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Occurrence and Transport of Waterborne Viruses in Surface Water in Michigan and Associated Public Health Risks

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OCCURRENCE AND TRANSPORT OF WATERBORNE VIRUSES IN SURFACE WATER IN MICHIGAN AND ASSOCIATED PUBLIC HEALTH RISKS

by

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ABSTRACT

OCCURRENCE AND TRANSPORT OF WATERBORNE VIRUSES IN SURFACE WATER IN MICHIGAN AND ASSOCIATED PUBLIC HEALTH RISKS

By

Theng Theng Fong

Enteric viruses are excreted in high concentrations by humans and are frequently detected in fecally-contaminated surface water. Enteric viruses, such as adenoviruses and enteroviruses, are capable of causing a wide spectrum of diseases and are the main cause of water-transmitted gastrointestinal diseases in swimmers. The overall objective of this study was to develop an understanding of and quantify human health risks associated with the presence of viral pathogens, particularly adenoviruses, contracted during recreational activities at beaches affected by sewage inputs in the lower Grand River, Michigan. In this research, it is hypothesized that human adenoviruses are present in high concentrations in sewage and rivers receiving sewage effluents because wastewater treatments do not efficiently remove these viruses and the risks for virus exposure at Great Lakes beaches are elevated above an acceptable risk for recreational waters during the swimming season. In addition, virus transport and attenuation in rivers were evaluated in a tracer study using bacteriophage P22 as a surrogate for waterborne viruses. Adenovirus distribution and loading in sewage contaminated water were evaluated by determining concentrations of human adenoviruses (HAdVs) in the lower Grand River during dry weather and after combined sewer overflow (CSO) events. Human adenoviruses were detected from 6/20 river water samples collected during dry weather with concentrations ranging between 8×10^1 and 6.6×10^4 viruses/L (average: 7.8×10^3

viruses/L). Concentration of HAdVs in samples collected after CSO events ranged between 6×10^4 and 1.3×10^6 viruses/L (average: 5.4×10^5 viruses/L). As a part of exposure assessment and to model virus transport and inactivation after CSO events, a dual tracer study was conducted on a 40-km reach of the lower Grand River in Grand Rapids. From the tracer study, it was concluded that bacteriophage P22 is a suitable tracer for the complex surface water system with inactivation rates between 0.27 and 0.57 day^{-1}. A model for estimating virus transport and attenuation after discharge was developed based on data from the tracer study. With the field survey data and virus transport model, a quantitative microbial risk assessment was performed to evaluate the probability of infection or gastrointestinal disease resulting from incidental ingestion of contaminated recreational water at Lake Michigan beaches that receive input from the lower Grand River. Monte Carlo simulations were used to characterize uncertainty associated with different discharge scenarios. Uncertainty analysis showed that river discharge and concentration of viruses in CSO discharge were the main contributing factors to differences in risk and duration of elevated risk. The duration of CSO discharge was identified as the key factor determining the duration of beach closure after a CSO event. Risk analysis shown that even in the best case scenario (i.e. with the lowest CSO virus concentration and CSO discharge volume), the risk of acute viral-induced gastrointestinal illness with swimming in Lake Michigan beaches after a CSO event in the lower Grand River in Grand Rapids is 2.4×10^{-1}, which is higher than EPA tolerable level for fresh recreational water of 8×10^{-3}. The high risk associated with recreating in water receiving CSO discharge determined from this study emphasizes the importance of proper wastewater treatment and retention.

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ABBREVIATIONS

Acoustic Doppler Current Profiler- ADCP

Analysis of variance- ANOVA

Areas of Concern- AOCs

Centers for Disease Control and Prevention- CDC

Colony forming unit- cfu

Combined sewer overflow – CSO

Combined sewer system – CSS

Contaminant candidate list – CCL

Cubic feet per second- cfs

Dissolved organic carbon- DOC

Dissolved organic matter- DOM

Double stranded DNA (/RNA)- dsDNA (/RNA)

Human adenoviruses- HAdVs

Human enteroviruses- HEVs

Humic acid- HA

International Committee on Taxonomy of Viruses- ICTV

Long-Term Control Plans - LTCPs

Michigan Department of Environmental Quality- MDEQ

Michigan State University- MSU

Microbiological source tracking- MST

National Research Council- NRC

Norovirus- NV

Ohio Department of Health- ODH

Ohio Environmental Protection Agency- OH EPA

Plaque forming unit- pfu

Polymerase chain reaction- PCR

Quantitative microbial risk assessment- QMRA

Reverse Transcriptase- RT

Rhodamine WT- RWT

Sanitary sewer overflow- SSO

Sanitary sewer system- SSS

Single stranded DNA (/RNA)- ssDNA (/RNA)

