and rural areas with much of the land used in agriculture and farming. The state is divided into 83 counties and 59 watersheds (EPA, 2006). Many of the waters of Michigan are used for recreational activities such as swimming and fishing in the warmer months.

There are 14,500 cattle operations in the state with 864 dairy farms (NASS, 2004).

County Selection

The sampling sites were chosen based on identification of Michigan counties and watersheds with high densities of farms and varying human incidences of reported *Campylobacter* spp. and or *Salmonella* spp. infections. Within these counties and watersheds, dairy cattle farms were selected for sampling. A letter describing the research project was sent to a random sample of cattle farms in the areas of interest and those interested in participating responded by returning a prepaid postcard. This resulted in the 6 counties to be sampled with 14 farms choosing to participate in the study (Isabella County, 5 farms; Livingston, 2; Mecosta, 1; Missaukee, 4; Ingham, 1; and Clinton County, 1 farm).

Sampling and Processing

Soil, environmental, and water samples were collected from all farms enrolled in the study and their surrounding draining waters. All samples were stored on ice and analyzed within 24 hours of collection using previously described methods.

5.5 RESULTS:

a. Validation Study

There was slight variation in results of the shaken and not shaken autoclaved soil samples (Table 5.1). There was also slight variation in outcome between the 90 ml pre-enrichment media volume as compared to 45 ml (Table 5.1). There was no difference in outcome when spiked autoclaved samples were diluted at the pre-enrichment phase when compared to diluting at the enrichment phase of culture (results not shown). Similar, results were achieved when spiked sample sets were replicated (triplicate) at the pre-enrichment phase and at the enrichment phase (results not shown). *Campylobacter* spiked samples were processed with varying incubation times to differentiate the recovery between a 24 hour and 48 hour incubation. Greater recovery was seen, with positive samples at 10^{-3} when incubated at 48 hours while no samples were positive at that concentration when incubated at 24 hours (Table 5.2). When *Campylobacter* and *Salmonella* spiked autoclaved samples were stored in the refrigerator and

processed every 12 hours for 48 hours to assess the maximum time till evaluation the greatest recovery occurs when samples are processed prior to 24 hours (Table 5.3 and Table 5.4). Methods used to evaluate autoclaved spiked water and soil samples showed sensitivity to *Salmonella* in the range of 0.1cfu/ml of water to a minimum of 2 cfu/g of soil. *Campylobacter* minimum limits were around 0.4cfu/.g in soil and 2cfu/g in water (Tables 5.5, 5.6, and 5.7). Non-autoclaved spiked samples showed inconsistent results.

b. Microcosm Study

Autoclaved spiked soil and water samples stored in the cool environment had the greatest recovery rates for both *Campylobacter* and *Salmonella* (all positive). Lowest recovery rates for both *Campylobacter* and *Salmonella* came from those stored at room temperature (Table 5.8).

c. Field Study

Study Area and Counties Selected

The sample farms in the study were located in Livingston, Missaukee, Isabella Mecosta, Ingham, and Clinton counties. These counties have varying reported human rates of *Campylobacter* and *Salmonella* infections (range in average incidence of 1.3 to 14.2 for *Campylobacter* and 1.8 to 13.6 for *Salmonella* infections), varying population densities (26 people per mi^2 in Missaukee to 499 in Ingham County), and vary with respect to the number of dairy farms in the counties (16 dairy farms in Livingston to 119 in Mecosta) (Table 5.9).

Processed Samples

All soil and water samples from all farms were negative for *Campylobacter* spp. One water sample from a farm in Livingston County was positive for *Salmonella* spp. (Table 5.9).

