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Waterborne Diseases: Linking Public Health And Watershed Data

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WATERBORNE DISEASES: LINKING PUBLIC HEALTH AND WATERSHED DATA

A Thesis Presented

By

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ABSTRACT

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Microbial contaminants in water are a major public health concern. Pathogens have been identified as a primary threat to river water quality in the United States, potentially impacting drinking and irrigation water sources and recreational waters. Agricultural runoff, feedlot operations, wastewater effluents, swimming activities, domestic and wild animals are potential sources of microbial contamination. This thesis presents Massachusetts as a case study for linking public health data of waterborne gastrointestinal diseases with sources of drinking water, potential recreational exposures, as well as hydrologic, climatic, and land use data. *Giardia sp.* has been chosen as a model organism. Information of reported human Giardiasis cases has been synthesized. Using Geological Information system and statistical software (SPSS and SAS) relationships of confirmed Giardiasis have been compared with available climate and hydrologic data. In this thesis the research finding suggest that there is no visible difference in disease occurrence related with amount of precipitation or extreme rain event. However human giardiasis in Massachusetts has been found related with temperature thus shows a seasonal trend in disease occurrence. Seasonal water related human activity likely have played a role in disease occurrence.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Microbial contamination of water is a major problem for human health, and has led to some major waterborne disease outbreaks (Mackenzie et al., 1994; O'Connor, 2002). Both drinking and recreational water can be highly susceptible to microbial contaminants, with pathogens frequently observed in surface and groundwater (Hancock, Rose, & Callahan, 1998; Lemarchand & Lebaron, 2003).

Zoonotic pathogens (that can be transmitted between animals and humans or transmission from livestock to humans and potentially wildlife) are of increasing concern. Almost three-quarters of the emerging infectious diseases are zoonotic. In recent decades, infectious pathogens from wild animals are becoming more problematic throughout the world. This not only impacts human health, but also agricultural production, wildlife-based economies, and wildlife conservation (Chomel, Belotto, & Meslin, 2007). In the United States, *Giardia*, *Campylobacter*, *Cryptosporidium*, *Salmonella*, and *Escherichia coli* have been the most commonly identified zoonotic agents of waterborne disease outbreaks (Craun, Calderon, & Craun, 2004).

1.1 Exposure to Pathogenic Microorganisms

Recent outbreaks of *E. coli* O157:H7, *Campylobacter,* and *Cryptosporidium* have the risk of contaminated water supplies (Thomas et al., 2006). In Milwaukee, Wisconsin, in 1993 approximately 400,000 gastroenteritis cases were linked to the city's drinking water source, where the etiologic agent was *Cryptosporidium parvum* (Mackenzie et al., 1994). In Walkerton, Canada in 2000, waterborne *E. coli* O157:H7 and *Campylobactor jejuni* caused more than 2,000 gastrointestinal disease cases, with seven deaths

(O'Connor, 2002). According to the FoodNet surveillance of the CDC in 2006 a total of 17,252 laboratory-confirmed infections were identified in 10 states: *Salmonella* (6,655 cases), *Campylobacter* (5,712), *Shigella* (2,736), *Cryptosporidium* (859), Shiga toxin (Vero cytotoxin)– producing Escherichia coli *O157* (590), Shiga toxin (Vero cytotoxin)–producing Escherichia coli non-O157 (209), *Yersinia* (158), *Vibrio* (154), *Listeria* (138), and Cyclospora (41) (CDC, 2007). When compared with the 1996--1998 baseline period, significant declines happened in 2006 in the estimated occurrence of *Campylobacter, Listeria, Shigella,* and *Yersinia* infections. "However, after substantial declines in 2003 and 2004, the incidence of STEC O157 infections increased in 2005 and again in 2006" (CDC, 2007).

Recreational water includes swimming pools, hot tubs, jacuzzis, fountains, lakes, rivers, springs, ponds, streams and oceans and it can become contaminated with sewage from humans or animals. Over the period of time water treatment distribution system deteriorate. Also sometimes as a result of excessive demand water supplies are overwhelmed (Ford, 1999). In 1986, the EPA examined the association between *E. coli* and *Enterococci* densities in recreational water and gastrointestinal illness in swimmers, and based bathing water quality standards on these data. *Enterococci* and *Escherichia coli* are commonly present in ocean water as well as fresh recreational water (Haack, Fogarty, & Wright, 2003). From 1999 to 2000, 59 diseases outbreaks in the U.S. were reported, and were related to recreational water exposure, with 61% involving gastroenteritis (Alm, Burke, & Spain, 2003).

Large multi-state outbreaks, such as the *E. coli* O157:H7 outbreak in freshly bagged spinach in September 2006, have occurred (CDC, 2006 a) mainly due to contaminated irrigation water. In the U.S. and Central American countries, 60% of the

total irrigation water (mainly for vegetables) has been found positive for *Giardia* cysts. *Giardia* cysts have frequently been found in crops, with detection dependent on the structure of harvest foliage (R. C. A. Thompson, 2002) *Giardia* cysts have been found on coriander, carrots, mint, radishes, and potatoes irrigated with untreated wastewater(R. C. A. Thompson, 2002). Contaminated fruits and vegetables in these outbreaks have also been frequently reported (Fayer, Dubey, & Lindsay, 2004). *Giardia* has also been detected in shellfish. In *Macoma* clams in the Rhode River, *Giardia duodenalis,* genotype A, was identified. Fayer et al (2004) suggested that these clams can be used as bio-indicators of water contamination (Fayer, Dubey, & Lindsay, 2004).

Human to human transmission can occur following the accidental ingestion of pathogens in water or food, or from direct contact with those with poor hygiene. Direct person-to-person transmission may be more common in certain communities or institutional settings, such as day care centers. Infectious diarrhea has been recognized as one of the most important health problems at day care centers, with its incidence being twice as high for children in day care versus children cared at home (R. C. A. Thompson, 2000).

Travel to regions of the world with inadequate access to clean water has long been associated with an increased risk of diarrheal illness For example, it has been reported that among travelers to Eastern European countries and the former Soviet Union, the risk of waterborne Giardiasis is well recognized (Dawson, 2005).

1.2 Water Quality Standards

According to National Primary Drinking Water Regulations of USEPA; the Maximum Contamination Level (MCL) standard for microbial contaminants in drinking

water is zero (EPA, n.d. -c). EPA's surface water treatment rules require 99% removal of *Cryptosporidium* and 99.99% removal/inactivation for *Giardia lamblia* from water (EPA, n.d. -c). Drinking water becomes contaminated when feces containing pathogens are deposited or flushed into the water. If treatment is insufficient, or if the water distribution system is inadequate, drinking water may contain sufficient numbers of pathogens to cause illness (O'Connor, 2002).

Pathogens are also a serious concern for recreational water resources. Waterborne pathogens are typically abundant, are deposited by infected hosts in that environment, and are then transmitted between hosts (Bolin, Brown, & Rose, 2004). Once in water, they are able to infect humans via contaminated organisms (like fish and shellfish), or by direct contamination such as skin contact or the ingestion of water. Section 303(c) of the Clean Water Act (originated in 1948 and amended in 1972) states that protection from pathogenic contamination is critical in recreational waters. Pathogen-contaminated recreational waters can result in gastrointestinal, respiratory, eye, ear, nose, throat, and skin infections (EPA, n.d. -c).

Most states failed to act on the requirements of the Clean Water Act until forced to do so by lawsuits. In 1999, the EPA signed a Consent Decree with the complainant the consent decree contained a TMDL development schedule through year 2010. Over 26,000 streams have been added to the EPA's impaired list, with 48,809 impairments. Of those impairments listed, 5,578 are for fecal microorganisms. Since 1996, the EPA has approved only 9,586 submitted TMDL plans (EPA, n. d.- b).

1.2.1 TMDL

In the U.S., Total Maximum Daily Load (TMDL) is used to determine the amount of pollution a stream can receive without being negatively affected. It has been suggested that "A TMDL or Total Maximum Daily Load is a calculation of the maximum amount of a pollutant that a water body can receive and still meet water quality standards, and an allocation of that amount to the pollutant's sources"(EPA, n. d.- b) and its purpose is to set a target for control measures.

TMDLs are often allocated using computer-based models of watersheds. As an example, the TMDL of Blackstone River in eastern Massachusetts and Rhode Island includes data on pathogens, nutrients, hypoxia, metals (Cr, Cu, Pb), and biodiversity impairments in the river. The TMDL in the Blackstone River for 1998 - 2001 required EPA/Massachusetts action against pollution levels (Rhode Island, DEM/Office of Water Resources)**.** However, the 2002 impairment list of the Federal Clean Water Act (CWA) identifies 11 segments in the Blackstone River watershed which should not be used because of excessive bacteria concentrations. Many models are available, which are selected for geographic extent, availability of data, and cost.

TMDLs are generally used for setting pollutant limits (specifically for fecal pathogen contamination). Watershed models are used to support TMDLs, but their use in simulating in-stream fecal bacteria concentrations is relatively underdeveloped (Benham et al., 2006). TMDL is like a threshold or upper limit, and must be established for both point source and non-point source pollutants; all parameters of water quality, including chemical, physical, and biological factors are considered.

Steps to develop a TMDL the guideline is-

- Required to list impaired waters on the 303(d) list their reason for impairment

- The waters are prioritized for TMDL development.
- Data collection
- Identify the sources of the contamination.
- Need to develop TMDL model
- Total of 3 public meetings need to be held
- TMDL will be submitted to the EPA for approval.
- TMDL is presented to the State Water Control Board (SWCB) for adoption as a regulation.

1.2.2 Limitations of TMDL

TMDLs are not appropriate for estimating risks from microbial contamination, as it is not the best technology. Quantitative Microbial Risk assessment is much more efficient process for the assessment. In the U.S., two watershed models frequently used to determine TMDLs are the Hydrological Simulation Program-FORTRAN (HSPF) and Soil and Water Assessment Tool (SWAT). However another method know as 'Load-duration method is being popular in different states ((NDEP, 2003). Both HSPF and SWAT generally describe a watershed temporally and spatially. These models cannot describe pathogen life cycles.

TMDLs cannot provide intra-watershed contributions, so it should be measured by supplemental sampling or modeling via land-use and hydrologic response data with bacterial concentrations. Bacteria source characterization procedures, supportive data, modeling that includes microbial contaminant life cycles, insertion of appropriate transport processes, and simulation of extreme weather conditions can be researched to develop TMDLs that are more effective (Benham et al., 2006).

1.2.3 Non Point Source Pollution (NPS):

Non Point Source pollution is different than industrial and sewage treatment plants. It comes from many disperse sources. In this type of pollution rainfall or snowmelt moves over and through the ground; collect and carries away natural and human-made pollutants. These runoffs finally depose them into lakes, rivers, wetlands, coastal waters, underground drinking water source and create non point source pollution. There are three types of NPS models: screening, simulation, and distributed process based models.

In 1972, Section 303(d) of the Clean Water Act required states to identify waters that did not meet water quality standards, to institute a schedule for developing TMDLs, and to establish TMDLs for each water body on the 303(d) list. The EPA revised their regulations in July 2000, requiring states to develop implementation plans for each TMDL (Copeland, 2005).

1.2.4 Quantitative Microbial Risk Assessment

Quantitative microbial risk assessment provides a tool for estimating pathogenic microorganism disease burden by using distribution and occurrence. The World Health Organization (WHO) in their 3rd edition of guideline for Drinking water quality strongly supported the use of risk assessment as well as risk management for water safety control in drinking water (WHO, 2004). Microbial Risk Assesment generate more robust data on microbial behavior/ survival/ transport/ persistence/ virulence/ and dose-responses in a broader range of environments which allows policy-makers to examine its usefulness. Microbial risk assessments are also used to assess potential exposure in food, agricultural infection control, and germ warfare preparedness (Howard G., Pedley S., & Tibatemwa,

S., 2006). QMRA methods have been started to be applicable and acceptable tools for first-responders and decision-makers to deal with microbiologically contaminated environments.

Like chemical risk assessment this assessment also includes steps of identifying hazards, exposure evaluation, assessment of dose response relationship and risk characterization (Chick S, Koopman J, Soorapanth S, & Brown M, 2001). But Quantitative microbiological risk assessment (QMRA) is more complex than chemical risk analysis, because, there are many more variables like fate, survival, transport, and changes in risk level over time, environmental conditions, at the time of dealing with microbiological agents.

1.3 Acceptable Risks

Any risk that is currently tolerated is considered as an acceptable risk. The annual risk of death from gastrointestinal disease is 1 in 20,000,000 people (Gerba, Rose, & Haas, 1996). Converting this time span to a 70-year lifetime risk to be comparable with rates cited for chemical contaminants results in a risk of 1 in 2 × 10–5, a figure that is similar to that measured acceptable by the WHO for carcinogenic risks (Gerba, Rose, & Haas, 1996; Hunter & Fewtrell, 2001).

Wyer *et al*. (1999) reported a dose–response relationship between the bacterial indicator fecal *streptococci* and gastroenteritis experienced by bathers. This was found to be independent of, and not confounded by, other predictors of gastroenteritis, including person-to-person transmission and a combined factor of non-water-related risk (Wyer et al., 1999). Each of these factors had a related probability in comparison to the dose–response to sea bathing (Hunter & Fewtrell, 2001).

1.4 Measurement & requirements

Colony-forming units (CFU) are a measure of viable bacterial numbers. The US EPA recommended 235 cfu/100mL of water for a single testing of *E. coli* and a maximum geometric mean of 126 CFU from 5 samples over a 30 day time period for recreational water (EPA, 2004: a). The acceptable risk of gastrointestinal illness is 8/1000 at freshwater sites and 19/1000 bathers at marine sites ((Hunter & Fewtrell, 2001).

According to the USEPA requirement for the drinking water using *Giardia* as a reference organism, acceptable microbiological risk is less than 1 infection per 10,000 people per year (Macler and Regli 1993). The current treatment obligation for all surface water systems is 2 logs (99%) removal (USEPA, 2001). However it's not logically impossible to reach that perfection.

1.4.1 Indicator Organisms

According to EPA a indicator organism is "a species, whose presence or absence may be characteristic of environmental conditions in a particular area of habitat"(EPA, n.d.-d). According to Bonde (1966) the criteria for indicators are related to occurrence and environmental resistance as pathogens, indicators should be correlated to health risk and have analogous fate and transport characteristics as pathogens. Bacteria such as *E. coli* and *Enterococci* will continue to be used for risk assessment of microbial and pathogenic contamination and to indicate the presence of fecal contamination. Using molecular tools the development of new rapid pathogen detection methods (Guy, Payment, Krull, & Horgen, 2003) will allow the monitoring of a greater number of pathogens and raises the question of the potential effectiveness of microbial indicators (Committee on Indicators for Waterborne Pathogens, 2004). Newer molecular methods

allow for the detection of pathogens in water that were not detected before, and other indicators of water are being considered for suitability. For example, Lucena et al. (2003) examined the occurrence and use of bacteriophages, enterococci , spores of sulphite reducing *clostridia*, somatic coliphages, F-specific RNA bacteriophages and bacteriophages infecting *Bacteroides fragilis* in 10 different climatic and socio-economic conditions in Argentina, Colombia, France and Spain (Lucena et al., 2003). Bosch (1998) proposed Bacteriophages as good indicator organism for their use as virus indicators to monitor human enteric viruses in waters. However, monitoring for all pathogens still remains impractical (Bosch, 1998)

1.4.2 LT2 Rules

The purpose of the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR or LT2 rule) is to reduce infirmity associated with *Cryptosporidium* and other disease-producing microorganisms in drinking water sources. The LT2 rule relates to all public water systems that use surface water or ground water which is under the direct influence of surface water. The rule includes further *Cryptosporidium* treatment requirements to high threat water bodies; it involves provisions to decrease risks from open finished water storage facilities. It also ensures that systems ensure microbial safety as they take steps to reduce the creation of disinfection byproducts. All unfiltered water systems require> 99 or 99.9 percent (2 or 3-log) inactivation of *Cryptosporidium* and all uncovered Finished Water Reservoirs treat the reservoir release to inactivate 4-log virus, 3-log *Giardia lamblia*, and 2-log *Cryptosporidium*(EPA, n. d.- a)

1.5 Factors Leading to Exposure

Understanding the contributions of land use and watershed protection measures is important for assessing microbial risks. In Ontario, *E. coli* O157:H7 cases were found to be more common in rural areas where direct and indirect contact with livestock sources of pathogens may be more common (Michel et al., 1999) Agricultural activities such as intensive livestock farming (such as concentrated animal feeding operations) do not exist in Massachusetts. However, urban land use may be associated with the presence of aging infrastructure that may contribute to pathogen contamination incidents. Approximately 772 cities in the U.S. have combined sewer overflow systems (CSOs) (EPA, 2007 -b). In Massachusetts, the city of Lowell has a CSO on the Merrimack River for which in 2006 the Clean Water State Revolving Fund had granted $14,000,000 for rehabilitations ("Commonwealth of Massachusetts", 2006). It is important to consider the effects of combined sewer overflow systems on numbers of gastrointestinal illnesses.