Soil and Water Assessment Tool- SWAT

Total suspended solid- TSS

Transient storage- TS

Tryptic soy agar- TSA

Trypticase soy broth- TSB

Ultraviolet- UV

United States Environmental Protection Agency – U.S. EPA

United States Geological Survey- USGS

Waste water treatment plant- WWTP

World Health Organization- WHO

CHAPTER 1

INTRODUCTION

Enteric viruses have been detected in various aquatic environments, i.e. ground water, surface water, estuarine, tap water and marine water (Girones et al. 1995; Castignolles et al. 1998; Chapron et al. 2000; Jiang et al. 2001; Xagoraraki et al. 2007). Because enteric viruses are excreted in high concentration by infected individuals and generally are transmitted through the fecal-oral route, water contaminated by fecal sources remains an important vehicle for the transmission of virus related waterborne diseases. Virus survival in the water environment is mainly controlled by temperature, microbial activity, and solar radiation (Hurst et al. 1980; Yates et al. 1987; Schijven and Hassanizadeh 2000; Song et al. 2005). Other factors such as attachment of viruses to solids, dissolved organic matters and pH may contribute to the survival and transport behavior of viruses, thus influencing human exposure via contaminated water.

The Great Lakes are important sources for drinking water and recreational activities. Enteric viruses including rotavirus, adenovirus and enteroviruses have been isolated from both surface water and groundwater of the Great Lakes (Fong et al. 2007; Xagoraraki et al. 2007); however, waterborne outbreaks related to these viruses may be underreported because they are not routinely looked for during outbreak investigations. In older communities of the Great Lakes and Northeast regions, overflows from combined sewer system (CSS) after heavy precipitation events are major sources of sewage/fecal pollution, deteriorating surface water quality and beach closure. The cost of beach closures is high; each beach closure in Michigan was estimated to cost would-be swimmers between between $1274 and $37030 per day (Rabinovici et al. 2004). The

actual health impact of a combined sewer overflow (CSO) event associated with illnesses in swimmers is difficult to assess; however, this may be evaluated through a new approach using a quantitative microbial risk assessment model.

Quantitative risk assessment is the process of integrating scientific and assessment data regarding an environmental hazard into a framework to address the risk of exposure and the potential health impacts (Rose and Grimes 2001). Risk assessment frameworks have been used extensively to examine human health risks associated with exposure to toxic chemicals in the environments. Quantitative risk assessment framework may also be used to assess risk related to exposure to microbial pathogens provided that information regarding microbial pathogenicity and exposures are available. The initial evaluation explores the hazards of waterborne disease and methods for assessing exposure, particularly for the Great Lakes.

Waterborne diseases and the sources of waterborne pathogens

Waterborne diseases have received widespread attention in recent years because of expanding spectrum of pathogens involved and increasing number of cases reported. Waterborne diseases may be transmitted through consumption of contaminated water, inhalation of water vapors/aerosols, dermal contact as well as ingestion during bathing or recreational activities. Waterborne pathogens are usually spread by the fecal-oral route with water as an intermediate; for example, an infected host may excrete for example, 10^{11} virus particles/g of feces for several weeks following initial infection (Wadell et al. 1987). In the United States, waterborne disease associated with fecal contamination of drinking water and community supplies as well as recreational waters remains a major public health problem. More than 50 % of waterborne disease outbreaks reported in the

United States between 1948 and 1994 were determined to be "acute gastrointestinal illness", viruses have often been ascribed to these illnesses (Curriero et al. 2001). In the Great Lake region, few waterborne outbreaks associated with viruses and other pathogens were reported, but the actual number of viral related outbreaks may be higher as they often go unreported because the symptoms are usually mild and without long-term complications (Mac Kenzie et al. 1994; Hrudey et al. 2003; Fong et al. 2007; O'Reilly et al. 2007). Waterborne pathogens, especially viruses, may cause a wide range of diseases. Major symptoms include but are not limited to diarrhea, vomiting, fever, respiratory infection and conjunctivitis (Bosch 1998). Although the complications of waterborne infections are usually mild and acute, they can cause severe illness and long-term complications in young children, immune-sensitive and older populations. In addition, some viral infections are easily transmissible and the resulting waterborne outbreaks often have huge economic effects. Norovirus, for example, has a secondary transmission rate of as high as 90% (Hoebe et al. 2004).