5.6 DISCUSSION:

a. Validation study

Given the limited budget for the study, we aimed to employ the most cost effective and time efficient yet still valid and reliable methods for the culture isolation and enumeration of *Campylobacter* and *Salmonella* from environmental soil and water samples (Carrique-Mas et al, 2009). As such, a process diagram was created and process evaluation was performed to identify areas for method modification and validation. Several process steps were identified for evaluation which included, a) the necessity of shaking soil samples during incubation, b) volume of media during pre-enrichment, c) replication and dilution (number of replicates and at which stage in the process to replicate and dilute the samples) for MPN, d) number of days for *Campylobacter* incubation, e) effects of background organisms, f) range of detection, and g) time till evaluation. The evaluation and analysis of these process steps made up the validation study.
In this validation study, we found that several steps could be minimized with respect to volume of media used without compromising the outcome. The results showed that 45 ml of pre-enrichment media could be used for sample processing and that samples can be replicated and diluted later in the process (where smaller volumes of media are required) (this data was not shown). In previous

studies with the processing of food samples, it was noted that samples should be shaken during the pre-enrichment incubation. In our evaluation of soil samples spiked with *Salmonella,* we found no difference in detection between the shaken vs. the non shaken samples when using 45ml pre-enrichment methods. We also found that at all steps of *Campylobacter* processing (pre-enrichment, enrichment, and plating), maximum detection occurs when samples are allowed to incubate for 48 hours. All samples should also be processed within 24 hours of collection for maximum detection.

The final variables addressed in the validation study included the range of detection for the methods employed and the effect of background organisms on recovery. To evaluate the effect of background organisms, autoclaved and non-autoclaved samples were processed and compared. It is suspected that background organisms play a major role in competition for nutrients during the culture processes as in very dilute spiked non-autoclaved samples there was some recovery of *Campylobacter.* While at higher concentrations there was growth of other organisms on the plates. This interference is also suspected to have influenced the *Salmonella* results, as recovery of *Salmonella* did not always decrease with dilutions. Due to these findings we determined that MPN techniques in conjunction with culture isolation methods for *Campylobacter* and *Salmonella* from environmental soil and water samples would be insufficient to allow for enumeration in raw samples, as such microcosm temperature and farm studies evaluated samples for recovery and detection.

b. <u>Microcosm Temperature Study</u>

It has been reported in the literature that *Salmonella* and *Campylobacter* survive for longer periods of time it colder temperatures (Buswell et al 1998). As expected, per the literature, the lowest recoveries of *Campylobacter* and *Salmonella* were associated with the warmer temperature.

c. <u>Farm Sampling Study</u>

It was expected that the sampling areas would have variation in the environmental presence of *Campylobacter* and *Salmonella* such that counties with higher incidences of these human diseases would have more environmental presence of the associated bacteria. This finding would begin to strengthen the possibility that environmental contamination may be a significant source of human infection. However, in this study there was low recovery of *Salmonella* (1 positive sample) and no recovery of *Campylobacter* from any of the farm samples. So our findings are inconclusive as to whether there is any correlation between county level human incidence and environmental prevalence.

5.7 CONCLUSIONS:

This study aimed to begin filling in a gap in an alternate route (environment) in the chain of Campylobacter and *Salmonella* transmission from animals to humans. The food route has been explained, and the water route has also been explained, but here the environmental contamination link between the dairy cattle and the abundance and transport of the bacteria through the environment was explored. The study employed a systematic approach to validate the methods, carry out an experimental microcosm study, and finally evaluate the natural farm environment.

This study found that due to the competition of background organisms, culture methods were insufficient to accurately and consistently evaluate environmental samples for the presence or absence of *Campylobacter* and *Salmonella*. Further research would have to be done, possibly repeating the study using quantitative real-time PCR techniques, to determine if there is a relationship between the prevalence (enumeration) of *Campylobacter* and *Salmonella* on Michigan dairy cattle farms (in the natural environment) and temperature (Hadjinicolaou et al, 2009).

Ideally, this study would lead to larger studies where tracers or molecular typing will be used to confirm the path of transport for these bacteria from animal, to the environment and water, and then on to humans. This would also significant public health policy potential, by confirming this path policies can be put into place to inform animal farming facilities of the biological waste contamination produced and make efforts to reduce them. Information can also be given to the residents of nearby communities where exposure to these contaminants is likely in efforts to reduce exposure and prevent infection.