Climate has been linked to infectious diseases, and the use of climate information has been recommended for early warning systems for epidemics (2005) There is growing evidence that weather is often a factor in waterborne disease outbreaks (Hrudey, Huck, Payment, Gillham, & Hrudey, 2002).

According to the 'US National Assessment on the Potential Consequences of Climate Variability and Change', (Patz et al., 2000) prediction of the role of weather in waterborne disease outbreaks is a major concern for public health research in USA.

With expected increases in precipitation in the Northeastern United States from climate change (Hayhoe et al., 2007) there is the possibility that there will be alterations in risk of waterborne illnesses associated with heavy precipitation. Increases in precipitation could intensify flooding, and increase the potential for surface and

groundwater contamination by enteric pathogens. Furthermore, flooding could decrease the effectiveness of water treatment.

1.6 Waterborne Pathogens of Concern

The microorganisms that generally cause disease are termed *pathogens*. A pathogen is any agent that causes disease in animals or plants. Pathogens include bacteria, protozoa, viruses, prions, fungi and helminthes (WHO, 2004). A waterborne disease outbreak is an outbreak in which epidemiologic evidence points to a drinking water source from which two or more persons become ill at similar times (Curriero, Patz, Rose, & Lele, 2001).

According to Centers for Disease Control and Prevention the definition Waterborne Disease Outbreak is "An incident in which two or more persons experience a similar illness after consumption or use of water intended for drinking, and epidemiologic evidence implicates the water as the source of the illness" (CDC, 1990).

Table 1: Names of Different Waterborne Disease and Their Symptoms

(Aldea-global, n. d.; CDC, 2006 b, , 2008a, , 2008b; EPA, 1993)

Disease	**Microbial Agent**	**Disease Symptoms**	**Chronic Effect**
Campylobacteriosis	Bacterium (*Campylobacter jejuni*)	Fever, abdominal pain, diarrhea	Chronic sequelae, such as reactive arthritis and Guillain-Barré syndrome (GBS
Cholera	Bacterium (*Vibrio cholerae)*	Watery diarrhea, vomiting, occasional muscle cramps	significant decrease in the pertussis-toxin-catalysed ADP-ribosylation, prolactin secretion increased
Cryptosporidiosis	Protozoan (*Cryptosporidium parvum*	Diarrhea, abdominal discomfort	the small intestine is most commonly affected, Cryptosporidium infections could possibly affect other areas of the digestive tract or the respiratory tract.
Giardiasis	Protozoan (*Giardia lamblia*)	Diarrhea, abdominal discomfort	leak flux, malabsorptive and secretory components

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Giardiasis	Protozoan (*Giardia lamblia*)	Diarrhea, abdominal discomfort	leak flux, malabsorptive and secretory components
Amebiasis	Protozoan (*Entamoeba histolytica*)	Abdominal discomfort, fatigue, diarrhea, flatulence, weight loss	Colitis, Appendicitis, Peritonitis, Liver abscess, Lung abscess
Hepatitis	Virus (hepatitis A)	Fever, chills, abdominal discomfort, jaundice, dark urine	Numbness in extremities. Mental confusion / 'brain fog Dizziness & peripheral vision problems. Cognitive dysfunction Shortness of Breath Visual Changes, Female Problems (irregular menses, severe PMS)

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Shigellosis	Bacterium (*Shigella* species)	Fever, diarrhea, bloody stool	seizures, confusion or coma, kidney failure, arthritis, rashes
Typhoid fever	Bacterium (*Salmonella typhi*)	Fever, headache, constipation, appetite loss, nausea, diarrhea, vomiting, appearance of an abdominal rash	Nosebleed, Chills, Delirium, Confusion Agitation Fluctuating mood attention deficit Hallucinations
Viral Gastroenteritis	Viruses (Norwalk, rotavirus and other types)	Fever, headache, gastrointestinal discomfort, vomiting, diarrhea	dehydration
Legionnaire's Disease (a type of pneumonia)	*Legionella pneumophila* and other *Legionella* species	Pontiac fever is an acute-onset, flu-like, non-pneumonic illness	Delirium Pulmonary complications Gastrointestinal tract complications Central nervous system complications Kidney insufficiency Pneumonia

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Hemolytic uremic syndrome	*E. coli O157:H7*	Bloody diarrhea and stomach pain,	Pallor, Petechiae, purpura and oozing, renal failure, ataxia, coma or seizures, infarction, intussusseption, perforation or hepatomegaly
Schistosomiasis (immersion)	*Schistosoma*	Rash or itchy skin. Fever, chills, cough, and muscle aches	according to species, i.e., S. japonica, S. mansoni, and S. mekongi primarily affect liver and intestines; while S. haematobium primarily affects the urinary tract
Salmonellosis (oral transmission)	Bacterium (*Salmonella* species)	Gastroenteritis, fever and rapid blood-poisoning.	Dehydrated, the infection spreads from the intestines
Toxoplasmosis	*Toxoplasma gondii*	"Flu" with swollen lymph glands or muscle aches, damage to the brain, eyes, or other organs	anemia, enlarged liver or spleen, seizures, limp muscle tone, feeding difficulties, hearing loss, mental retardation

1.7 Climate and Waterborne Disease Outbreaks

Rainfall and surface runoff have been a concern for different waterborne disease outbreaks in the United Kingdom and the United States (Patz et al., 2000). Curriero et al. (2001) found a statistically significant association between rainfall and disease in the United States (Curriero, Patz, Rose, & Lele, 2001). 51% of waterborne disease outbreaks

were preceded by precipitation events above the 90th percentile (P=0.02) and 68% of waterborne disease outbreaks were preceded by precipitation above the 80th percentile (P=0.01) A recent study of precipitation and waterborne illness in the United States found that more than half the waterborne disease outbreaks in the United States during the last half century followed a period of extreme rainfall (Curriero, Patz, Rose, & Lele, 2001).

Weather has often played a significant role in a many reported waterborne disease outbreaks (Hrudey, Payment, Huck, Gillham, & Hrudey, 2003). The relationship between high impact weather events and the occurrence of waterborne disease outbreaks has been described by Thomas et al. (2006) They reported 'total maximum degree-days' above 0 degrees C and cumulative rainfall percentiles were associated with risk of waterborne disease outbreak. Their results suggest that in Canada warmer temperatures and extreme rainfall are factors in waterborne disease outbreaks (Thomas et al., 2006).

In 1993 in Milwaukee, Wisconsin there were around 400,000 gastroenteritis cases caused by *Cryptosporidium.* In 2000 in Walkerton, Canada more than 2000 waterborne gastrointestinal illness cases were caused by *E. coli* O157:H7 and *Campylobactor jejuni.* Both incidences have been related with previous heavy rainfall period (Hrudey, Payment, Huck, Gillham, & Hrudey, 2003). In Australia in different seasons gastroenteritis disease shows a statistically significant difference (P=0.02) (Hall, Kirk, Ashbolt, Stafford, & Lalor, 2006). The likelihood of gastroenteritis in Australia shows seasonal peak mainly in summer, though exceptions such as campylobacteriosis (in spring) or Rotavirus infection (in winter) occurs. For gastroenteritis greater odds have been reported in summer as compared to the spring and winter (OR 1.2); and there is a lower odds ratio in autumn (OR 0.7) (Hall, Kirk, Ashbolt, Stafford, & Lalor, 2006)

In the northeastern USA, peak rates of clinical cryptosporidiosis in late summer have been observed (Naumova et al., 2000). In Russia a cross sectional study in city of Cherepovets, has reported higher seropositivity in November–December than in June. This suggests a peak in *Cryptosporidium* infections in the summer–fall in Russia (Egorov et al., 2004).

Escherichia coli, also considered a foodborne pathogen, has been reported to be linked to rainfall events. In the state of New York in September 1999 the biggest reported outbreak of *E coli* O157:H7 occurred at a fairground, which included approximately 800 suspected cases. This event has been reported to be associated with infected well water (CDC, 2007). A drought followed by an extraordinarily heavy amount of rainfall, were both associated with this large outbreak (Patz et al., 2000). In a 10- year summary of *E. coli* O157:H7 surveillance in Scotland over 60% of the reported cases occurred between May and September (Coia, Sharp, Curnow, & Reilly, 1994).

In the Province of Ontario, Canada, in a 72 month time series based study on 3001 reported cases of verocytotoxigenic *Escherichia coli* (VTEC) demonstrated a marked seasonal pattern for occurrence of verocytotoxigenic *Escherichia coli* (VTEC) with peaks in July (Michel et al., 1999).

The reason for the most frequent occurrence of verocytotoxigenic *Escherichia coli* incidences during the summer months is unknown. However it is most likely related with increased ambient temperature (Michel et al., 1999). It is possible that high environmental temperatures increase reproduction of VTEC on the farm and on food products during handling and preparation for consumption.

A waterborne cryptosporidiosis outbreak in Milwaukee, Wisconsin in 1993 was one of the largest reported waterborne disease outbreaks with approximately 403,000

cases of intestinal illness and 54 deaths. It was also reported to be related to rainfall. In this outbreak a period of heavy rainfall and runoff followed by a high turbidity load affected the potency of local drinking water treatment plant (Hoxie, Davis, Vergeront, Nashold, & Blair, 1997).

Recent analyses continue to support conclusions that an increase in the frequency and severity of extreme precipitation events from climate change will result in an increased risk of waterborne and food borne illnesses. The most vulnerable groups in this condition are the very young (< 1 year of age), older adults (> 65 years of age) and immunocompromised individuals (Ebi, Mills, Smith, & Grambsch, 2006).

1.8 Land Use and Waterborne Disease Outbreaks

Literature supports the concept that waterborne disease outbreaks are somewhat related with the land use of the area. The infection rates for *Giardia* vary by geographic location (Laupland & Church, 2005). A study supported by a consequent GIS spatial scan statistical investigation of clusters of giardiasis in southern Ontario confirmed a relationship between Giardiasis and rural location (Odoi et al., 2004). Another study by Odoi et al (2003) has shown significant ($P < 0.05$) associations of giardiasis rates with fertilizer use on farming land and livestock (Odoi et al., 2003). In a study by Parra and co-workers (1991) verocytotoxigenic *Escherichia coli* (VTEC) isolation rates of diarrheic patients living in urban and rural regions of Mexico was compared to reveal the impact of living in an agricultural area on the risk of verocytotoxigenic *Escherichia coli* (VTEC) cases. This study confirmed (as seen by (Michel et al., 1999)) a higher verocytotoxigenic *Escherichia coli* (VTEC) isolation rate in patients who lived in rural regions compared to those in urban areas. In another study in Ontario, Canada by Michel et al. (1999), a

relatively high incidence of the verocytotoxigenic *Escherichia coli* (VTEC) was reported in rural regions in comparison to the urban areas. The spatial association of cattle density and human verocytotoxigenic *Escherichia coli* (VTEC) incidence proposes that living in an agricultural (rural) region with high cattle density could be a potential risk factor for the infection of VTEC disease (Michel et al., 1999).

1.9 Occurrence of Pathogens in Environmental Waters

1.9.1 Relationship of pathogen and indicators

Waterborne disease is usually spread through fecal contamination. It is important to determine if fecal contamination is present in order to determine whether there is potential for exposure to pathogens. Worldwide *E. coli*, coliform bacteria and enterococci have served as the indicator organisms for fecal contamination (Anderson, Whitlock, & Harwood, 2005).

As beach closure decisions are typically based on measured densities of fecal coliforms, *E. coli* and enterococci, a detailed literature study has been done to determine the appropriateness of these decisions. Our research is based upon studies examining the relationship between indicators and pathogens. Of the studies examined, among 150 pairs of indicator-pathogen comparisons (Supportive material, Table 11), 49% confirmed significant correlations. In a comparison of established indicators in fresh and saline water environments, generally correlations ranged from 50 to 70%, suggesting that classical indicators continue to be suitable, albeit imperfect predictors for the presence of pathogens.

1.10 *Giardia* as a model organism for waterborne diseases

In order to understand the importance of our research it is essential to look at the literature background of the organism. *Giardia* has been known as a major cause of gastrointestinal illness for a long time. In 1681 *Giardia* was initially described by Van Leeuwenhoek when he was examining his own diarrheal stools under the microscope.

Giardia and *Cryptosporidium* are the two most important intestinal parasites infecting North Americans (Laupland & Church, 2005). The waterborne *Giardia intestinalis* is the most frequent protozoan agent of intestinal disease, which causes about $2.8*10^8$ cases yearly across the world (Lane & Lloyd, 2002). This is sometimes also referred to as *Giardia lamblia* or *Giardia intestinalis* (Dawson, 2005).

Giardia is a waterborne zoonotic protozoan parasite. Fecal material from humans and domestic animals causing environmental pollution is an important pathway for wildlife infections Wild animals are frequently considered to be potential reservoirs of zoonotic disease. It is found all over the world and is one of the most frequently reported parasites of humans and animals. Wild mammals have been found to be potential reservoirs of *Giardia*. Beavers have often been suggested as the source of waterborne contamination for *Giardia*. For this reason in North America, giardiasis is commonly referred to as 'beaver fever'. It has been demonstrated that some of the genotypes of *Giardia* are zoonotic and some are host specific (R. C. A. Thompson, 2000). *Giardia* has two important stages in its life cycle which affect its host specificity – the trophozoite and the cyst.

Fayer *et al.* (2004) points out "*Giardia* cysts are transmitted by the fecal–oral route of humans and animals and are associated with outbreaks of infection from

contaminated surface water drinking". So, water is the most important route of its transmission. *Giardia* poses a risk to water supplies because of it's resistance to conventional chlorine disinfection than other pathogens such as bacteria (Fayer, Dubey, & Lindsay, 2004) However, the larger size of *Giardia* cysts facilitates their removal by filtration as compared to *Cryptosporidium* oocysts (Dawson, 2005).

1.10.1 Symptoms of *Giardia*

Pathogenic *Giardia sp.* cause the disease called giardiasis, which can be characterized by diarrhea and malabsorption (R.C.A Thompson & Robertson, 2003) In humans, giardiasis symptoms start with severe stomach cramps, sickness and diarrhea, nausea, fatigue and weight loss. Stools may be pale, greasy, and malodorous and foul smelling. Weight loss may be significant. The incubation period is 7 to 14 days. Depending on vulnerability, the sickness can last from two weeks onwards. For children and immune-compromised individuals, it can pose a greater threat (EPA, 1999; Sullivan, Linneman, Clark, & Walzer, 1987).

1.10.2 Sources of *Giardia*

A common source of *Giardia* is sewage effluent and it has been found frequently in water supplies throughout the world. *Giardia* is generally found in the feces of domestic animals, livestock and wild animals. Usually, it is not considered as a significant animal disease. The cysts in animal and environmental samples have been demonstrated to be infective to humans (R. C. A. Thompson, 2000).

With regard to sources of *Giardia* in coastal regions, marine mammals may be important sources of *Giardia. Giardia* cysts have been found in feces from a California

sea lion, ringed seals in arctic Canada, and harp, grey and harbor seals in the Gulf of St Lawrence, Canada (Fayer, Dubey, & Lindsay, 2004).

Table 2: *Giardia* Detected in Marine Mammals

Parasite	Host (common name)	Location	No. infected	Detection method	Reference
Giardia	*Phoca hispida* (Ringed seal)	Arctic Canada	3	Microscopy	M.E. Olson *et al(1997)*
Giardia	*Phoca groenlandica* (Harp seal)	Gulf of St Lawrence	15	Microscopy	L.N. Measures and M.E. Olson (1999)
	Halichoerus grypus (Grey seal)		4		
	Phoca vitulina (Harbor seal)		1		
Giardia	*P. hispida*	Ungava Bay, Canada	43	Flow cytometry	
Giardia	*Zalophus californianus* (California sea lion)	Humboldt Bay, USA	1	Microscopy	M.Q. Deng *et al (2000)*

Modified from (Fayer, Dubey, & Lindsay, 2004)

1.10.3 Exposure to *Giardia* through drinking water

Water is one of the major transmission routes of *Giardia* infection (Laupland & Church, 2005). Drinking water sources become contaminated when feces containing the parasites are deposited or flushed into water. If treatment is insufficient, drinking water may contain sufficient numbers of *Giardia* cysts to cause illness. The infectious dose of *Giardia* is less than 10 cysts when given orally and may even be as low as 1 cyst depending on the host immunity (PHAC, n. d.). The comparative importance of these various routes of exposure is unknown (CDC, 1990). In an international study by Fayer et al. among selected eight countries over the world almost 21–100% of the examined

samples (> 2350) of surface water, contained 5/L *Giardia* cysts (Fayer, Dubey, & Lindsay, 2004).