Globally, waterborne disease is also a major problem, especially in less developed regions. According to the World Health Organization (WHO), water-related diarrheal diseases cause more death than cancer, and are responsible for the deaths of 1.8 million people every year in less developed regions of the world (WHO 2004; WHO 2005). In developing countries, up to 13 million deaths annually may be attributed to consumption of contaminated water (WHO 1996). Of the three important categories of waterborne pathogens (viruses, bacteria, and protozoa), viruses are among the most resilient and infectious, yet difficult to isolate because of the lack of sensitive and standard monitoring protocols. In the United States, viral gastroenteritis is identified as the second most

common cause of illness (NIH 2006). Despite this, viral outbreaks are likely to be underreported because improved technology for detection of viruses in stool and water samples is still not widely practiced and in most cases, outbreak samples are only screened for specific types of viruses.

More than 140 types of pathogenic viruses may be present in high concentrations in sewage-contaminated waters. Four groups of waterborne viral pathogens that have been most extensively studied and remain a public health concern are adenoviruses, noroviruses and enteroviruses and rotaviruses (Moe et al. 1994; Gerba et al. 1996; Crabtree et al. 1997; Parshionikar et al. 2003; Widdowson 2004; Turcios et al. 2006). Recent advancement in molecular detection assays for viruses has brought to the public's attention some emerging potential waterborne viruses, viruses that recently have been isolated from waters, such as polyomaviruses, torovirus, coronaviruses, Torque Teno virus (TT virus) and picobirnaviruses (Theron and Cloete 2002; Vaidya et al. 2002; Myrmel et al. 2004; Haramoto et al. 2005). Although their transmission through water has not been fully elucidated, these viruses have been shown to persist through wastewater treatment processes and in aquatic environments. A list of some of the important waterborne viral pathogens, their characteristics and implications to human health are summarized in Table 1.1.

Table 1.1 Waterborne viral pathogens and emerging waterborne viral pathogens and related diseases.

Viruses	Size (nm)	Nucleic Acid	Diseases	Concerns	References
*Adenoviruses	68-85	dsDNA	Conjunctivitis, pneumonia, gastroenteritis	60 times more resistant to UV irradiation than RNA viruses	Jiang 2006
Enteroviruses (poliovirus, *coxsackievirus, *echovirus, numbered enteroviruses)	20-30	ssRNA	Paralysis, meningitis, fever, gastroenteritis, myocarditis, rash, respiratory diseases, diabetes	A diverse group of viruses that cause a wide range of diseases	Kocwa-Haluch 2001
*Noroviruses	27	RNA	Gastroenteritis, fever	Can withstand freezing and heating to 60 °C; short-lived host immunity to infection permit re-infection	Embrey et al. 2002
Polyomaviruses			Progressive multifocal leukoencephalopathy (PML), colon cancer	Resistant to heat inactivation	Okamoto et al. 1998
Rotavirus	dsRNA		Infantile gastroenteritis		Kocwa-Haluch and Zalewska 2002

Of all waterborne enteric viruses, human adenoviruses are recently receiving renewed attention and study. Human adenoviruses are frequently isolated in high concentrations in environmental waters; ranging between 10^4 and 10^8 viruses/liter in sewage, 10^0 and 10^4 viruses/liter in river, and 10^{-1} to 10^3 viruses/liter in seawater (Pina et al. 1998; Jiang et al. 2001; Choi and Jiang 2005; He and Jiang 2005; Bofill-Mas et al. 2006). Quantification of human adenoviruses from different environmental matrices are summarized in Table 1.2. There are 51 serotypes of adenoviruses (tentative serotype 52 has just been isolated and sequenced) that cause a wide spectrum of illnesses in human, from conjunctivitis, respiratory infection, gastroenteritis to haemorrhagic cystitis (Gu et al. 2003; Jones et al. 2007). The 51 serotypes of human adenoviruses are grouped into 6 species (A–F) based on their oncogenicity in newborn hamsters, hemagglutinating, and DNA sequence properties (Walls et al. 2003). Adenoviruses species F (serotypes 40 and 41), which attack the gastrointestinal system of hosts are the adenovirus species most frequently isolated from environmental waters (Cho et al. 2000; Lee and Kim 2002; van Heerden et al. 2005; Bofill-Mas et al. 2006; Xagoraraki et al. 2007) (Table 1.3). Adenoviruses are the second most important etiologic agents of childhood gastroenteritis after rotavirus; together, adenoviruses type 40 and 41 accounted for 50% of adenoviruses isolated from stool specimens (Wigand et al. 1983; Brown 1990; Soares et al. 2002; Li et al. 2004). In 1998, adenoviruses were identified by the U.S. EPA as one of nine microorganisms and one of four viruses (the three others are caliciviruses, coxsackieviruses and echoviruses) on the Drinking Water Contaminant Candidate List (CCL) (U.S. EPA 1998). The CCL identifies contaminants of concern, which may require