Table 5.1 Media Volume and Shaking of Soil Samples

Media volume	Concentration N** (5cfu/g of *Salmonella*$*10^N$)	Soil Results (positive/total*)	
		shaken	not shaken
45ml	-2	3/3	3/3
	-4	3/3	3/3
	-6	3/3	3/3
	-8	0/3	0/3
90ml	-2	3/3	3/3
	-4	3/3	3/3
	-6	3/3	0/3
	-8	0/3	0/3

** The concentration of each sample is the initial spiking volume multiplied by 10^N, where N is the value listed above in the concentration column.

*Results are given as the number of positive samples out of the three replicates.

Table 5.2 *Campylobacter* Processing: Days in Incubation

Concentration N** (500cfu/g of Campylobacter*10^N)	1 day	2 days
0	0/3	0/3
-1	3/3	3/3
-2	3/3	3/3
-3	0/3	3/3

** The concentration of each sample is the initial spiking volume multiplied by 10^N, where N is the value listed above in the concentration column.

Table 5.3 Time Till Evaluation of Soil Samples with Background Organism Consideration

	Time till Evaluation (hrs)	Soil Results (positive/total*)					
		Autoclaved soil amount**			Non-Autoclaved soil amount		
		5g	1g	.5g	5g	1g	.5g
Campylobacter	0	6/6	6/6	6/6	6/6	6/6	6/6
	12	6/6	6/6	6/6	2/6	3/6	6/6
	24	6/6	6/6	6/6	0/3	3/3	3/3
	36	3/3	3/3	3/3	0/3	3/3	3/3
	48	3/3	3/3	0/3	0/3	0/3	3/3
Salmonella	0	3/3	1/6	0/6	5/6	6/6	0/6
	12	6/6	1/6	0/6	0/6	0/6	0/6
	24	6/6	0/6	0/6	0/6	0/6	0/6
	36	6/6	0/6	1/6	2/6	3/6	1/6
	48	6/6	2/6	6/6	2/6	3/6	2/6

*Results are reported as the number of positive samples out of the total number. The six samples include triplicate samples spiked with 218 CFU/ml of *Campylobacter* or 2cfu/g of *Salmonella* and processed in triplicate and each diluted 1:10 ratio.

**Soil samples were evaluated using 5g, 1g, and .5g amounts of spiked soil.

Table 5.4 Time Till Evaluation of Water Samples with Background Organism Consideration

Time till Evaluation (hrs)	*Salmonella* Water Results (positive/total*)					
	Autoclaved water volume			Non-Autoclaved water volume		
	25ml	10ml	1ml	25ml	10ml	1ml
0	9/9	9/9	2/9	6/9	7/9	5/9
12	9/9	9/9	4/9	9/9	9/9	6/9
24	9/9	9/9	0/9	8/9	7/9	5/9
36	9/9	6/9	0/9	4/9	9/9	7/9
48	9/9	6/9	7/9	5/9	7/9	9/9

*Results are reported as the number of positive samples out of the total number. The nine samples include triplicate samples spiked with 50 CFU/ml of *Salmonella* and processed in triplicate and each serially diluted 1:10 and 1:100 ratios.

Table 5.5 Range of Detection

Sample type	*Campylobacter*		*Salmonella*	
	Concentration (CFU)	Result (positive/total)	Concentration (CFU)	Result (positive/total)
Soil (per g)*	102	11/12	25.6	12/12
	10	11/12	2.2	12/12
	1	12/12	1.7	6/12
Water (per ml) *	102	12/12	25.6	12/12
	10	12/12	2.2	12/12
	1	12/12	1.7	12/12

Results are reported as the number of positive samples out of the total number.

*The twelve samples include autoclaved samples spiked with *Campylobacter* or *Salmonella* and processed in triplicate and each diluted 1:10 ratio. This was done for each concentration and for two soil amounts (5g and 1g) and for two water volumes (25ml and 10ml).