1.10.4 Exposure to *Giardia* through food

In the United States and Central American countries, 60% of the total irrigation water (mainly for vegetables) has been found positive for *Giardia* cysts (Fayer, Dubey, & Lindsay, 2004). *Giardia* cysts have been found on wastewater irrigated coriander, carrots, mint, radishes and potatoes. Contaminated fruits and vegetables related to outbreaks have been reported frequently (Fayer, Dubey, & Lindsay, 2004).

Giardia has been detected in shellfish. The high prevalence of *Giardia* contamination in mussels (41.8%, n = 184) has been reported by Gómez-Couso et al (2005) both in surface and discharged waste water. This leads to *Giardia*'s waterborne transmission and also food borne transmission through the consumption of contaminated shellfish (Gomez-Couso, Mendez-Hermida, Castro-Hermida, & Ares-Mazas, 2005). In a study by Schets et al. (2007) in an oyster farm in Yerseke 13.0% (6 of 46) commercial oysters have been found infected with *Cryptosporidium* and/or *Giardia* in their intestines. The detection of *Cryptosporidium* and *Giardia* in oysters intended for human consumption with human pathogenic (oo) cysts present in marine environment is an important public health concern (Schets, van den Berg, Engels, Lodder, & Husman, 2007).

1.10.5 Exposure to *Giardia* through contaminated coastal recreation water

Human and animal feces contain encysted *Giardia* that are transported through agricultural runoff, suburban and urban land surfaces, wastewater discharges and other

sources to rivers and streams. These streams carry contaminated sediments to estuaries and eventually to coastal waters. In many countries disposal of raw sewage and sediments from shipping lanes in coastal waters is a common practice. Literature by Fayer (2004) includes studies measuring the presence of *Giardia* cysts in marine waters such as sewage outfalls in Mamala Bay, a few kilometers from Waikiki bathing beach in Hawaii and off the coast of Panama (Fayer, Dubey, & Lindsay, 2004).

1.10.6 Cycle of transmission of *Giardia*

1.10.6.1 In Humans

Like *Cryptosporidium*, *Giardia* infection occurs when cysts infect through ingestion by contaminated hands, food, contaminated water, human-to-human contact, or directly in environments with compromised hygiene levels (Odoi et al., 2004; Welch, 2000). In high frequency transmission environments and direct person-to-person transfer conditions (such as localized endemic communities or institutional settings such as day care centers), *Giardia* transmission occurs.

Giardiasis outbreaks as well as individual cases had proven to be associated with inappropriate food management, exposure to contaminated water (i.e. swimming pools, surface and groundwater including those found in beaver ponds and springs), travel to less developed countries or close contact with a case (i.e. families, day care centers) (Isaac-renton & Philion, 1992).

Enteric parasitic infection with either *Giardia sp.* or *Cryptosporidium sp.* may have been reported to be transmitted through sexual contact and immunocompromised persons (acquired immunodeficiency syndrome) who are particularly at risk of

developing severe constant infection (Griffiths, 1998).

1.10.6.2 Cattle

Infection of *Giardia* in young livestock is common and occurs at exceptionally high levels. Throughout the world, *Giardia* has been frequently reported in beef and dairy products. According to the longitudinal studies the prevalence rate is 100% (Ralston, McAllister, & Olson, 2003). Between the ages of 4 and 12 weeks, the highest excretion intensity is 10^5–10^6 cysts/ gram of feces. The chronic giardiasis in calves may reduce growth, rate of weight gain, hamper feed efficiency and decrease skeleton weight (Ralston, McAllister, & Olson, 2003). However, it isn't generally considered an important animal disease. The main threat of *Giardia* in cattle is its cross host contamination through animal protein (milk, beef) products. In a follow up study by Ralston et. al (2003) of 20 cow calves from birth to weaning, the results showed a 100% infection rate (Ralston, McAllister, & Olson, 2003). The high prevalence of *Giardia* in newborn and young calves is well known (Xiao, Herd, & Rings, 1993).

1.10.6.3 Dogs and cats

In the USA as well as in other countries *Giardia* is also widely common in dogs and cats. In Australia it was found that *G. duodenalis* was the most common enteric parasite of domestic dogs and cats. Even though *Giardia* is common in dogs and cats, it is rarely associated with clinical disease in these animals. Molecular epidemiological studies proved dogs may be infected with their own, host-adapted (canid) genotype of *Giardia*, as well as with zoonotic genotypes.

Giardia is a common parasite in cats world-wide (Collins, Pope, Griffin, Walker, & Connor, 1987). A survey of dogs and cats in the Perth metropolitan area revealed 21% prevalence of *Giardia* in dogs and 14% of giardiasis in cats (Swan & Thompson, 1986).

1.10.6.4 Wildlife

Although wildlife is susceptible to infection with zoonotic genotypes of *G. duodenalis*, the limited evidence collected under natural, pristine conditions suggests that wildlife harbors their own genotypes/species of *Giardia*.

As example, genotypic characterization of *Giardia* from native marsupials in Australia has revealed that they are infected with a new, genetically distinct genotype of *Giardia*. In North America animals like beavers, nutria and deer are also frequently infected with *Giardia* and often the prevalence rates are over 50% (Dixon et al., 2002; Dunlap & Thies, 2002; Heitman et al., 2002; Rickard, Siefker, Boyle, & Gentz, 1999).

1.10.7 *Giardia* Outbreaks

Between 1965 and 1984, 90 outbreaks with 23,776 cases were reported in the United States (however it is not understood whether it was waterborne or not). Between 1979 and 1988, *Giardia* was the most frequently implicated organism in waterborne disease in the US (Flanagan, 1992, as cited by (Dawson, 2005). From 1984 to 1994, 18 drinking-water-Giardiasis outbreaks including 3994 individuals were reported (Fayer, Dubey, & Lindsay, 2004). The National Giardiasis Surveillance System reported from 1992 to 1997 among 43 states of United States annually 2.5 million cases of giardiasis occur (Furness, Beach, & Roberts, 2000).

The WHO reported an estimated 2.8×10^8 cases/ year of *Giardia duodenalis* globally (WHO, 1996). In developed countries it is the most frequent intestinal parasite of humans. In developing countries like Asia, Africa and Latin America, about 200 million people have indicative giardiasis. Every year globally almost 500,000 new cases are reported. (R. C. A. Thompson, 2004).

Table 3: Some Examples of Outbreaks of Waterborne and Foodborne *Giardia*

Outbreaks	Location	Water Type	Cases	Reference
Giardia **(Waterborne)**				
1985	Bristol (UK)	Treated reservoir	108 laboratory confirmed cases	Browning and Ives (1987)
1992	Sweden	Drinking water at ski resort	More than 3000 cases estimated	Hunter (1997)
1985–1986	Massachusetts (USA)	Unfiltered water supply	703 reported cases	Hunter (1997)
Giardia **Foodborne**				
1979	Minnesota (USA)	Prepared salmon	29	Rose and Slifko (1999)
1985	Connecticut (USA	Noodle salad at picnic	13	Rose and Slifko (1999)
1986	New Jersey (USA)	Fruit salad at party	10	Rose and Slifko (1999)
1986	Minnesota (USA)	Sandwiches (nursing home)	88	Rose and Slifko (1999)

(Modified from (Dawson, 2005)

1.10.7.1 Populations at risk

In Canada *Giardia lamblia* has been reported as one of the primary etiologic agents of outbreaks in recent decades. A significant association between development of giardiasis and age was observed (Laupland & Church, 2005). Apparently harmless dose to a healthy individual could be potentially fatal to immuno-compromised and elderly population (Ford, 1999). In the United States one population-based surveillance study confirmed increasing rates for giardiasis from 1992–97 where the highest national rates

of giardiasis has been found among children aged 0–5 years and closely followed by persons aged 31–40 years (Furness, Beach, & Roberts, 2000). Children under 5 years of age have been reported with the highest incidence of giardiasis (Greig et al., 2001).

A statistically significant difference ($P<0.001$) in gastroenteritis risk across age groups was identified in an Australian study by Hall et al (2006). In comparing children 0–4 years the odds of gastroenteritis in most adult age groups is OR 0.5 or less where female had an OR of 1.3 ($P=0.01$). This was possibly due to a higher rate of gastroenteritis among women aged 20–40 years, with a higher chance of having a young child with gastroenteritis in the house (Hall, Kirk, Ashbolt, Stafford, & Lalor, 2006).

It is possible that community exposure and behavioral factors likely play a role in young children's susceptibility to giardiasis (Greig et al., 2001). The activities of young children may enhance their exposure to pathogens via environmental or secondary (person-to-person) transmission (Hall, Kirk, Ashbolt, Stafford, & Lalor, 2006). Young children are more susceptible to infection with *Giardia sp.* and *Cryptosporidium sp.* because of their exposure to infected water sources such as swimming pools and communal contact (like day care centers) (Laupland & Church, 2005).

Studies report different susceptibility rates between two genders. In comparison to females in all age groups males had a higher mean annual Ggiardiasis incidence (Greig et al., 2001). In 2001-2002 in a National Survey in Australia males reported less gastroenteritis prevalence at 6.3% (95% confidence interval (CI) 4.7–7.8) compared to females at 7.7% (95% CI 6.1–9.4) ((Hall, Kirk, Ashbolt, Stafford, & Lalor, 2006)). In contrast, a study by Laupland and Church (2005) reported that males were at higher risk for development of giardiasis infection as compared to females (21.2 vs. 17.9 per

100,000/yr; relative risk (RR). Additionally there was a significant decrease in risk associated with an increasing age (Laupland & Church, 2005).

1.10.7.2 Genotype specificity

More than 50 species of *Giardia* have been discovered. *Giardia* has been observed in the gastrointestinal tracts of all classes of vertebrates. In humans and the majority of domestic and wild mammals, the common *Giardia* species is *Giardia duodenal.*

Table 4: Recognized Species in the Genus *Giardia*

Species	**Hosts**	**Morphological characteristics**	**Trophozoite dimensions: length/width (µm)**
G. duodenalis	Wide range of domestic and wild mammals including humans	Pear-shaped trophozoites with claw-shaped median bodies	12–15/6–8
G. agilis	Amphibians	Long, narrow trophozoites with club-shaped median bodies	20–30/4–5
G. muris	Rodents	Rounded trophozoites with small round median bodies	9–12/5–7
G. ardeae	Birds	Rounded trophozoites, with prominent notch in ventral disc and rudimentary caudal flagellum. Median bodies round-oval to claw-shaped	~10/~6.5
G. psittaci	Birds	Pear-shaped trophozoites, with no ventro-lateral flange. Claw-shaped median bodies	~14/~6

(R. C. A. Thompson, 2004)

1.10.7.3 Hosts specificity/ Cross host transmission

The *Giardia* parasite has a broad host range. The host specificity of *Giardia* not only influences the taxonomy but also its contradictory multi-host zoonotic nature. It has

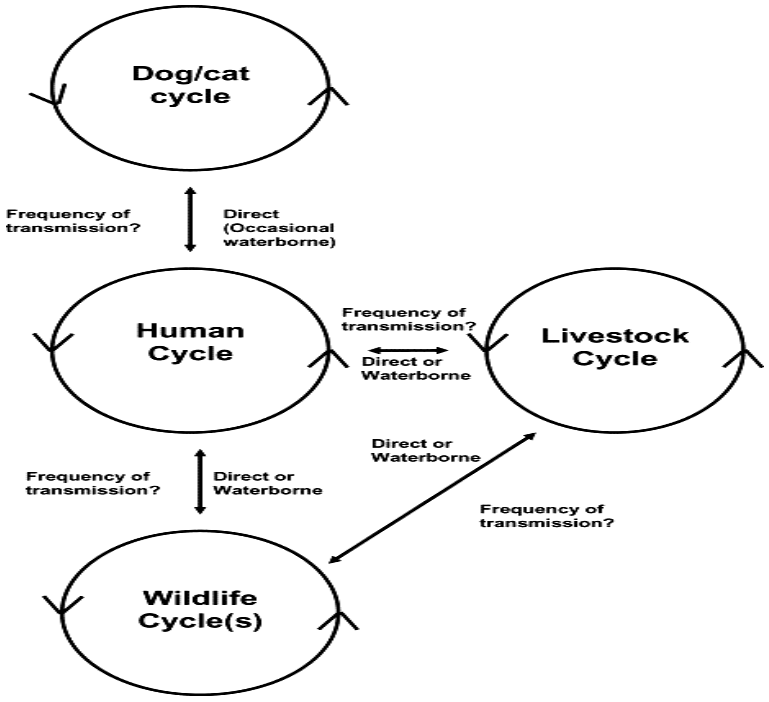
been found that a few species are host specific while others have a broad range of host species.

Table 5: Genetic Groupings and Host Range of Isolates within the *Giardia duodenalis* (Appelbee, Thompson, & Olson, 2005)

Assemblage	**Genotype**	**Host range**
A	Zoonotic	Human, livestock, dog, cat, beaver, guinea pig, slow loris, mountain gorilla, rock hyrax, harp seal, hooded seal, deer, prairie dog, bobcat, groundhog and domestic mouse
B	Zoonotic	Human, cattle, dog, cat, beaver, musk rat, slow loris, siamang, chinchilla, rat, coyote and domestic mouse
C and D	Dog	Dog, coyote and domestic mouse
E	Livestock	Cattle, alpaca, goat, sheep and pig
F	Cat	Cat
G	Rat	Domestic rat
Vole	Muskrat	Muskrat and vole
Novel	Marsupial I	Quenda (bandicoot), mouse and sheep
Novel	Marsupial II	Tasmanian devil

Through the advancement of genotyping studies, assemblages of *G. intestinalis* with different host ranges have been recognized. Large-scale studies are needed for better identification of sources and transmission routes.

Some *Giardia* strains are zoonotic and can be transmitted from humans to beavers, dogs and muskrats which can be proven by similar gene sequences among isolates. Assemblages C and D are found primarily in canines and assemblages E, F and G found primarily in hoofed livestock, cats and rats. However it has not been found in human infections respectively. So assemblages A and B are the most important because their cross host transmission is related to human health all over the world (Fayer, Dubey, & Lindsay, 2004).



((R. C. A. Thompson, 2004)

Figure 1: *Giardia* Transmission among Different Hosts

Thompson (2004) reported a large number of cross host *Giardia* transmission between human and wildlife. The same with cat and dog or livestock animals. These could be primary or direct transmission or could be transmitted by water media.

Livestock animals also can be affected by wild life animals like beaver, wild goose or vice-versa. However there is no known transmission reported between cat/dog and livestock. The frequency or the rate of these transmissions is not known yet (R. C. A. Thompson, 2004)

Lab based experimental cross transmission studies are not reliable because there is a lot of uncertainty about the *Giardia*-free status of experimental animals and the common use of high doses of cysts which is unlikely to represent a natural infection. Cross transmission studies have also used uncharacterized isolates, limiting their usefulness in determining the host specificity of the different genotypes.

1.10.8 Environmental persistence

Bingham (1979) examined the temperature resistance of *Giardia sp.* by using excystation. Storage at 8 ^{o}C led to greatest cyst survival whereas at 37 ^{0}C and over survival rates of *Giardia* cyst reduced. Freezing and thawing cysts resulted in an almost complete loss of viability. Cysts exposed to boiling water immediately lost excystation ability (Bingham & Meyer, 1979).

Cysts are infectious when shed in the feces and their pathogenicity continues for prolonged periods in cool, damp environments. Also the presence of giardiasis infections in marine mammals suggests it is resistant to exposure of low salinities (Appelbee, Thompson, & Olson, 2005)

1.10.9 Social factors related to *Giardia* exposures

Recent social changes in developed countries have led to a large number of young children spending time outside the family in group care. Thompson (2000) reported

infectious diarrhea (giardiasis) has been recognized as one of the most important health problems among young children who attend day care centers. It has been proven in a study by Thompson (2000) that the children at day care are twice susceptible to the incidence of diarrhea in compared to children at home" (R. C. A. Thompson, 2000). Diarrhea and other clinical symptoms of *Giardia lamblia* infect children in day care at a higher rate than the general population (Cody, Sottnek, & Oleary, 1994). Not only the children but also working adults are under the threat of *Giardia* infection at care centers. According to the EPA, an estimated infection risk from 5-20% of household contacts and 9-35% of care-center staff can occur.