regulation in the future. One key objectives of the CCL is the need to identify the occurrence of the contaminant in water.

Adenoviruses belong to the family *Adenoviridae*. Adenovirus has double-stranded DNA and ranges in size from 60 to 90 nm (Embrey et al. 2002). The viruses are shed for extended periods (up to months or years) in feces, urine and respiratory secretions of infected persons (Crabtree et al. 1997). Adenoviruses have been found to be more resistant to UV disinfection, fluctuations in temperature and humidity than other enteric viruses (Wasserman 1962; Meng and Gerba 1995; Mahl and Sadler 1975; Hara et al. 1990). They are extremely resistant to UV irradiation because of their high molecular weight and their undamaged DNA strand may serve as a template for repair by host enzymes (Roessler and Severin 1996). They have been shown to be up to 60 times more resistant to UV irradiation than RNA viruses, such as enteroviruses and hepatitis A virus (Gerba et al. 2002). Several studies have suggested that adenoviruses outnumbered other enteric viruses in sewage-contaminated waters and may survive longer than other viruses in water (Enriquez et al. 1995; Pina et al. 1998). The number of waterborne outbreaks caused by adenoviruses might have been underestimated because they are not being routinely screened for in outbreak samples.

Table 1.2 Detection and quantification of human adenoviruses from different environmental matrices.

Number of Samples*	Volume (L)	Serotype	Mean (gc/L)	Min (gc/L)	Max (gc/L)	Geographical Region	Reference
Sewage							
5/5	0.05	All	$1.4x10^7$	$4.71x10^5$	$2.52x10^7$	Spain	Albinana-Gimenez et al. 2006
17/17	0.1	All	$8.5x10^4$	$5.1x10^3$	$1.8x10^7$	Tokyo, Japan	Haramoto et al. 2007
17/17	0.1	Enteric	$6.7x10^4$	$7.3x10^3$	$1.5x10^6$	Tokyo, Japan	Haramoto et al. 2007
6	0.04	All	$3.87x10^7$	sigma:$3.78x10^6$		Barcelona, Spain	Bofill-Mas et al. 2006
10/10	0.05	All	$2.27x10^7$	$1.1x10^6$	$1.8x10^8$	Rome, Italy	Muscillo et al. 2008
Primary effluent							
3/3	0.01	All		$6.6x10^5$	$7.4x10^5$	CA, USA	He and Jiang et al. 2005
Secondary effluent							
4/4	0.01	All		$6.1x10^5$	$14x10^5$	CA, USA	He and Jiang et al. 2005
14/17	1	All	$3.9x10^2$	$4.6x10^0$	$5.4x10^3$	Tokyo, Japan	Haramoto et al. 2007
13/17	1	Enteric	$3.2x10^2$	$6x10^{-1}$	$4.1x10^3$	Tokyo, Japan	Haramoto et al. 2007

Table 1.2 cont'd

7	0.04-1	All	$4.69x10^3$	$4.69x10^3$		Barcelona, Spain	Bofill-Mas et al. 2006
Sludge							
5/5	0.1	All	$1.9x10^5$	$1.3x10^2$	$7.96x10^5$	Spain	Albiana et al. 2006
8		All	$1.83x10^2$/g	$8.64x10^4$		Barcelona, Spain	Bofill-Mas et al. 2006
Biosolids							
7		All	$1.59x10^4$/g	$2.24x10^7$/g		Barcelona, Spain	Bofill-Mas et al. 2006
River							
5/5	95-105	All	$2.9x10^2$	$9.1x10^1$	$6.9x10^2$	Spain	Albiana et al. 2006
8/9	200-300	All	$4x10^2$	$1.4x10^1$	$1.7x10^3$	Spain	Albiana et al. 2006
1/61	100	Enteric	$2.3x10^0$			CA, USA	Rajal et al. 2007
18/114	10	All		10^2	10^4	CA, USA	Choi et al. 2005
10/145	25	All	$3.29x10^3$	$<8.33x10^0$	$1.05x10^4$	Pretoria, S. Africa	van Heerden et al. 2005
29/36	0.5	All	$\sim 1x10^3$	$>1.8x10^0$	$>7x10^3$	Tokyo, Japan	Haramoto et al. 2007
29/36	0.5	Enteric	$<1x10^3$	$1.8x10^0$	$7x10^3$	Tokyo, Japan	Haramoto et al. 2007
Treated Drinking Water							
10/188	200	All	$7.84x10^2$	$<1.04x10^0$	$5.46x10^3$	Pretoria, S. Africa	van Heerden et al. 2005