Table 5.6 Range of Detection in Water with Background Organism Consideration

	Concentration (CFU/ml)	Water Results (positive/total*)					
		Non Autoclaved water volume			Autoclaved water volume		
		25ml	10ml	1ml	25ml	10ml	1ml
Campylobacter	384	0/9	0/9	0/9	9/9	7/9	9/9
	38	0/9	0/9	0/9	0/9	0/9	0/9
	4	0/9	0/9	0/9	0/9	0/9	0/9
	0.4	9/9	3/9	0/9	0/9	1/9	0/9
Salmonella	143	9/9	8/9	4/9	9/9	9/9	9/9
	14	6/9	7/9	6/9	9/9	9/9	2/9
	1	5/9	7/9	1/9	9/9	9/9	5/9
	0.1	4/9	3/9	5/9	1/9	6/9	3/9

*Results are reported as the number of positive samples out of the total number. The nine samples include triplicate samples spiked with *Campylobacter* or *Salmonella* and processed in triplicate and each diluted 1:10 and 1:100 ratios.

Table 5.7 Range of Detection in Soil with Background Organism Consideration

	Concentration (CFU/g)	Soil Results (positive/total*)					
		Non Autoclaved soil amount			Autoclaved soil amount		
		5g	1g	.5g	5g	1g	.5g
Campylobacter	218	6/6	6/6	6/6	6/6	6/6	6/6
	20	1/6	6/6	3/6	6/6	6/6	6/6
	2	3/6	0/6	0/6	5/6	0/6	6/6
Salmonella	2	5/6	6/6	0/6	6/6	1/6	0/6
	0.2	0/6	0/6	0/6	0/6	0/6	0/6
	0.02	0/6	0/6	0/6	0/6	0/6	0/6

*Results are reported as the number of positive samples out of the total number. The six samples include triplicate samples spiked with *Campylobacter* or *Salmonella* and processed in triplicate and each diluted 1:10 ratio.

Table 5.8 Recovery of *Campylobacter* and *Salmonella* with Respect to Length of Time at Varying Storage Temperatures

Sample Type	Time	Temperature Microcosm					
		Campylobacter (+/total)			*Salmonella*(+/total)		
		Freezing	Cool	Room Temp	Freezing	Cool	Room Temp
Soil*	24	12/12	0/12	12/12	11/12	12/12	12/12
	48	1/12	12/12	0/12	12/12	12/12	12/12
	72	1/12	12/12	0/12	1/12	12/12	12/12
Water*	24	12/12	12/12	12/12	11/12	12/12	12/12
	48	12/12	12/12	12/12	12/12	12/12	0/12
	72	12/12	12/12	12/12	12/12	12/12	12/12

Results are reported as the number of positive samples out of the total number.

*The twelve samples include autoclaved samples spiked with 10cfu/g or ml of *Campylobacter* or 2.2 cfu/g or ml *Salmonella*, processed in triplicate, and each diluted 1:10 ratio. This was done for each soil amount (5g and 1g) and for each water volume (25ml and 10ml).

Table 5.9 Sample Farm Results and County Characteristics

Counties	Farm #	Farm Sampling Results		Human Incidence average cases/100,000 people (range)		Human density People/mi^2	# of dairy farms
		Campylobacter soil/water	*Salmonella* soil/water	*Campylobacter*	*Salmonella*		
Isabella	1	-/-	-/-	13.0 (0-37)	7.8 (0-14)	116.1	80
	2	-/-	-/-				
	3	-/-	-/-				
	4	-/-	-/-				
Missaukee	5	-/-	-/-	14.2 (0-31.3)	13.6 (0-44.4)	26.4	63
	6	-/-	-/-				
	7	-/-	-/-				
	8	-/-	-/-				
	9	-/-	-/-				
Livingston	10	-/-	-/+	7.8 (2.6-26.1)	8.3 (3.9-23.6)	322.3	16
	11	-/-	-/-				
Mecosta	12	-/-	-/-	1.3 (0-7.3)	1.8 (0-4.8)	75.7	119
Ingham	13	-/-	-/-	4.1 (0-17)	5.8 (0-10.6)	499.4	43
Clinton	14	-/-	-/-	4.8 (0-13.3)	7.8 (0-10.2)	121.9	78