It has been reported that giardiasis is particularly associated with foreign travel. Among travelers to Eastern European countries and in the former Soviet Union, waterborne giardiasis is well recognized (Dawson, 2005).

1.10.10 *Giardia* Detection Methods

In cases of *Giardia* the infective dose is generally between 10 and 100 cysts (MADPH, 1996). However according to EPA the maximum contaminant level for *Giardia* in drinking water is zero(EPA, n.d. -c). EPA has suggested membrane filtration method by using mo TEC which is membrane thermo tolerant *Escherichia coli* agar, method 1103.1(EPA, 2002 a) and a modified membrane filtration method by using mo TEC (membrane thermo tolerant *Escherichia coli* agar, method 1603) for the quality control measurement of *E. coli* in recreational water (EPA, 2002 b). However Noble et al (2004) reported rising recognition of new methods based on chromogenic substrate (CS) technology (Noble, Leecaster, McGee, Weisberg, & Ritter, 2004). EPA policy as updated in the BEACH Act of 2000 (EPA, 2000b) recommended that beaches needed to

be sampled once a week or more often if they are high use or there is evidence of pathogen related illness.

1.10.10.1 Concentration and Separation from Environment

The current EPA approved method for detection in environmental samples is by Membrane Filtration Using Modifiedmembrane-Thermotolerant Escherichia coli Agar (Modified mTEC) also know as Method 1623 (EPA, 2002 b) Several improvements to methods have been reported in literature for the successful detection of *Giardia* cysts in environmental samples as well as in feces (Noble, Leecaster, McGee, Weisberg, & Ritter, 2004). However, direct immunofluorescence microscopy is the best method to confirm the presence of *Giardia* in sewage sludge and in surface water. However over the period of development polymerase chain reaction (PCR) has become a specific and sensitive method of detecting detection of a variety of microorganisms analysis in environmental samples (EPA, 2004: b)

1.10.10.1.1 In Surface Water

During the 1980-1990s, a large volume of water (100-1000 L) water was measured by the 'yarn wound cartridge filtration' method. The recovery efficiency was 12-28% for cysts (Nieminski, Schaffer, & Ongerth, 1995) depending on techniques and inoculation level.

Later a new method, immunomagnetic separation (IMS) based protocols uses paramagnetic bead coated with antibody against *Giardia*. This procedure (EPA, 2005) can separate and identify up to 85% of cysts.

Membrane filtration methods have a higher recovery rate and more sensitive detection limit (Hsu, Huang, Hsu, Jiang, & Hsu, 2001); however this method is only possible with low turbidity water (Lane & Lloyd, 2002).

Also for detection of *Giardia* (as well as for *Cryptosporidium* and *E. intestinalis*) portable continuous flow centrifuge (PCFC) shows substantially high recoveries that EPA approved filtration method (method 1623) (Zuckerman & Tzipori, 2006).

1.10.10.1.2 Sewage sludge

Oocyst sedimentation in Phosphate buffered saline (PBS) and Immunomagnetic Separation (IMS) process has potential for *Giardia* identification. The recovery efficiency for this technique is 40-60% (Rimhanen-Finne, Ronkainen, & Hanninen, 2001).

1.10.10.1.3 Feces

Immunomagnetic Seperation (IMS) technique is most successful to measure oocyst of *Giardia* from animal and human faeces. However zinc sulfate flotation and formalin-ethyl acetate sedimentation techniques are also similarly effective for *Giardia* separation (Rimhanen-Finne, Ronkainen, & Hanninen, 2001).

1.10.10.2 Identification

1.10.10.2.1 Immunofluorescence (IF) microscopy

Fluorophore-labeled polyclonal (pAb) and monoclonal (mAb) antibodies become attached to cell wall antigens of cysts. Thus, the shape and size of cysts is emphasized (Rose, Landeen, Riley, & Gerba, 1989). For IF microscopy, the detection limits in human and animal faeces vary between 10–50 000 cysts/g

1.10.10.2.2 Enzyme-linked immunosorbent assay (ELISA)

Several antigens are known to be associated with *Giardia* infection. ELISA is a cost-effective, rapid, and sensitive method for detecting the presence of *G. lamblia* in fecal specimens. Two types of ELISA assays are used for *Giardia* detection (Rosenblatt, Sloan, & Schneider, 1993)

1) pAb-based ELISA reacts with multiple antigens,
2) The mAb-ELISA cannot detect different species of *Giardia*.

1.10.10.2.3 Molecular identification techniques

In the 'sample purification density gradient centrifugation technique' highly processed cysts are needed. Presently, commercial DNA purification kits for direct DNA isolation from feces are widely being used. The benefit of molecular identification techniques is that it is able to detect genus, species or genotype-specific nucleic acid sequences in *Giardia* (Rimhanen-Finnea, Enemarkb, Kolehmainena, Toropainena, & Hänninen, 2007).

1.10.11 Treatment

Though *Giardia* is resistant to common disinfection using chlorine treatment, it can be inactivated by long contact with chlorine or UV light which exposure between 16 mJ/cm2 to 40 mJ/cm2 (NSF, n.d) . Commonly used water disinfectants can effectively inactivate *Giardia* cysts depending on the disinfectant concentration and contact time. Cysts are relatively more resistant to disinfectants than bacteria and viruses, and high doses and lengthy contact times may be needed (EPA, 2000a). This may result in high levels of disinfection byproducts which are regulated by the EPA (EPA, 2000a).

When operated under appropriate conditions, filtration technologies can effectively remove *Giardia* cysts from water. The highest removal is possible with Membrane filtration' and 'granular filtration techniques' (EPA, 2000a) (EPA, 2002 a) .

CHAPTER 2

GOALS AND OBJECTIVES

An overall goal of the study is to determine the extent of waterborne exposures to pathogenic microorganisms. This can be accomplished through the analysis of the spatial and temporal variability of confirmed reported human cases of a microorganism such as Giardia. *Giardia* is a good reference pathogen for several reason: (1) it is one of the most commonly identified etiologic agents in waterborne disease outbreaks; (2) it has a multitude of environmental sources that may be influenced by watershed hydrology; (3) it is more resistant to conventional treatment (Hoff & Akin, 1986) than the bacterial pathogens. Thus confirmed human cases are expected to be more likely to occur from a waterborne route (as compared to other pathogens that are more easily removed by treatment processes). Hence the relationships between precipitation, streamflow, broad watershed characteristics and confirmed human cases of *Giardia* for Massachusetts will be examined.

The hypotheses and specific aims of the follow research are the following:

A) Infection rates for waterborne pathogens are due to contact with untreated water and will be related to recreational behaviors, seasonal access and use of recreational water.

Specfic Aim (1) To determine if seasonal trends in confirmed human cases of *Giardia* infections coincide with seasonal recreational water use,

(B) Characteristics and conditions of watersheds influence the temporal and spatial abundance of waterborne pathogens and associated gastrointestinal illness.

Specific Aim (2) To examine public health data from Massachusetts from a variety of watersheds to determine if a link exists between waterborne diseases and watershed conditions and characteristics (land use distribution of the watersheds, existence of any specific features in

Specific Aim (3) To determine the impact of land use (urban versus rural) on the frequency of confirmed *Giardia* cases

(C) Older engineering technologies such as Combined Sewer Overflows (CSO's) allow untreated water to contaminate drinking water sources resulting in increased exposure to waterborne pathogens.

Specific Aim (4) Evaluate the differences in frequency of confirmed *Giardia* cases in watersheds with and without Combined Sewer Overflows (CSOs) upstream of drinking water sources.

(D) High runoff induced by heavy precipitation causes a greater influx of pathogens to drinking water sources leading to higher infection rates from waterborne pathogens after these precipitation events

Specific Aim (5): To examine the temporal association between high rainfall events and outbreaks of *Giardia* cases.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Areas

Three watersheds in Massachusetts were chosen that represent different watershed and water management characteristics and were studied in detail. They are: (1) the Blackstone River watershed, (2) the Deerfield River watershed, and (3) the Merrimack River watershed.

3.1.1 Blackstone River Watershed

This watershed is a series of streams originating in the hills of Worcester, Massachusetts. The Blackstone River flows 48 miles in Massachusetts south into Rhode Island. It has a total drainage area of 640 square miles among which about 382 square miles are in Massachusetts. The Blackstone River watershed also encompasses 1300 acres of lakes, ponds, and reservoirs. Worcester and Providence, the second and third largest population centers in New England, are in the Blackstone River watershed. In the early 19th Century, immigrants to the region took advantage of the natural water power of the Blackstone River, which became the "Birthplace of America's Industrial Revolution"(EOEEA, 2007-a). The Blackstone River watershed was selected as being representative of an urban, highly contaminated watershed.

3.1.2 Deerfield River Watershed

The Deerfield River is one of the coldest and cleanest rivers in Massachusetts. It drops approximately 2000 feet from its headwaters to its convergence with the Connecticut River. Its drainage area is approximately 665 square miles; most of its headwaters are located in the Green Mountains of southern Vermont. The Deerfield River

watershed includes more than 149 streams, 21 lakes and ponds (EOEEA, 2007 - b). It is renowned for its whitewater and high water quality, which have encouraged multiple recreational uses of the river such as sport fishing, kayaking and canoeing. The Deerfield River watershed was selected as being representative of a rural watershed with low contamination.

3.1.3 Merrimack River Watershed

The Merrimack River watershed is the fourth largest watershed in New England. The river flows south through central New Hampshire for 78 miles and into Massachusetts. The total drainage area of the Merrimack River watershed is 5,010 square miles among which 1,200 square miles are in Massachusetts. It includes all or part of 24 Massachusetts municipalities (EOEEA, 2007-C). Lowell is one of the major cities of this watershed. Several communities along the Merrimack River obtain their drinking water from the river. The drinking water sources are potentially impacted by combined sewer overflows (CSOs). In a CSO, storm water is mixed with untreated wastewater and discharged to the river prior to complete treatment. In Lowell, nine CSOs can discharge more than 10 million gallons of sewage and storm water during a one-inch rainstorm (EPA, 2007 -b). The Merrimack River was selected as it is representative of a watershed with important sources of drinking water contamination.

3.2 Land Use Data

ArcGIS 9.2 (ESRI, Boston, MA) software was used for GIS analysis for processing land use, census population, and watershed delineation data files. These Geological Information System data were collected from the Office of Geographic and

Environmental Information (mass.gov, 2006), Commonwealth of Massachusetts Executive Office of Environmental Affairs ("Commonwealth of Massachusetts", 2006).

The base map was selected as town boundary layers, downloaded as Census 2000 Tiger Town polygon layer (cencus2000towns_poly) from MassGIS (mass.gov, 2006). MA town boundaries were added as a layer in the new Arc map document. Georeferencing (a relation between raster or vector images to map projections or coordinate systems) of the map was verified. A “Major watersheds” layer was downloaded and overlapped onto the same map (mass.gov, 2006). Adding both layers provides the location of different watersheds in Massachusetts.

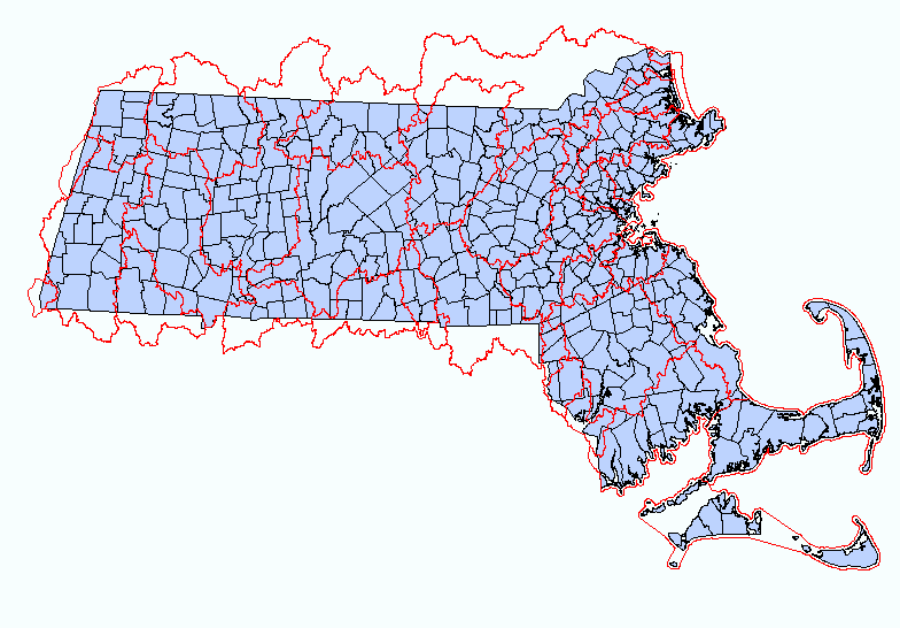


Figure 2: All Watersheds over Town Boundary census 2000

Based on this map, after receiving information of number of towns in watersheds a query based on town names was made to select the three preferred watersheds from statewide watershed data from MassGIS. For this purpose a permanent selection function was made.

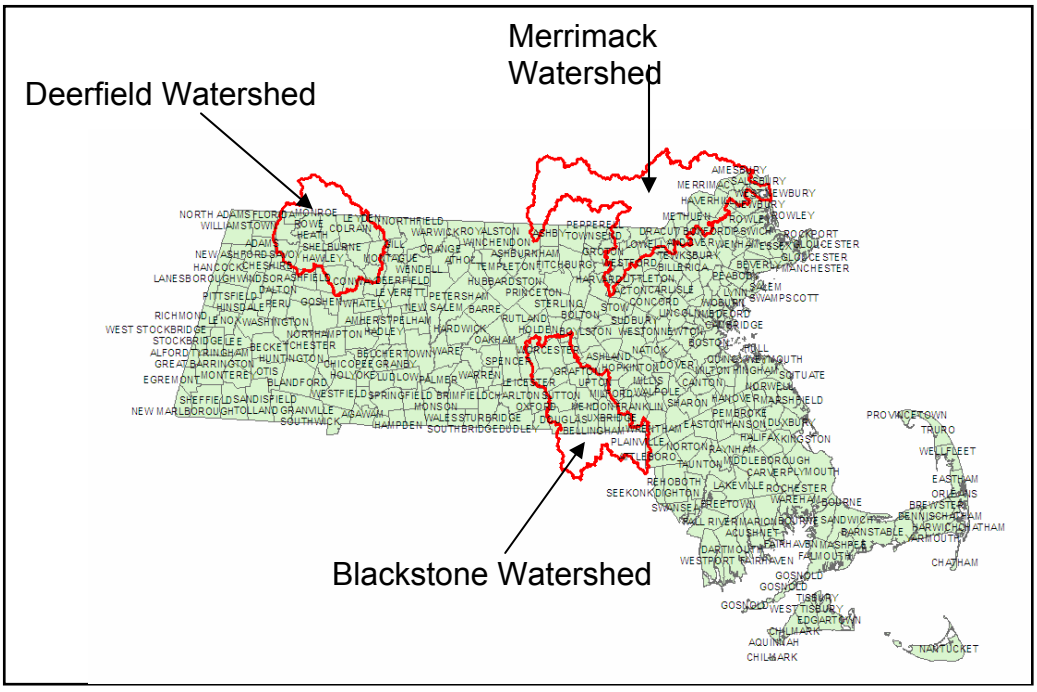


Figure 3: Selecting Three Watersheds using GIS

Land used of all individual towns (those located in Massachusetts within the watershed) were downloaded one by one from MassGIS.