Table 1.2 cont'd

Lake Beach (fresh water)							
8/30	250-350	All		$1.7x10^{1}$	$3.4x10^{2}$	MI, IN, USA	Xagoraraki et al. 2007
6/28	250-350	All		$7x10^{0}$	$3.8x10^{3}$	MI, IN, USA	Xagoraraki et al. 2007
3/58	250-350	Enteric		$4.8x10^{1}$	$4.6x10^{2}$	MI, IN, USA	Xagoraraki et al. 2007
Seawater							
15/18	1	All	$<5.8x10^{2}$	$6.1x10^{1}$	$6.6x10^{3}$	Tokyo, Japan	Haramoto et al. 2007
15/18	1	Enteric	$5.8x10^{2}$	$3.2x10^{1}$	$6.1x10^{3}$	Tokyo, Japan	Haramoto et al. 2007
20/26	10	All	$1.19x10^{2}$	$4x10^{-1}$	$7.7x10^{2}$	Rome, Italy	Muscillo et al. 2008
Estuarine River							
3/7	10	All	$4.41x10^{4}$	$3.4x10^{4}$	$2.3x10^{5}$	Rome, Italy	Muscillo et al. 2008

* number of positive samples/number of sample analyzed.

Table 1.3 Adenovirus serotypes isolated from environmental samples.

Matrices	No. of +ve samples/Total no. of samples	Serotype	Serotyping Assay	Geographical Region	Reference
Sewage		11, 12, 31, 34, 35, 40, 41	Cloning	Spain	Bofill-Mas et al. 2006
Sewage	10/10	2,41	PCR product/Cloning	Rome, Italy	Muscillo et al. 2008
Sewage	13/13	3, 12, 40, 41	Direct sequencing of PCR products	MI, USA	This Study
Primary Effluent	13/13	12, 41	Direct sequencing of PCR products	MI, USA	This Study
River	1/61	40/41	Real-time PCR	CA, USA	Rajal et al. 2007
River	10/45	2, 40, 41, species D	PCR product/Cloning	Pretoria, S Africa	van Heerden et al. 2005
Treated Drinking Water	10/188	2, 40, 41, species D	PCR product/Cloning	Pretoria, S Africa	van Heerden et al. 2005
Tap Water	4/4	5, 40, 41	PCR product/Cloning	Korea	Cho et al. 2000
Tap Water				Korea	Lee and Kim 2002
Lake Beach	14/58	40,41	Real-time PCR	MI, IN, USA	Xagoraraki et al. 2007
Seawater	18/26	2, 41	PCR product/Cloning	Rome, Italy	Muscillo et al. 2008
Estuary/river	3/7	2,41	PCR product/Cloning	Rome, Italy	Muscillo et al. 2008
Acanthamoeba	34/236	1, 2, 8, 37	PCR-based typing assay	Spain	Lorenzo-Morales et al. 2007

Other key viruses of concern in water are the norovirus and enteroviruses. Noroviruses cause gastroenteritis and are associated with multiple recreational and drinking water outbreaks (Parshionikar et al. 2003; Hoebe et al. 2004; Widdowson 2004; Maunula L et al. 2005). Between 2003 and 2004, the Centers for Disease Control and Prevention (CDC) estimated that the norovirus was the etiologic agent in 4.8% and 16.7% of gastrointestinal illness outbreaks of drinking water and recreational water in the US, respectively (Dziuban et al. 2006; Liang et al. 2006). Noroviruses (NVs) are formerly classified as Norwalk-like viruses. The virus was first identified in a gastroenteritis outbreak in Norwalk, OH in 1968. Norovirus consists of small, circular and single-stranded RNA. They belong to the family of *Caliciviridae* and are approximately 23-35 nm in diameter (Embrey et al. 2002). Noroviruses have low infectious dose and can cause prolonged asymptomatic shedding in infected individuals for up to two weeks (Murata et al. 2007).