OVERALL DISCUSSION AND CONCLUSIONS

The five major sections of this dissertation included the Literature Review: Drivers of *Campylobacter* and *Salmonella* Infections: Known and Suspected (Chapter 1),an analysis of the Epidemiology of Campylobacteriosis and Salmonellosis in Michigan (Chapter 2), the Evaluation of Seasonal and Geographic Trends in Reporting (Chapter 3), the modeling of Environmental Factors Influencing Incidence of Campylobacteriosis and Salmonellosis in Michigan (Chapter 4), and Culture Isolation and Enumeration of *Campylobacter* and *Salmonella* from Michigan Dairy Farm Environmental Soil and Water (Chapter 5). These sections attempted to make logical connections incorporating the model of thought (illustrated in figure 1.1) that environmental factors could be influencing and possibly driving human rates of *Campylobacter* and *Salmonella* infections in Michigan.

Published literature has shown the relationship of climate to health and disease. Large climatic events affect global and local weather patterns resulting in increased precipitation and runoff. Based on the type of land use there can be significant amounts of runoff containing pathogens such as *Campylobacter* or *Salmonella*. These pathogens are able to persist in natural waters, where humans may be exposed and their survival is related to environmental conditions. The source of the drinking water may also be a key factor in the transmission of the disease. The literature review detailed the background literature around the environment and links to *Campylobacter* and *Salmonella* human infections, paying particular attention to routes of transmission that may be influenced by the environment (Figure 1.1). This section concluded by reporting on the series of recent articles evaluating the consistent associations of climate and geography to variability in rates of these diseases.

To further establish the foundation for the necessity of evaluating environmental factors, chapters 2 and 3 examine historical case data to describe the epidemiological trends for Michigan (a state that has not been included in the national extrapolations). The seasonal peak in reporting for both of these infections had already been noted in the literature but, Chapter 3 goes on further defining and describing the parameters associated with these reporting trends (seasonality) and evaluating possible geographic relationships. It has been suggested that in sporadic cases of *Campylobacter* and *Salmonella* infections when evaluated by geography should occur randomly in space. However, the reported clustering of cases that do not occur around outbreaks but are sustained clusters suggests that factors other than food, possibly geographic or environmental may be driving these clusters. Chapter 2 highlighted these areas of consistently high and consistently low rates of disease and Chapter 3 began to associate geography with parameters of the high reporting periods.

Chapter 4 continues to build on the previous work by incorporating environmental factors that have been described in detail in chapter 1, where the logic around these specific factors and their relation to variation in rates of disease is explained. This study found that indeed, in Michigan some of the variation in rates of campylobacteriosis and salmonellosis can be explained (in various portions of the state) by combinations of these meteorological and environmental variables.

The final step in logic was covered in chapter 5. After the detailed evaluation of historical *Campylobacter* and *Salmonella* infection data and relating the trends to environmental factors (Chapters 2-4), Chapter 5 targets at the environmental prevalence. This chapter evaluated methods for the culture isolation and enumeration of these bacteria from environmental soil and water samples and found that future studies should employ alternative techniques when performing environmental *Campylobacter* and *Salmonella* culture isolation and enumeration due to competition from indigenous microorganisms in environmental soil and water sample cultures.

This research has been conducted with the goal of describing particular trends and geographical patterns with the hopes that this research can be used to predict variability in incidence of campylobacteriosis and salmonellosis in Michigan so that in future public health measures can be put into place to lessen the transmission. This study also aimed to add to the literature by explaining and filling a gap in the chain from animals to humans. By focusing on the environmental connections that may explain some of the variation in human rates of these diseases, the study was able to begin evaluating a missing link. The food route has been explained, and the water route has also been explained, but here the environmental contamination link between animals and the abundance of the bacteria through the environment was explored. By modeling the environmental associations with rates of disease and beginning to evaluate environmental prevalence this has been an informative step towards understanding trends in prevalence and will potentially provide insight into how to lessen transmission between these animals and on to humans.

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