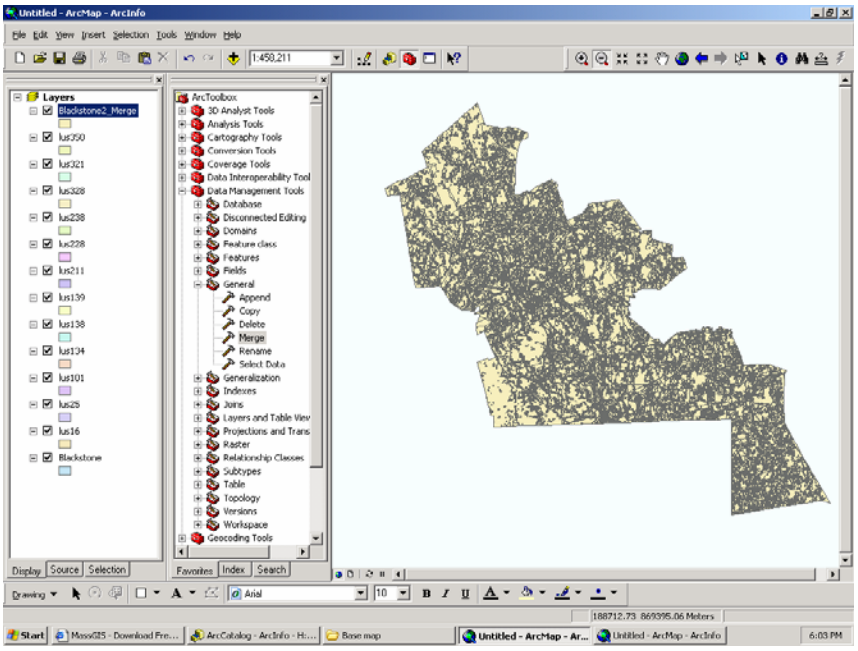


Figure 4: Merging all Town Layers under Blackstone

The merging of town layers was repeated individually for each one of the three watersheds and merged layers were clipped out (in the manner of cookie cutting) according to the watershed boundary. These land use layer with the Unique Value LU21_1999 which represents GIS land use distribution of 1999 and categorized into 21 categories. They are following

1. Cropland
2. Pasture
3. Forest
4. Nonforest wetland
5. Mining
6. Openland
7. Participation Recreation
8. Spectator Recreation
9. Water based Recreation
10. Multifamily residency
11. High Density Residency
12. Medium Density Residency
13. Low density residency
14. Salt water wetland
15. Commercial
16. Industrial
17. Urban open
18. Transportation
19. Waste Disposal

20. Water and

21. Woody Perennial

According to the purpose of our research these layers were selected by attribute, reclassified and divided into only 5 different categories. They are named as

1. Agriculture

2. Water

3. Wetland

4. Urban/industry

5. Forest/ openland.

A selection query was performed on land use layers. Total area per town was obtained from the attribute table of 'town layer' by performing a selection query and copied into a spreadsheet. A ratio was made of agricultural area with the total area per town. From attribute table I determined the area of agriculture land and water for each town of the 3 watersheds to sum them.

Based on the statistics and geographic distribution of the area that Deerfield is an high agricultural based rural watershed and Blackstone is an industry based, highly populated, also high agricultural and large natural water body containing watershed. Merrimack is also a highly industrial based, very less agricultural watershed but with a large volume of water.

3.3 Watershed populations

The population living within each watershed was calculated using the census data and watershed layers. Census_2000 data (US_Census_Bureau, 2000) from the attribute table of Arcmap provided the total population of towns in the watershed but do not give

the information of population of the towns which are partially present in the watersheds in Massachusetts. Thus considering the population directly from the Arcmap attribute table, creates a bias in watershed population. So the population of each of the watersheds needed to be calculated.

Since the chosen watersheds extended beyond the borders of MA, this population of watershed information was important. Mainly because our health data from MADPH was Massachusetts based. For each watershed, the clipped watershed area was compared with previously watershed-based merged town layer area. This gives information about which towns in what ratio were within or adjacent to the watershed and thus in Massachusetts.

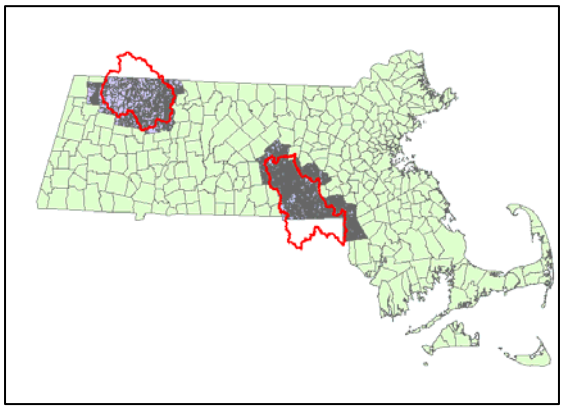


Figure 5: Merging Watershed Based Town Layer

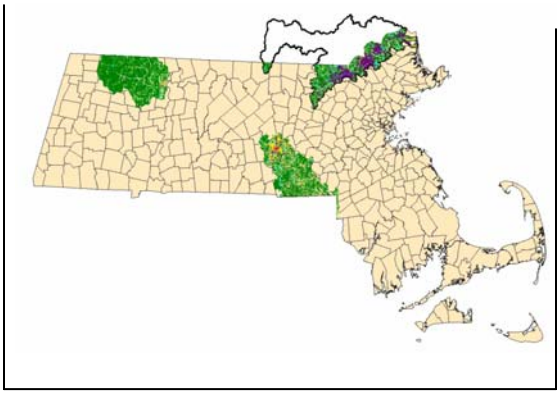


Figure 6: Clipped Watersheds

This ratio was multiplied with town based population of Census_2000. This gives each watershed based population in Massachusetts. Based upon U.S. Census data for the year 2000, the calculated total Massachusetts population of the Blackstone River watershed was 340,297, Deerfield River watershed is 31,337 and Merrimack watershed is 390,887. We were unable to control bias due to uneven distribution of population density. Although the population of each watershed changed over the duration of the study, the

2000 population was used for per capita estimates. The reason for doing these calculations was that our town based health data was only for Massachusetts and so it was necessary to know exactly what part of the watershed population lived in Massachusetts. The other reason for doing it is that we need to know the exact ratio of actual population in watersheds and reported *Giardia* cases.

3.4 Precipitation and Streamflow Data

The base maps were acquired from MassGIS (mass.gov, 2006). Hydrometric data were downloaded from the U.S. Geological Survey (USGS, 2006) database from a gauge in each of the study watersheds. Daily precipitation and temperature were downloaded as ASCII character type data from the NOAA database archive at the National Climatic Data Center (NCDC) from cumulative mean of 3 station for each of the study watersheds ("National Oceanic and Atmospheric Administration", 2006b).

The downloaded data included a summary of daily measurements such as maximum/minimum temperatures, precipitation, and snowfall/snow depth. Some stations had additional data such as evaporation and soil temperature. These data generally undergo automated and manual quality control.

Station based information was collected from noaa.gov; station locator accessed on April, 2007 ("National Oceanic and Atmospheric Administration", 2006a). For the Merrimack River watershed, precipitation and temperature information were collected for the station located in city of Lowell in Middlesex county (42°39'N / 71°22'W), Haverhill of Essex county (42°46'N / 71°04'W)and Lawrence of Essex county (42°42'N / 71°10'W). This information was collected as digital ASCII files either on a daily or

monthly basis. Date range was selected from 1st January 1988 to 31st October, 2006. The period of selection was made to match the available health data.

3.5 Public Health Data

Reported cases of gastrointestinal illness were requested from the Commonwealth of Massachusetts, Department of Public Health (DPH). Under the Epidemiology Program of DPH; Reportable Communicable Diseases, Office of Integrated Surveillance and Information Service a request for reported gastrointestinal illnesses for the last two decades was submitted. The reason for choosing such a long period of time was to understand disease trends for a longer period of time. Due to the limitation in the availability of digital data from the Public Health Department, only data from January 1988 to October 2006 (almost a 19 year time period) was available. The personally identifiable data was de-identified manually, and used in subsequent analysis after review and approval by the Human Research Protection Office (HRPO), IRB at the University of Massachusetts. Datasets of confirmed human cases of giardiasis, shigellosis, cryptosporidiosis, campylobacteriosis, and shiga toxin-producing *E. coli* were obtained from the Massachusetts Department of Public Health with city/town and zip code for the years 1988 to 2006.

The information on infectious disease surveillance by the Department of Public Health is conducted by local health departments, including but not limited to public health nurses, health agents, sanitarians, and administrative staff. In some cases no case report form is submitted. Missing health data over a large period of time increased the chance of bias in our total number of reported cases because we assumed that no data

meant there were no cases during that specific time period. However, there is no opportunity to correct the missing information.

Original reports of disease come from laboratories, physicians, etc (LaPorte, 2007). In addition, reports of all identified waterborne disease outbreaks for the same period were obtained. Laboratory results are entered into the surveillance system and forwarded to local boards of health for investigation. Outbreaks investigations are conducted by state epidemiologists and local boards of health. Of the thousands of confirmed cases of illness, very few are associated with documented waterborne disease outbreaks. No information on quality control was reported between 1988-2006 (personal communication from Surveillance Epidemiologist, Office of Integrated Surveillance and Informatics Services, Massachusetts Department of Public Health, September 2007).

Confirmed etiologic agents from the outbreaks included *Legionella pneumophila*, *Giardia*, *Cryptosporidium*, and *Shigella sonnei*. Public health data were imported into MS Access (Microsoft Corp., Redmond, WA). Following the identification of cities or towns within the watershed, a query was run to determine the numbers of cases of illness for all cities or towns in each watershed over the period of study.

3.6 Statistical Analysis

Statistical analysis has been performed using SPSS 14.0 (SPSS Inc., Chicago, Illinois). Student's t test was used to compare differences between watersheds that are characterized as urban as compared to agricultural. The Merrimack River Watershed was compared with the Blackstone River watershed using a t test to determine the effect of the CSOs on numbers of *Giardia* cases. Cross correlation is a function in SPSS software

which allows comparing correlation between date specific climate data and reported disease data.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Comparison of three watersheds (Urban Vs Rural)

Of interest in this study was a comparison of distribution of cases of confirmed giardiasis among 3 specific watersheds: Blackstone, Deerfield and Merrimack. These sites were of interest for their representation of urban (Blackstone), rural (Deerfield) and CSO in drinking water system(Merrimack) respectively. Unlike Merrimack watershed the CSO in Blackstone watershed is not in drinking water system.

These groups were compared two at a time to permit assessments of rural versus urban, rural versus CSO and urban versus CSO. For these analyses, two sample t-test were performed. The dependent variable for these analyses was the number of confirmed cases per 100,000.

4.1.1 Student t-test

Using SPSS, student's t-tests were performed to evaluate the effects of land use and CSOs on human cases of giardiasis. It was found that there was no significant difference (P = 0.546) between the urban watershed (Blackstone River watershed) and the rural watershed (Deerfield River watershed) with regards to pathologically confirmed cases of giardiasis.

However, the Merrimack River watershed, which is a watershed with drinking water supplies impacted by combined sewer overflows, had significantly higher numbers of confirmed cases of *Giardia* infection (P=0.003) as compared to the urban watershed (Blackstone River watershed). Figure 8 represents the total annual confirmed *Giardia* cases in the Blackstone (BS), Deerfield (DF), and Merrimack (MMc) River watersheds.

This figure visually shows higher number of giardiasis cases in the Merrimack watershed. But there is a possibility that a higher number of cases may be related to a higher population density within the watersheds. In order to overcome that bias we calculated annual Giardiasis cases in per 100,000 populations for each of the watersheds. Figure 9 represents the total annual number of cases per 100,000 people for the three watersheds. As seen in the figure, there is an increase in the number of giardiasis cases in the Deerfield watershed in comparison to the Blackstone watershed when calculated per 100,000 populations which could be due to some reporting bias. So, the raw number of giardiasis cases (before normalizing with 100,000 populations) might have been influenced by the larger watershed area and population density (Figure 8). Also there is a possibility of reporting bias. However, the Merrimack Watershed continues to show high number of giardiasis cases even after normalized per 100,000 populations.

4.1.2 Chi square test of equality of proportion:

In this study, the days of interest were January 1, 1988 through October 31st, 2006. Available data for this period were comprised of the number of reported cases on those days for which number of reported cases is 1 or more. Thus, days for which cases are either zero or not reported are indistinguishable. Therefore, for these analyses, it was assumed that not reported was equivalent to zero cases.

The number of total monitoring days for all 3 of the watersheds is 6879. The days of zero or no report of giardiasis are 6168 for Blackstone, 6801 for Deerfield and 5724 for Merrimack watersheds. Respectively the numbers of days with report of 1 or more cases are 711 (10.33%), 78 (1.13%), 1155 (16.79%) for Blackstone, Deerfield and Merrimack watersheds respectively.

Our null hypothesis was there is no difference between the number of monitoring days with 0/no reporting and 1 or more reporting days in 3 watersheds. The result shows Chi square value of 998.2272 and the P <.0001 with the degree of freedom 2. So the null hypothesis can be rejected. That means monitoring days with 0 or no reporting and 1 or more reported cases are different in the 3 watersheds.

4.1.3 Influence of climatic conditions on giardiasis occurrence in the Merrimack River watershed

The Merrimack River watershed has the highest incidence of giardiasis, therefore it was chosen as our final case study. Detailed analysis of the influence of precipitation, temperature and stream flow on human *Giardia* cases was performed for the Merrimack River watershed. Both long term stream flow and long term average precipitation data of Merrimack River watershed are presented respectively in Figures 10 (from USGS) and Figure 11 (from NOAA) for gauges at Lowell, a city within the watershed. As can be seen from Figure 10 stream flow is greatest in the spring when snowmelt occurs, declines during the summer, and then increases in the fall when precipitation increases. October is the month with the highest average total monthly precipitation. To see the nature and significance of variations in confirmed cases of *Giardia* with season by calendar month we observed long term (1988-2006) averages of total monthly confirmed cases (Figure 12). The result shows that the month of August has the highest numbers of reported cases of *Giardia*. The peak of *Giardia* cases in the summer is consistent with the hypothesis that recreational waters are a primary route of transmission for the parasite although it is not possible to determine the actual sources of illness.

It is also interesting to note that among months for which no outdoor waterborne recreational exposure will likely occur, October has the highest number of confirmed

Giardia cases, and February, the lowest which is comparable with Figure 12. October has the greatest amount of precipitation, and February, the least. Streamflow at a monthly time scale is not related to incidences of confirmed *Giardia* cases. The reasons for a lack of relationship between streamflow and illness appears to be that exposure to pathogens in the environment are greatest during the summer months when streamflow is lowest. Furthermore, illnesses are low in the spring, when streamflow is highest. However, it is possible that some of these infections were acquired by other routes of transmission such as food or person to person contact. A cross correlation was performed between monthly precipitation and *Giardia* cases in the Merrimack River watershed (Table 7). The +- lag 12 represents 12 months. Very little positive correlation was found (Fig: 13). No significant cross correlations between precipitation and *Giardia* cases were observed for daily or weekly values (Table 8). A possible reason may be that too many days and weeks have zero *Giardia* cases or amount of precipitation. If more than 70% of the data is zero then it could bias the data and change the strength of the data.

When a correlation was performed between monthly temperature and *Giardia* cases in Merrimack watersheds a periodic rhythmic positive correlation was found (Table 9 and figure 15) which was consistent with our expectation of seeing seasonality in *Giardia* cases. Auto correlation of monthly *Giardia* cases also shows a seasonal trend over the year (Table 10, Figure 16).

4.1.4 Regression model

To get a better sense if any significant relationship exists between precipitation and giardiasis cases in the Merrimack River watershed, a regression model was created using the SAS software. The data showed a high degree of scatter and the relationship

between precipitation and *Giardia* cases was non-significant (Fig: 17) (P = 0.9590; R^2 =0.00000). However a regression model between temperature and giardiasis data in the Merrimack River watershed had a significant P value (Fig: 18) (P = 0.0001; R^2 is 0.0623). This suggests that the occurrence of *Giardia* cases are related with temperature, so more *Giardia* cases were observed when temperatures were higher.

It is interesting to note that *Giardia* dies off more rapidly at higher temperatures (Olson, Goh, Phillips, Guselle, & McAllister, 1999) and thus temperature is not related to the better survival of the pathogen. Rather, the higher number of cases may be related to differences in human activities when temperatures are higher such as being more likely to make use of water bodies for recreation.

Among three of the watersheds, the Merrimack has the highest frequency of disease. Combined sewer overflows in a drinking water source may have an impact on the number of cases of gastrointestinal illnesses. Additional cases may also be related to the urbanization of the Merrimack watershed. The CSO effect in drinking water and higher number of giardiasis can be confirmed if we can compare another identical watershed with CSO in a rural structure. This is virtually impossible because CSOs are urban constructs.