Noroviruses are extremely stable in the environment, they are stable in less than 10 parts per million (ppm) chlorine and can withstand freezing and heating to 60 °C (Nwachcuku and Gerba 2004). Substantial strain diversity leads to short-lived host immunity to infection, permits re-infection and makes the development of a vaccine that offers lifelong protection impossible (Glass et al. 2001). Recent data suggested that humans may not be the only host group for NV, reservoir hosts of NVs may include calves and pigs (van der Poel et al. 2000).

Enteroviruses comprise of a large group of viruses that include poliovirus, coxsackieviruses, echoviruses, and the numbered enteroviruses. At least 89 serotypes of enteroviruses have been identified and ratified by the Executive Committee of the

International Committee on Taxonomy of Viruses (ICTV) (Büchen-Osmond 2002). Two members of the enverovirus group - coxsackievirus and echovirus have also been included in the contaminant candidate list (CCL) of the Safe Drinking Water Act Amendments of 1996 by the US Environmental Protection Agency (U.S. EPA) (Roessler and Severin 1996).

Enteroviruses are single-stranded RNA viruses with an icosahedral capsid ranging from 20 to 30 nm in diameter. Because of their ability to grow in cell culture and be quantified as plaque forming units (PFU), enteroviruses are the most studied group of viruses in water and had been included by the European Union regulations governing water quality as a parameter for evaluating viral pollution in waters (Rao 1986; Pina et al. 1998). Enteroviruses can cause a wide spectrum of diseases in humans. Polioviruses usually infect their host by attacking the central nervous system and cause paralysis in victims (poliomyelitis). Coxsackieviruses have not only been associated with respiratory system infections and gastroenteritis, but also insulin-dependent diabetes and heart diseases, such as myocarditis and pericarditis (Kocwa-Haluch 2001). Echoviruses are usually associated with the common cold and respiratory diseases. The numbered enteroviruses (type 68 – 71) have not been studied extensively but have been isolated from patients with bronchiolitis, conjunctivitis, meningitis and paralysis resembling poliomyelitis (Kocwa-Haluch 2001).

Viral pathogens, especially those that have high prevalence in human populations, have been suggested as one of the most promising alternative tools to evaluate fecal pollution as well as public health risk. Viral indicators may be used in conjunction with bacterial indicators to assess risks relating to water consumption and recreational

activities (Pina et al. 1998; McQuaig et al. 2006). As for tracking the source of fecal contamination, the host specificity of viruses makes them ideal candidates for source tracking purposes. In a comparative study of microbiological source tracking (MST) methods, molecular detection of human enteric viruses reliably identified sewage, though yielded some false negative results when analyzing individual human feces (Griffith et al. 2003). Human and animal enteric viruses have been tested in several studies to track fecal contamination of difference origins (Ley et al. 2002; Maluquer de Motes et al. 2004; Hundesa et al. 2006).

Of all waterborne human enteric viruses, adenoviruses, with their high prevalence and stability, and little seasonal variation in shedding, stand out as the most promising index virus for assessing viral contamination in water (Allard et al 1990, Enriquez et al 1995, Irving and Smith 1981, Krikelis et al. 1985). In addition, because of their clinical importance and high occurrence in environmental waters, different molecular assays for detection and quantification of these viruses have been developed. By detection and quantification of adenoviruses in water, the exposure to viruses from contaminated surface water may be assessed and evaluated.

Transport and Survival of Viruses in Surface Water

Information regarding the transport and survival of viruses in aquatic environments is critical for assessment of exposure and alternately risk from waterborne transmission, especially as a result of recreational water contact. The transport and survival of viruses depend largely on their genetic makeup (i.e. either DNA or RNA) and structures (i.e. shape, enveloped or nonenveloped). Viruses are protected by their protein coat or lipid envelope; thus, they are extremely sensitive to factors that can cause protein

degradation. The environmental factors controlling the survival of viruses in aquatic environments depend largely on the type and physical-chemical characteristics of the water (i.e. surface water, groundwater, marine etc).

The three most important factors affecting virus survival in surface water are temperature, microbial activity, and solar radiation (Hurst et al. 1980; Yates et al. 1987; Schijven and Hassanizadeh 2000; Song et al. 2005). Other factors that may affect the virus inactivation in surface water include agitation, pH, salinity, organic matter, and suspended solids and, turbidity (Babich and Stotzky 1980). The combinations of some of the factors above have shown antagonistic effects on virus survival.