Outside of the summer outdoor water recreation period, the month of October has the highest number of *Giardia* cases which may be related to peak precipitation (not stream flow). Amin (2002) reported no seasonality in *Giardia sp.* infections in the United States (Amin, 2002). However, a significant seasonal variation was observed in Canada by Laupland and Church (2005) in *Giardia sp.* with a peak in late summer to early fall (Laupland & Church, 2005), which is similar to our results Greig et al. (2001)

found a higher mean rate of Giardiasis in urban populations and an increased incidence that peaks in late summer or early fall which is similar to our results (Greig et al., 2001).

Recreational activities such as camping go beyond the summer period, and individuals who are camping may be more likely to drink untreated water. Consultation with the Department of Conservation and Recreation (DCR) website confirms that most of the camping sites in Massachusetts are open through October. That might have some influence in the number of giardiasis cases in the early fall.

4.2 Extreme Events

In the last century, mean daily temperatures in the US have increased about 1°F. Warmer air holds more moisture, and has changed the hydrologic cycle in the United States. This increases in the cloud cover and also the total precipitation as a result causing extreme precipitation events to increase (Curriero, Patz, Rose, & Lele, 2001). The extreme event increase chances of surface runoff, inadequate water treatment and thus increase the possibility of more microbial Giardia cases outbreak.

4.2.1 Extreme Rain days

In order to analyze the effect of extreme precipitation events on the number of reported giardiasis cases was studied by statistically. From the total of 6860 data points (from Jan ‘88 to Oct ’06) only the upper 10% precipitation dates were selected. The reason to choose upper 10% was support from the literature study.

Since a large number of days had no rain I decided to choose our control group as the days without any rain events. Despite the large number of days without rain, when came to use no rain days, there are only 2 week runs with total precipitation under 30cm.

So I decided to ignore the days with out rain and choose the extreme event (top 10% precipitation) only within the days which have rain events (whether big or small). Now calculating the top 10% precipitation gives the "extreme" rain events which are 95 mm or more in a single day. The number of dates in upper 10% precipitation is 228.

Thus for the case group we decided to choose **15 days after** big rain periods. The reason I choose 15 days is because that the time length prime period for giardiasis incubation. Though some of the literature supports incubation period up to 25 days (EPA, 2000a; Furness, Beach, & Roberts, 2000).

4.2.2 Control group / Non extreme rain days

The control group of data was selected from the bottom 10% of the precipitation percentile. As mentioned previously 2/3 of the original non consecutive days are without any rain Therefore, choosing the bottom 10% as a control group may not make any sense.

So for the control group it was decided to choose **15 days before** the largest days of rain (extreme rain event), only if that period of time doesn't overlap with incubation period of another extreme rain day. The 15 days before and after extreme rain days come around 4244 data point. But as arrived from the data there is a high proximity of extreme rain days followed by yet another extreme rain day. So there are too many overlapping periods. They were cleaned manually looking before and after two follow-up periods. For example, if two rain events occurred back to back then the second one is overlooked. The reason is because in that case the first event and its follow-up period remain unhampered (regardless of the rain amount). But the second event has to be removed because then its 15 days previous no rain control period is actually the follow up period of the first event.

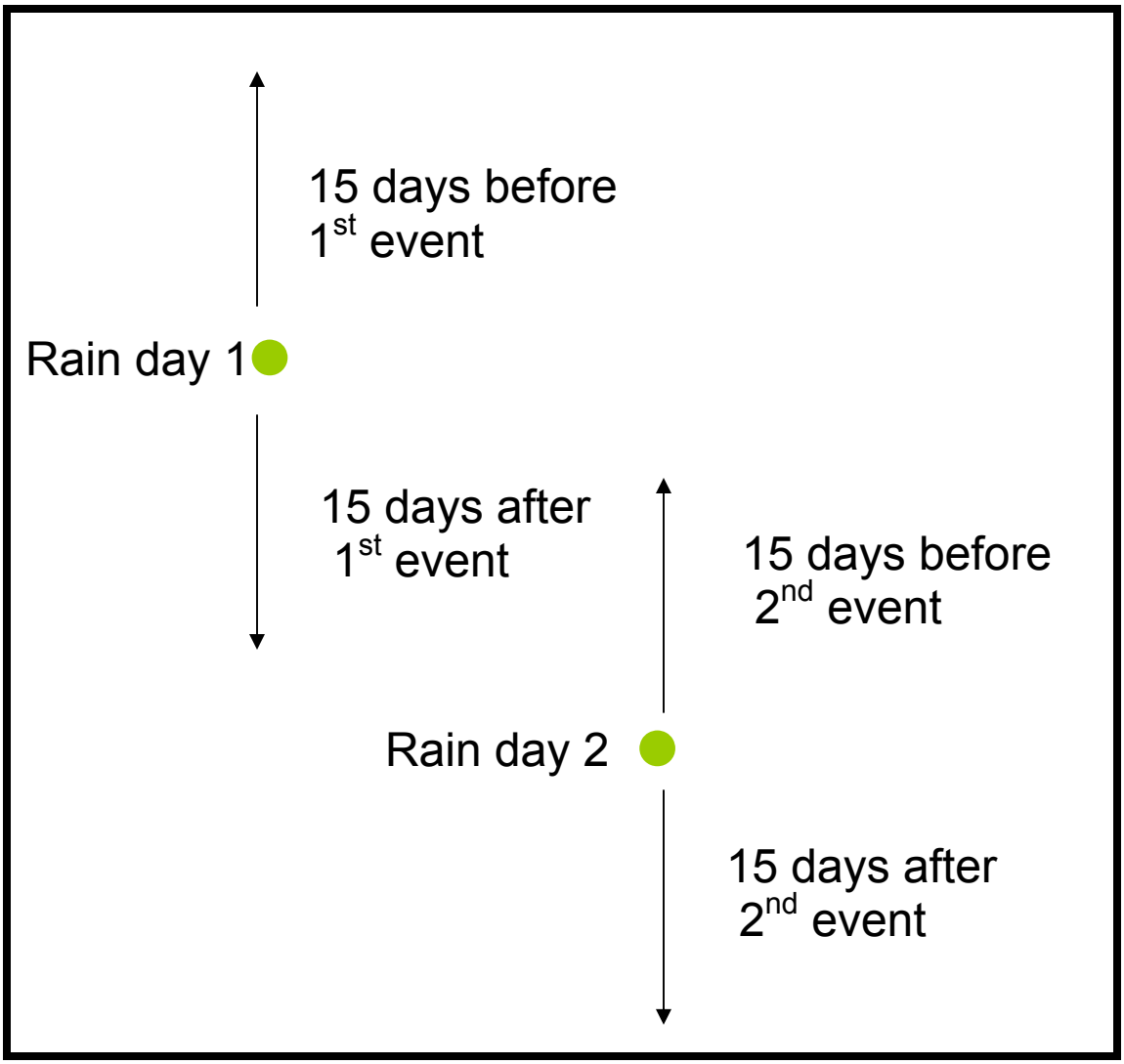


Figure 7: Overlapping Period between Two Extreme Rain Days

Ultimately, 50 extreme rain events were found of which before and after remain unaffected by another rain event.

A summary of total precipitation before and after period of the each extreme rain day and total giardiasis cases during the before and after follow-up period was calculated and compared. These data were exported to SPSS and a paired T test was run to compare the two groups of *Giardia* cases (total before and after 15 days of an extreme rain day). The p value of the two tailed paired T test is 0.899 which is not significant. That

concludes that an extreme rain day doesn't influence the occurrence of giardiasis in the following 2 week of period of time.

There is NO correlation between precipitation period before extreme rain day & precipitation period after the extreme rain day. The significant difference between precipitation before and after because some observations, where the total rain before is quite high - even higher than the total rain after the event.

After sorting two group according to the total amount of precipitation before an extreme rain day we eliminate the days where the total amount of rain before the extreme day is higher than the total amount of rain after. So any difference between these two sets of data where cumulative total of dataset earlier than extreme rain event is bigger than the cumulative total of dataset later than extreme rain event has been eliminated from consideration. The reason for eliminating these days (where the total amount of rain in previous 15 days is higher than the later 15 days) is so that they are not considered as extreme events. Then the high precipitation days are part of a bigger rain event. From our 50 extreme event days only 7 were eliminated for this reason. Then running a 'paired t test' between *Giardia* cases before rain events and cases after rain events gives the result of 'two tailed p value' 0.74 and correlation of 0.601. These results say that there is a strong correlation between cases before & after, but no difference between number of cases before and after. That means the data are strongly correlated so that when statistically significant differences are being tested, there are none. These two suggest that reported cases are somehow related to time, rather than to specific amounts of rain at that time – i.e. that during certain time periods more (or fewer) cases reported, regardless of rain events.

As seen from the data table, total precipitations of previous groups of data are often higher than the total precipitation of extreme rain and follow up 15 day data periods. The reason might be that instead of a single high rain day there are several moderate rain days in a single period of time which creates an extreme rain period. At the same time, the giardiasis data might have been underreported to Department of Public Health. For precipitation data we solely relied on the NCDC data. These data are being collected from different weather stations in MA. In the last two decades, innovations of science have improved climate measurement procedures and instruments in various ways (gillesen.nl, n.d). Since our data includes data from 1988, we can't eliminate the possibility of reporting bias from weather data as well. However, since precipitation measurements are fairly standard and easy to measure. Too much difference in measurements is not expected.

4.3 Figures and Tables

Table 6: In Three Watersheds Agriculture and Water, Land Use Area Distribution (in acer) from Attribute Table of Arcmap

	Total Area	Total area of Agri. in Acer or % of total area	Total area of water in Acer or % of total area
Blackstone watershed	214659.700	22848.123 or (10.64%)	4868.491 or (~2.26%)
Deerfield watershed	221807.700	19601.284 or (~8.83%)	1797.562 or (~0.81%)
Merrimack watershed	284334.9138	58.0409375 or (~0.02%)	6643.3 or (~2.33%)

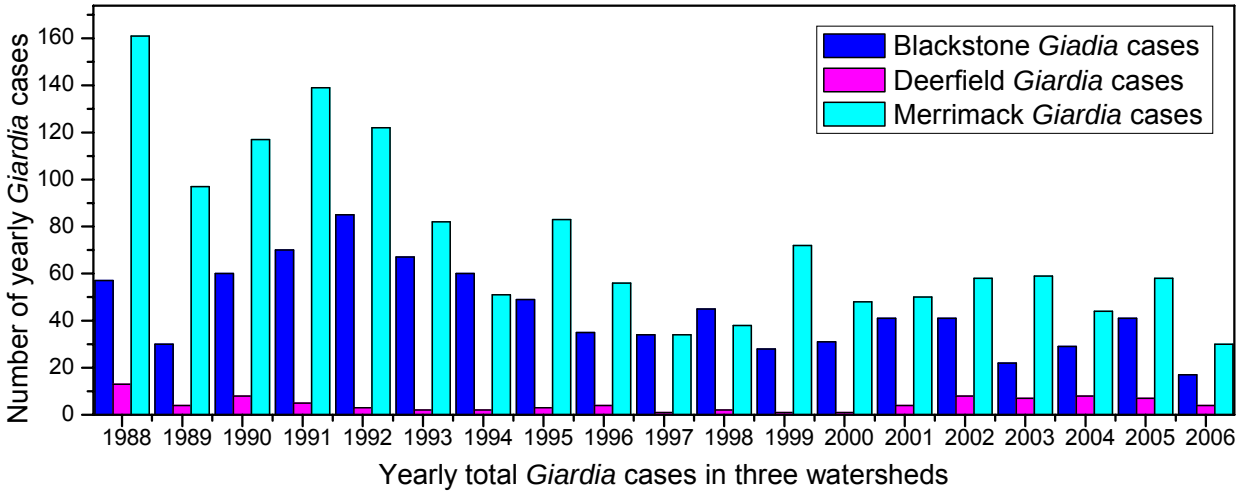


Figure 8. Before Normalization Total Annual Confirmed *Giardia* Cases in the Blackstone (BS), Deerfield (DF), and Merrimack (MMc) River watersheds.

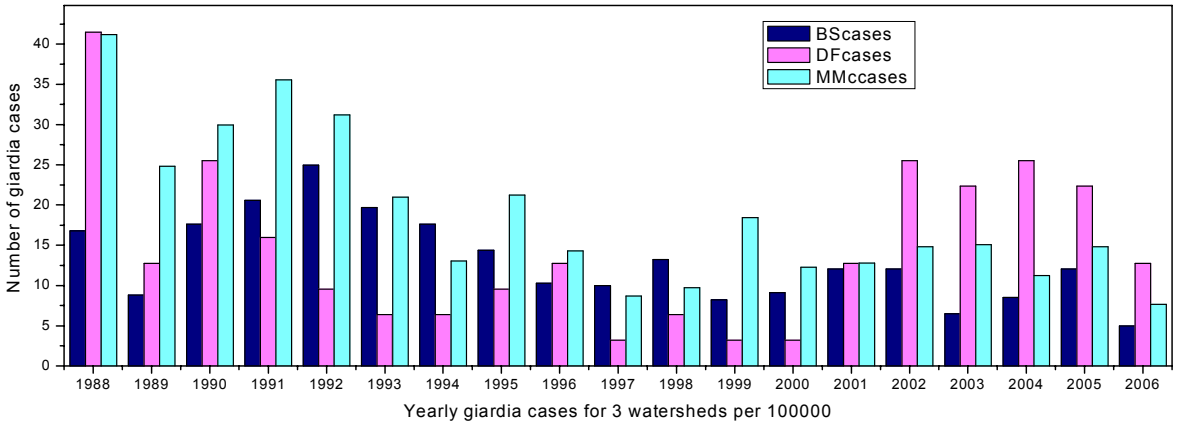


Figure 9. Total Annual Confirmed *Giardia* Cases per 100000 Populations in the Blackstone (BS), Deerfield (DF), and Merrimack (MMc) River watersheds.

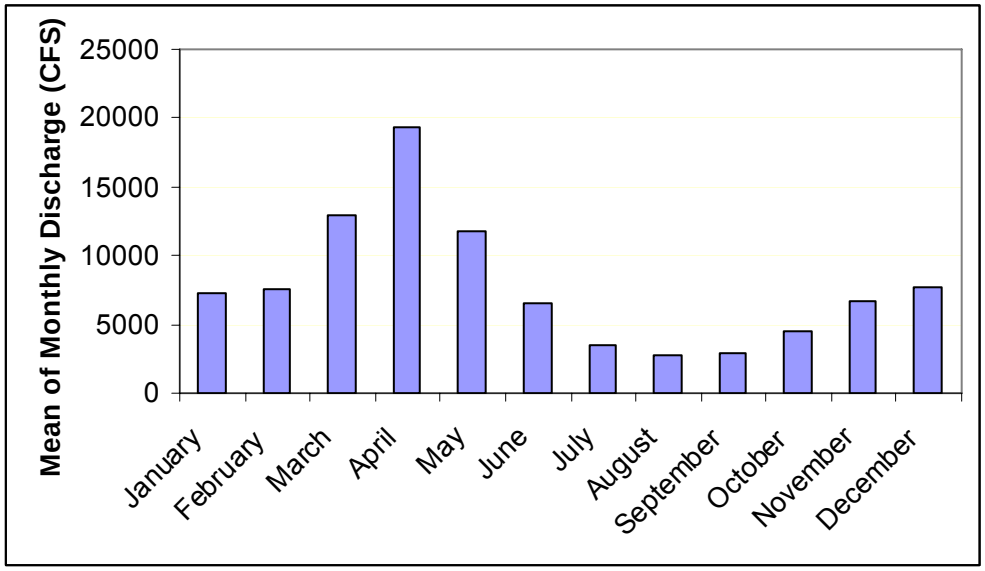


Figure 10. Mean of Monthly Discharge of the Merrimack River at Lowell, Massachusetts (USGS 01100000, 1924-2006).

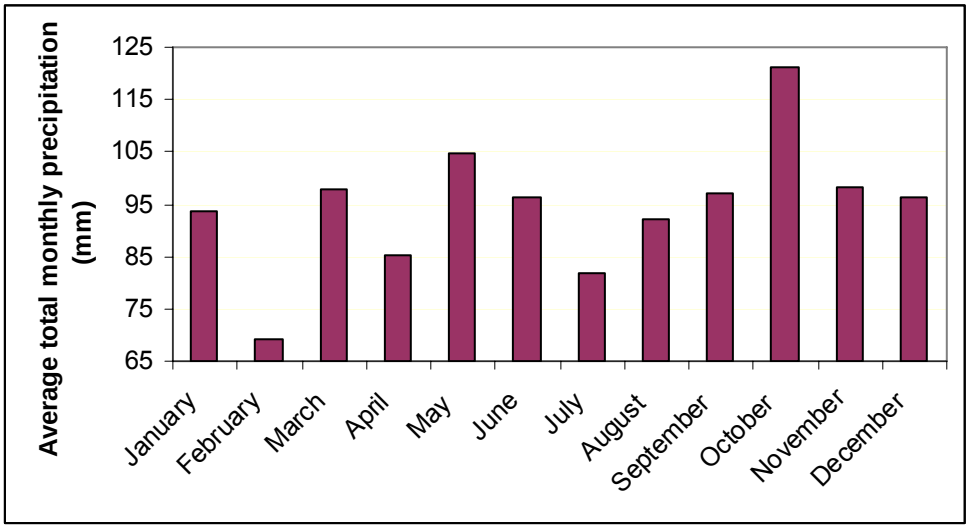


Figure 11. Average Total Monthly Precipitation in Lowell, Massachusetts (NOAA 194313, 1988-2006).

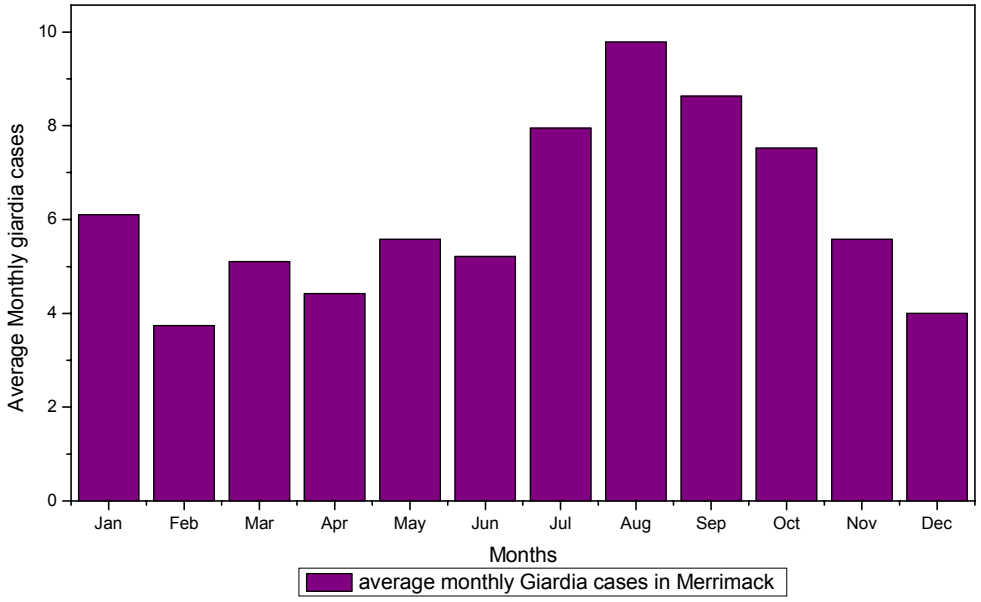


Figure 12. Average Confirmed Monthly Cases of *Giardia* in the Merrimack River Watershed (1988-2006). However source (whether food borne/waterborne) is unknown.

Table 7: Cross Correlations of Monthly Precipitation and Monthly *Giardia* Cases in Merrimack Watershed

Series Pair: Monthly Total precipitation with Merrimack Watershed Monthly *Giardia* cases. Lag +/- 12 represents 12 months in each year. Positive value in Cross correlation represents positive correlation which here very few in number.

Lag	Cross Correlation	Std. Error(a)
-12	.034	.068
-11	.104	.068
-10	.045	.068
-9	.041	.068
-8	.020	.068
-7	-.082	.068
-6	-.085	.067
-5	-.113	.067
-4	-.143	.067
-3	-.004	.067
-2	.063	.067
-1	-.001	.067
0	-.003	.067
1	-.087	.067
2	-.091	.067
3	-.104	.067
4	-.023	.067
5	-.112	.067
6	-.139	.067
7	-.098	.068
8	-.060	.068
9	-.097	.068
10	.024	.068
11	-.001	.068
12	-.018	.068

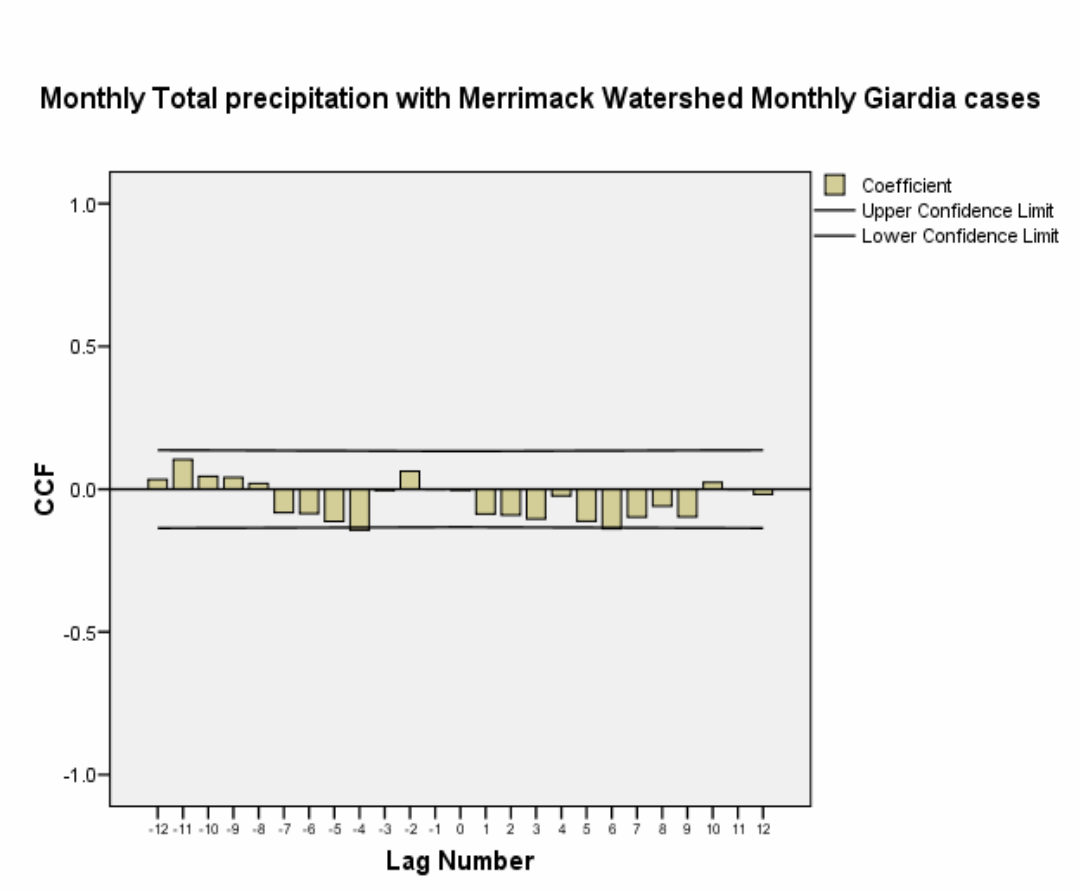


Figure 13: Cross Correlation between Monthly Precipitation and *Giardia* Cases in Merrimack Watersheds.
(very little positive correlation)

Table 8: Cross Correlations between Daily Precipitation and Daily Reported *Giardia* Cases in Merrimack Watershed

Series Pair: Precipitation with Reported case in Merrimack Series Pair: Daily Total precipitation with Merrimack Watershed daily *Giardia* cases. Lag +/- 15 represents 15 days incubation period. Positive value in Cross correlation represents positive correlation which here also very few in number.

Lag	Cross Correlation	Std. Error(a)
-15	-.001	.012
-14	.005	.012
-13	.000	.012
-12	.004	.012
-11	.010	.012
-10	-.008	.012
-9	.001	.012
-8	-.006	.012
-7	.001	.012
-6	.018	.012
-5	-.005	.012
-4	-.002	.012
-3	-.025	.012
-2	-.017	.012
-1	.008	.012
0	-.003	.012
1	-.020	.012
2	-.007	.012
3	.010	.012
4	-.006	.012
5	-.012	.012
6	-.009	.012
7	.017	.012
8	.016	.012
9	.002	.012
10	.006	.012
11	.007	.012
12	-.014	.012
13	-.009	.012
14	-.019	.012
15	.004	.012

Table 9: Cross Correlations of Monthly Temperature and Monthly *Giardia* Cases in Merrimack Watershed

Series Pair: Monthly mean temperature with reported monthly *Giardia* cases in Merrimack Watershed. Lag +/- 14 represents 14 days of incubation period. Positive value in Cross correlation represents positive correlation which here is significant in number.

Lag	Cross Correlation	Std. Error(a)
-14	-.062	.069
-13	.107	.069
-12	.240	.068
-11	.282	.068
-10	.271	.068
-9	.146	.068
-8	.013	.068
-7	-.159	.068
-6	-.293	.067
-5	-.350	.067
-4	-.319	.067
-3	-.211	.067
-2	-.042	.067
-1	.112	.067
0	.250	.067
1	.320	.067
2	.292	.067
3	.168	.067
4	.026	.067
5	-.132	.067
6	-.251	.067
7	-.313	.068
8	-.294	.068
9	-.193	.068
10	-.041	.068
11	.119	.068
12	.234	.068
13	.296	.069
14	.253	.069

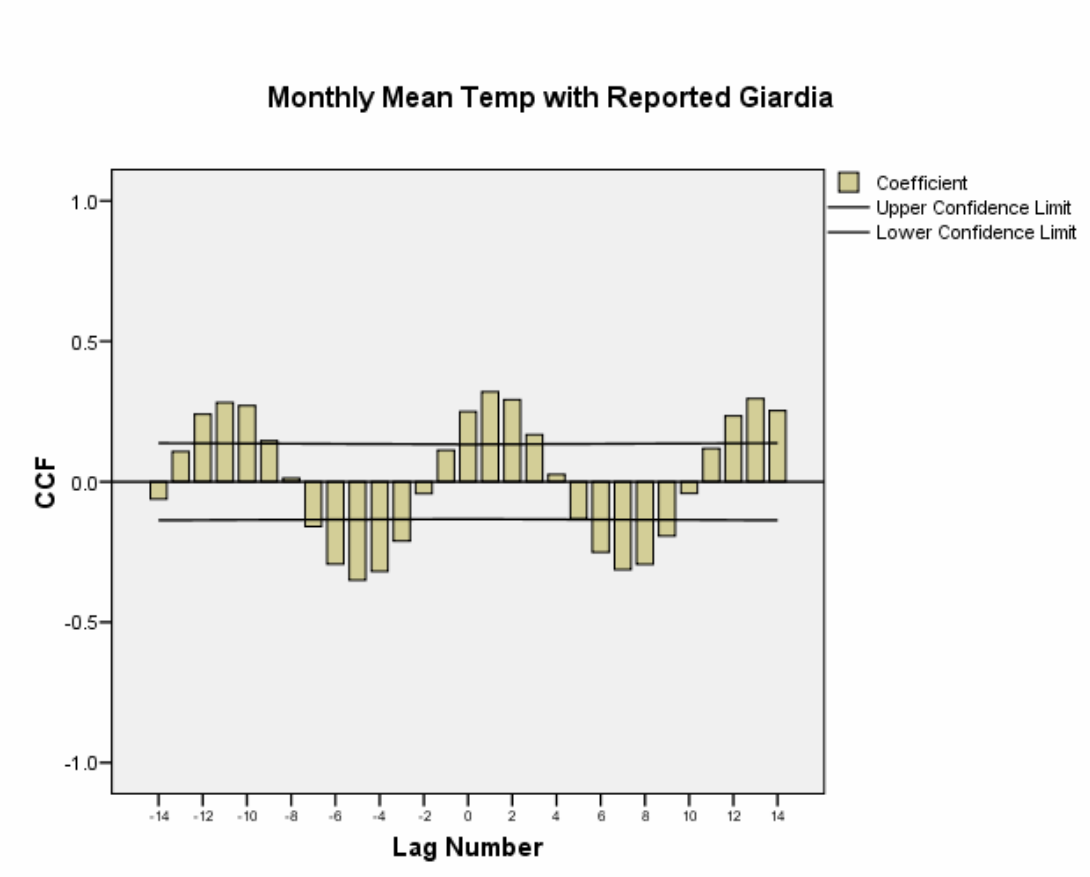


Figure 14: Cross Correlation between Monthly Temperature and *Giardia* Cases in Merrimack Watershed
(Rhythmic positive correlation)

Table 10: Autocorrelations of Monthly Reported *Giardia* Cases in Merrimack Watersheds

Series: Monthly reported *Giardia* cases in Merrimack Watershed. Lag + 16 represent 1 day of exposure + 15 days of incubation period. Positive value in Cross correlation represents positive correlation which here is significant in number.

	Autocorrelation	Std. Error(a)	Box-Ljung Statistic		
Lag	Value	df	Sig.(b)	Value	df
1	.559	.066	71.601	1	.000
2	.442	.066	116.564	2	.000
3	.344	.066	143.877	3	.000
4	.283	.066	162.446	4	.000
5	.204	.065	172.133	5	.000
6	.205	.065	182.014	6	.000
7	.163	.065	188.233	7	.000
8	.228	.065	200.473	8	.000
9	.245	.065	214.728	9	.000
10	.286	.065	234.255	10	.000
11	.257	.065	250.139	11	.000
12	.323	.064	275.256	12	.000
13	.257	.064	291.230	13	.000
14	.213	.064	302.237	14	.000
15	.124	.064	306.000	15	.000
16	.096	.064	308.257	16	.000

a The underlying process assumed is independence (white noise).
b Based on the asymptotic chi-square approximation.

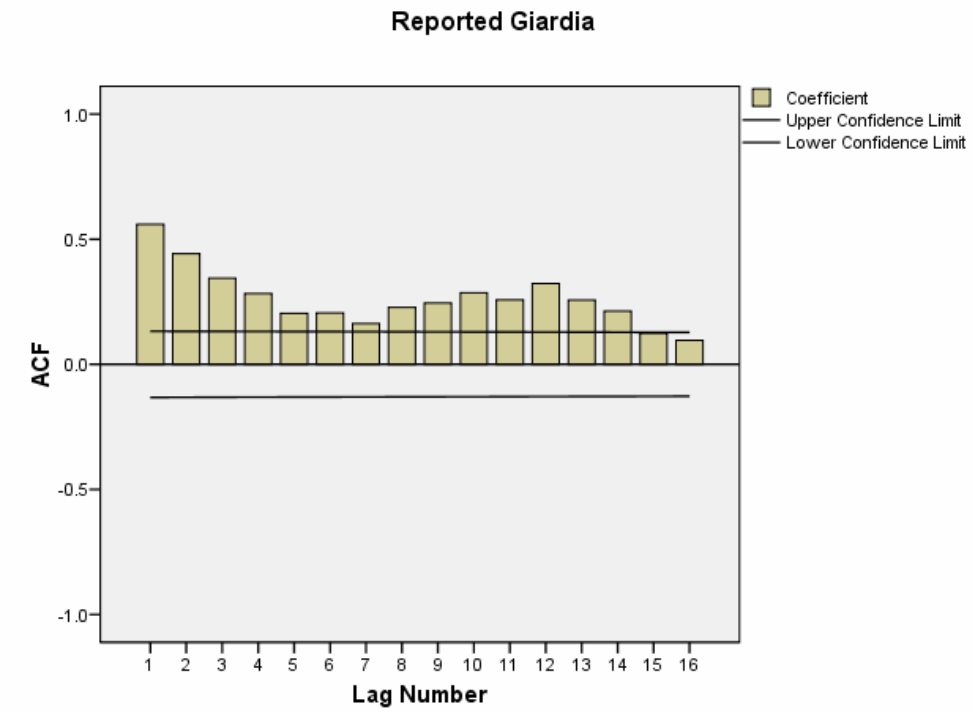


Figure 15: Auto Correlation of Monthly Reported *Giardia* Cases in Merrimack Watershed