Temperature is one of the most important factors affects viral survival (Hurst et al. 1980; Yates et al. 1985; Blanc and Nasser 1996). Both human and animal viruses have been shown to persist longer and occur more frequently at lower temperatures in natural environments (Pesaro et al. 1995; Lipp et al. 2001).

High temperatures can damage the virus capsid and nucleic acids, may cause protein denaturation and prevent adsorption of the virus to its host, as well as inactivate enzymes required for replication (Bitton 1980). Under subtropical climate, Lipp et al. (2001) detected enteroviruses from an estuary in southwest Florida only in winter (when water temperature was below 23 °C). In an *in vitro* study, enhanced poliovirus survival and detection was observed at 22°C as compared to 30 °C (Wetz et al. 2004). Viruses were detected by RT-PCR for at least 60 days at 22 °C, compared to only 30 days at 30 °C in artificial and filtered seawater. Similarly, Tsai et al. (1993) reported that poliovirus virions could not be detected by RT-PCR after seven days of incubation at 25 °C, compared to after 21 days when incubated at 4 °C.

In addition to water temperature, microbial activity is another important factor controlling survival of viruses in surface water. Wetz et al. (2004) showed that poliovirus survival in unfiltered natural seawater was much shorter than survival in filtered seawater or artificial seawater regardless of incubation temperatures (i.e. 22 °C and 30 °C). In cases where seasonal water temperature fluctuation is apparent (e.g. subtropical climate), elevated water temperature and microbial activity has a synergistic effect on virus survival (Gordon and Toze 2003). As temperature increases, bacterial and protozoan metabolic processes accelerate, predation increases and degradative enzymes (i.e. extracellular proteases and nucleases) produced by plants, flagellates or bacteria can degrade viral capsids and damage viral DNA or RNA (Tsai et al. 1995; Noble and Fuhrman 1999). Wait and Sobsey (2001) showed that the decreased survival of poliovirus incubated in the laboratory at 6 °C in water collected during summer was significantly compared to survival in water collected during other seasons. When comparing *in vitro* and *in situ* survival in seawater, Wait and Sobsey (2001) reported viruses survived significantly longer at lower temperatures in laboratory conditions but there was no significant difference in virus survival between seasons in natural seawater (*in situ*).

Next to temperature and microbial activity, ultraviolet (UV) radiation may also be a significant factor affecting virus inactivation. UV irradiation inactivates viruses by causing cross-linking among virus nucleotides (Gerba 2007). Photosensitivity is virus type-dependent and related to the structure of viruses (i.e. nucleic acid type-double-stranded DNA or double-stranded RNA, guanine and cytosine content, enveloped), and virion size as well as the molecular structure of the specific virus (Meng and Gerba. 1996). In general, non-enveloped viruses (poliovirus, adenoviruses) are more

resistant to UV light than enveloped viruses (vaccinia, herpes simplex, influenza) (Jensen 1964; Meng and Gerba 1996). Virus nucleic acid type may be an important determinant of its UV resilience; dsDNA viruses such as adenoviruses are extremely stable when exposed to UV because their undamaged DNA strand may serve as a template for repair by host enzymes (Gerba et al. 2002; Thurston-Enriquez et al. 2003). Meng and Gerba (1996) concluded that adenovirus 40 was 1.2, 1.8, 4.1 and 5.6 times more resistant that adenovirus 41, bacteriophage MS-2, bacteriophage P22* and poliovirus 1, respectively. Rotaviruses, which consist of double-stranded RNA have been shown to be more resistant to UV inactivation than hepatitis A virus, coxsackievirus B5 and poliovirus type 1 (Battigelli et al. 1993; Wilson et al. 1993). Sinton et al. (2002) found that bacteriophage inactivation rates in sunlight are ten times higher than their inactivation rates in the dark. This is consistent with the findings by Johnson et al. (1997), who observed 1 $\log_{10}$ inactivation of polioviruses in marine water after 24 h incubation in dark compare to 3 $\log_{10}$ inactivation of polioviruses incubated under the same condition but exposed to sunlight.