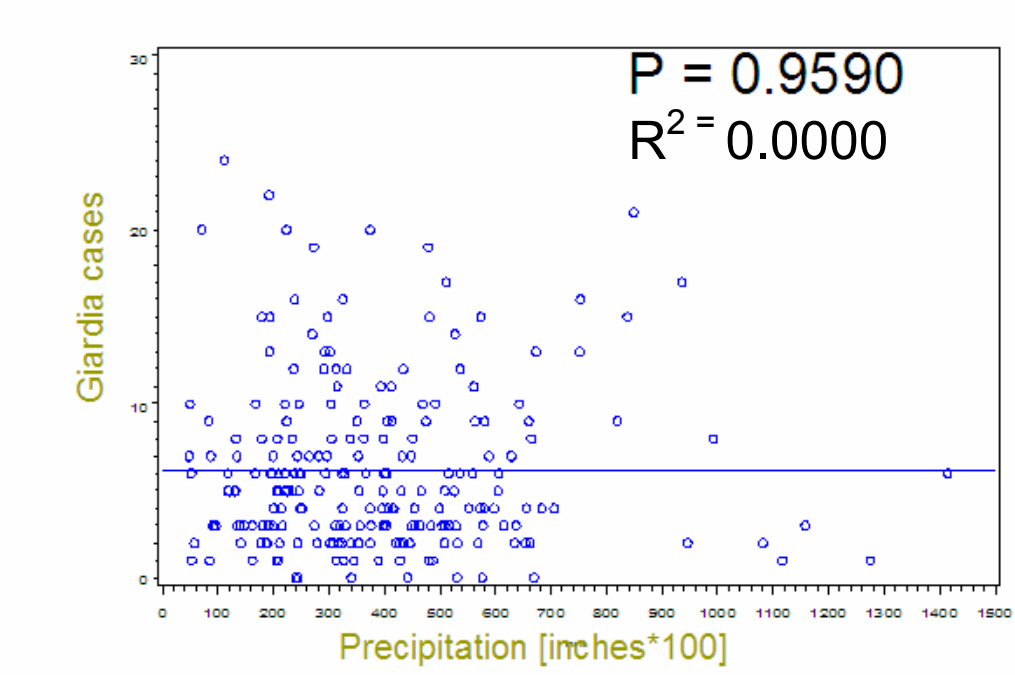


Figure 16: Regression Model between Monthly Precipitation and *Giardia* Cases in Merrimack Watershed

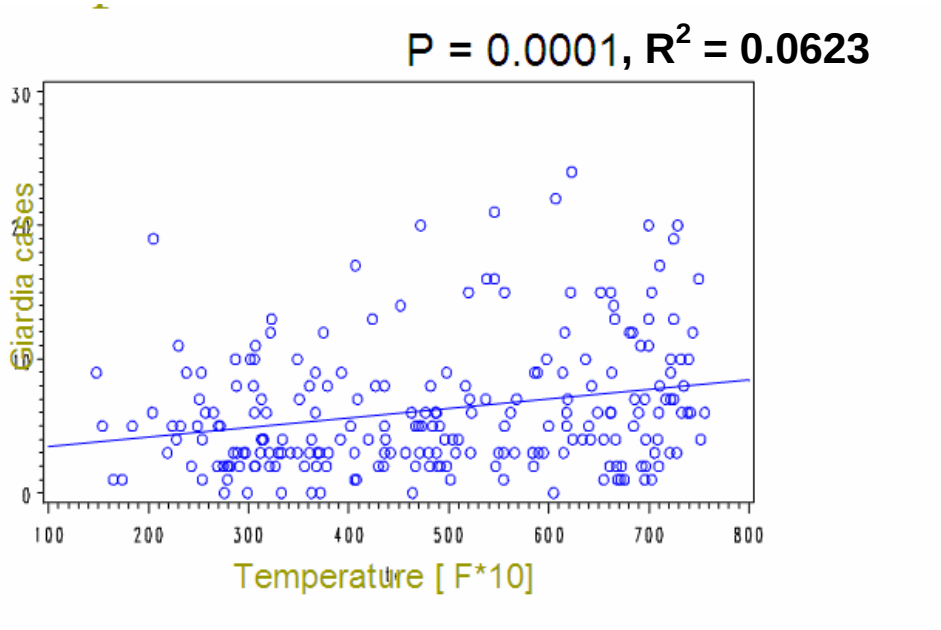


Figure 17: Regression Model between Monthly Temperature and *Giardia* Cases in Merrimack Watershed

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Most of the documented waterborne disease outbreaks in Massachusetts were from recreational waters that included both fresh lake/pond water or swimming pool/hot tub waters. A limitation of our data set is that the majority of the reported disease data is without any information with regards to the causative media or source of the infection. Very little information is available with regards to the origin of these reported illnesses, such as whether these cases are food borne or waterborne. However, these kinds of limitations in health department data are common. Health Canada reported 4200 cases of giardiasis and 1600 cases of cryptosporidiosis in Canada in the year of 2001, but the proportion of cases that was waterborne is unknown (CCDR, 2002; Charron et al., 2004). Human cases of gastrointestinal illnesses are typically underreported (Andersson & Bohan, 2001). Therefore, the information bias may have an influence on the results. Individuals may also acquire illnesses outside of their watershed boundaries.

Another major problem of getting accurate data of gastrointestinal disease is under reporting. In most of the cases the available disease data from Massachusetts Department of Public Health is collected from self reporting methods. Therefore, the chance of reporting biases can not be avoided. Such type of bias is not very uncommon with gastrointestinal disease related research. Mohanty (1997) reported in 1997 in Hyderabad, a city of India the original number of gastrointestinal cases were two times higher in compare to under reporting of Disease (Bartram, Fewtrell, & Stenstrom, 2001).

No significant difference ($P = 0.546$) between the urban watershed (Blackstone River watershed) and the rural watershed (Deerfield River Watershed) has been found with regards to confirmed cases of giardiasis. It is possible that urban wastewater

pollution of the Blackstone River watershed and rural farm practices and animal husbandry are putting same amount of stress on their water resources.

The Merrimack River watershed had significantly higher numbers of confirmed cases of *Giardia* infection (P=0.003) as compared to the Blackstone River watershed which may have come from its contaminated drinking water source ths is impacted by CSOs. The confirmation of the CSO impact has not been influenced by the urbanization of Merrimack, the confirmation is only possible if it can be compared with another watershed in rural area with CSOs and all the same criteria. However this kind of watershed is not available in Massachusetts and therefore could not be tested. But one part of our objective is confirmed that a link exists between waterborne diseases and watershed conditions and characteristics and impact of land use has some relation with of reported *Giardia* cases in Massachusetts.

Seasonal trends are one of the major characteristics of gastrointestinal illnesses (Kuhn, Campbell-Lendrum, Haines, & Cox, 2005). There is evidence of seasonal trends in microbial pathogen occurrence in the environment (Ong, Moorehead, Ross, & IsaacRenton, 1996), the public health significance of which is unknown. However, because of the costs associated with pathogen monitoring, data are often not collected for long enough periods to properly determine the seasonality of pathogen occurrence.

However while October has the highest average total monthly precipitation but the long term (1988-2006) averages of total monthly cases show that the month of August has the highest numbers of reported cases of *Giardia.*

Between monthly temperature and *Giardia* cases in Merrimack watersheds a periodic correlation was found (Table 9 and figure 15) that is consistent with our expectation of seeing seasonality in *Giardia* cases. The peak of *Giardia* cases in the

summer is consistent with the hypothesis that recreational waters are a primary route of transmission for the parasite.

Our results show there is a confirmed strong correlation between *Giardia* cases before & cases after an extreme rain event (two tailed p value 0.74 and correlation of 0.601), but no difference between number of cases before and after. This suggests that reported cases are somehow related to time, rather than to specific amounts of rain.

As human behavior (winter-summer differences) and recreational patterns change over the seasons, seasonal differences of human behavior may be contributing to exposures to waterborne pathogens. This research enlightens the seasonal trends of reported gastrointestinal diseases depending on seasonal use of water in selected watersheds in Massachusetts. Furthermore, results show that the human population in watersheds with drinking water supplies impacted by combined sewer overflows is at a greater risk for exposure to *Giardia*.

APPENDIX

SUPPORTIVE MATERIAL : INDICATOR – PATHOGEN RELATIONSHIP

Table 11: Indicators and Pathogens Relation in Fresh Water
(A collaborative work with Jianyong Wu)

Indicator	Pathogen	Water Type	Correlation	Correlation Method	Source
Thermotolerant coliforms	*Giardia*	Drinking water (source water)	Significant ($P<0.05$)	Spearman correlation	Hachich *et al.* (2004)
Fecal streptococci	*Giardia*	Drinking water (source water)	Significant ($P<0.01$)	Spearman correlation	Hachich *et al.* (2004)
C. perfringens	*Giardia*	Drinking water (source water)	Significant ($P<0.01$)	Spearman correlation	Hachich *et al.* (2004)
Total coliforms	*H. pylori*	Groundwater	No significant correlation	χ^2 test	Hegarty *et al.* (1999)
E. coli	*H. pylori*	Groundwater	No significant Correlation	χ^2 test	Hegarty *et al.* (1999)
Fecal coliforms	*Giardia*	Source water	Significant ($P<0.01$)	Regression	LeChevallier et al. (1991)
Total coliforms	*Giardia*	Source water	Significant ($P<0.01$)	Regression	LeChevallier et al. (1991)
Fecal coliforms	*Cryptosporidium*	Source water	Significant ($P<0.05$)	Regression	LeChevallier et al. (1991)
Total coliforms	*Cryptosporidium*	Source water	Significant ($P<0.01$)	Regression	LeChevallier et al. (1991)
Total coliforms	*Giardia*	Drinking water	Significant ($P<0.01$)	Spearman correlation, Logistic regression	Payment et al. (2000)
Total coliforms	*Cryptosporidium*	Drinking water	Significant ($P<0.01$)	Spearman correlation, Logistic regression	Payment et al. (2000)
Total coliforms	Human enteric viruses	Drinking water	Significant ($P<0.01$)	Spearman correlation, Logistic regression	Payment et al. (2000)

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Fecal coliforms	*Giardia*	Drinking water	Significant (P<0.01)	Spearman correlation, Logistic regression	Payment et al. (2000)
Fecal coliforms	human enteric viruses	Drinking water	Significant (P<0.01)	Spearman correlation, Logistic regression	Payment et al. (2000)
C perfringens	*Giardia*	Drinking water	Significant (P<0.01)	Spearman correlation, Logistic regression	Payment et al. (2000)
C perfringens	*Cryptosporidium*	Drinking water	Significant (P<0.01)	Spearman correlation, Logistic regression	Payment et al. (2000)
C. perfringens	Human enteric viruses	Drinking water	Significant (P<0.01)	Spearman correlation, Logistic regression	Payment et al. (2000)
C. perfringens	Human enteric viruses	Drinking water (Raw)	Significant (P<0.01)	Spearman correlation	Payment and Franco(1993)
C. perfringens	*Giardia*	Drinking water (Raw)	Significant (P<0.01)	Spearman correlation	Payment and Franco(1993)
C. perfringens	*Cryptosporidium*	Drinking water (Raw)	Significant (P<0.01)	Spearman correlation	Payment and Franco(1993)
Coliphages	Human enteric viruses	Drinking water (Raw)	No significant correlation	Spearman correlation	Payment and Franco(1993)
C. perfringens	Human enteric viruses	Drinking water Settled	Significant (P<0.01)	Spearman correlation	Payment and Franco(1993)
C. perfringens	*Giardia*	Drinking water Settled	No significant correlation	Spearman correlation	Payment and Franco(1993)
C. perfringens	*Cryptosporidium*	Drinking water Settled	No significant correlation	Spearman correlation	Payment and Franco(1993)
Coliphages	Human enteric viruses	Drinking water Settled	Significant (P<0.01)	Spearman correlation	Payment and Franco(1993)
Coliphages	*Giardia*	Drinking water Settled	No significant correlation	Spearman correlation	Payment and Franco(1993)
Coliphages	*Cryptosporidium*	Drinking water Settled	No significant correlation	Spearman correlation	Payment and Franco(1993)

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C. perfringens	Human enteric viruses	Filtered drinking water	Significant (P<0.01)	Spearman correlation	Payment and Franco(1993)
C. perfringens	*Giardia*	Filtered drinking water	No significant correlation	Spearman correlation	Payment and Franco(1993)
C. perfringens	*Cryptosporidium*	Filtered drinking water	Significant (P<0.01)	Spearman correlation	Payment and Franco(1993)
Coliphages	Enteroviruses	Drinking water	Significant (P<0.01)	Spearman correlation	Stetler (1984)
Total coliforms	*Campylobacter*	Pond water	No significant correlation	N/A	Carter *et al.* (1987)
Fecal coliforms	*Campylobacter*	Pond water	No significant correlation	N/A	Carter *et al.* (1987)
Fecal streptococci	*Campylobacter*	Pond water	No significant correlation	N/A	Carter *et al.* (1987)
Thermotolerant coliforms	Noroviruses	River and lake water	Significant (P<0.05)	Spearman correlation	Hörman et al. (2004)
E. coli	*Cryptosporidium*	River and lake water	Significant (P<0.05)	Spearman correlation	Hörman et al. (2004)
E. coli	Noroviruses	River and lake water	Significant (P<0.05)	Spearman correlation	Hörman et al. (2004)
F-RNA phages	*Giardia*	River and lake water	Significant (P<0.05)	Spearman correlation	Hörman et al. (2004)
C. perfringens	*Campylobacter*	River and lake water	Significant (P<0.01)	Spearman correlation	Hörman et al. (2004)
Thermotolerant coliforms	*Cryptosporidium*	River water	Significant (P<0.05)	Spearman correlation	Lemarchand and Lebaron (2003)
Heterotrophic bacteria	*Giardia*	River water	No significant correlation	N/A	Hsu *et al.* (1999)
Total coliforms	*Giardia*	River water	No significant correlation	N/A	Hsu *et al.* (1999)
Fecal coliforms	*Giardia*	River water	No significant correlation	N/A	Hsu *et al.* (1999)
Heterotrophic bacteria	*Cryptosporidium*	River water	Significant (P=0.047)	N/A	Hsu *et al.* (1999)
Total coliforms	*Cryptosporidium*	River water	Significant (P=0.057)	N/A	Hsu *et al.* (1999)
Fecal coliforms	*Cryptosporidium*	River water	Significant (P=0.058)	N/A	Hsu *et al.* (1999)
Thermotolerant coliforms	*Salmonella*	River water	No significant correlation	Spearman correlation	Lemarchand and Lebaron (2003)

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Enterococci	*Cryptosporidium*	River water	Significant (P<0.05)	Spearman correlation	Lemarchand and Lebaron (2003)
Enterococci	*Salmonella*	River water	No significant correlation	Spearman correlation	Lemarchand and Lebaron (2003)
Total coliforms	*Salmonella*	Fresh water	Correlated	N/A	Sharma and Rajput (1996)
Fecal coliforms	*Salmonella*	Fresh water	Correlated	N/A	Sharma and Rajput (1996)
Fecal streptococci	*Salmonella*	Fresh water	Correlated	N/A	Sharma and Rajput (1996)
E. coli	*Cryptosporidium*	Lake and reservoirs	Significant (P<0.05)	Spearman correlation	Brookes et al.(2005)
Enterococci	*Cryptosporidium*	Lake and reservoirs	Significant (P<0.05)	Spearman correlation	Brookes et al.(2005)
Aerobic spores	*Cryptosporidium*	Lake and reservoirs	No significant correlation	Spearman correlation	Brookes et al.(2005)
Somatic bacteriophages	*Cryptosporidium*	Lake and reservoirs	Significant (P<0.05)	Spearman correlation	Brookes et al.(2005)
C. perfringens spores	*Cryptosporidium*	Lake and reservoirs	Significant (P<0.05)	Spearman correlation	Brookes et al.(2005)
E. coli	*P. aeruginosa*	Bathing water	Significant (P<0.05)	Pearson correlation	Wiedenmann et al 2006
E. coli	*Aeromonads*	Bathing water	Significant (P<0.01)	Pearson correlation	Wiedenmann et al 2006
Enterococci	*P. aeruginosa*	Bathing water	Significant (P<0.05)	Pearson correlation	Wiedenmann et al 2006
Enterococci	*Aeromonads*	Bathing water	Significant (P<0.05)	Pearson correlation	Wiedenmann et al 2006
C. perfringens	*P. aeruginosa*	Bathing water	No Significant	Pearson correlation	Wiedenmann et al 2006
C. perfringens	*Aeromonads*	Bathing water	Significant (P<0.01)	Pearson correlation	Wiedenmann et al 2006
Somatic coliphages	*P. aeruginosa*	Bathing water	No significant	Pearson correlation	Wiedenmann et al 2006
Somatic coliphages	*Aeromonads*	Bathing water	Significant (P<0.01)	Pearson correlation	Wiedenmann et al 2006

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