Attachment of viruses to solids, particularly to sediment with high clay and organic matter content is generally believed to increase their persistence and stability in natural environments by offering protection from protein degrading enzymes, other degrading factors and UV inactivation (Babich and Stotzky 1980; Davis et al. 2006; Gerba and Schaiberger 1975; Green and Lewis 1999; Lipson and Stotzky 1986; Straub et al. 1992). Sediment has been suggested as a reservoir and a sink for viruses, Green and Lewis (1999) reported that enteroviruses and hepatitis A could be detected throughout the year in sediment in the immediate vicinity of a sewage outfall even though enterovirus

* bacteriophage P22 was misidentified as bacteriophage PRD1 in this study

concentrations peaked in the wastewater during winter months. Likewise, Ferguson et al. (1996) reported that during wet season, enteric viruses were isolated from both water and sediment samples, but during the dry season, viruses were isolated from the sediment only. On the other hand, some researchers that studied the effect of attachment on virus survival suggested that the outcome of virus attachment depends on the degree of binding (Schijven and Hassanizadeh 2000). Schijven et al. (1999) found that the inactivation rate of attached viruses exceeded the inactivation rate of viruses in the solution.

However, soil organic matters, such as humic acid (HA) have been shown to have some protective effects on viruses (Foppen et al. 2006). Foppen et al. (2006) reported that in conditions without HA and in the absence of sand, the inactivation rate of PRD1 at 5±3°C was 0.014 day^{-1}, which was 15.5 times higher than in the presence of HA (0.0009 day^{-1}. Although humic acids have been shown to be protective of viruses, Babich and Stotzky (1980) observed that the survival of bacteriophage Φ11M15 was not affected by particulate humic acids without the presence of clay minerals such as attapulgite, vermiculite, and kaolinite and there was no significant difference in the survival of the bacteriophage between different types of water amended with heat-killed bacterial cells (Babich and Sotozky 1980). Thus, it was suggested that the presence of clay minerals helped the adsorption of viruses to soil organic matters, which has a protective effect on viruses.

In addition, Foppen et al. (2006) observed that in the presence of dissolved organic matter (DOM), viruses could be transported for long distances in surface water and survive longer because DOM usually out-compete viruses in occupying favorable attachment sites on solid particles; at the same time, anionic surfactants in DOM has

found to be protective of viral particles (Blanford et al. 2005; Lefler and Kott 1974; Ryan et al. 2002; Zhuang and Jin 2003).

Most viruses are stable between pH 5 and 9. In general, most non-enveloped enteric viruses are stable at pH levels as low as 3 and as high as 10 to 10.5; pH affects the survival of viruses in water by changing the aggregation status of viruses and adsorption of viruses to the surrounding strata. Higher ionic concentrations (low pH) increase the virus aggregation as well as adsorption of viruses to particles (Langlet et al. 2007). Yates et al. (1985) found that inactivation of MS2, poliovirus 1 and echovirus was not significantly affected by pH ranged between 6.0 and 8.2 (Yates and Gerba 1985). Van Elsen and Boyce (1966) suggested that only pH higher than 10.5 may cause structural change in viruses (Van Elsen and Boyce 1966). In addition to pH, the presence of other chemicals may have an effect on the survival of viruses. Van Elsen and Boyce (1966) observed that under alkaline condition (pH between 8.5 and 9.5), the presence of free ammonia shows synergistic effects on the survival of poliovirus (van Elsen and Boyce 1966). Yates et al. (1985) found that ammonia, hardness of water (calcium, magnesium and total hardness), nitrate, total dissolved solids and turbidity did not significantly affect the inactivation of MS2, poliovirus 1 and echovirus 1. While it was clear that, globally, waterborne viruses remain a risk, the evidence for the waters in the Great Lakes needed to be summarized and assessed.

The Economic and Human Health Effects Caused by Deteriorating Water Quality in the Great Lakes and in Michigan

The States bordering the Great Lakes have the nation's longest coastline (5,500 miles) which includes over 1000 beaches for recreational activities (Dorfman and Stoner

2007). At the same time, the Great Lakes also provide drinking water to over 40 million U.S. and Canadian citizens and thus, water quality is an important concern for the region (Government of Canada and U.S. EPA 1995). The Great Lakes is one of the largest sources of fresh surface water, holding 90 % of the fresh surface water in United States (U.S. EPA 2007). The deteriorating water quality in the Great Lakes caused by waterborne pathogens, associated with both recreational and drinking water exposures has been identified as one major threat to public health (Dorfman and Stoner 2007). The latest reports on the Great Lakes ecosystem demonstrate that although chemical contamination has been greatly reduced, microbial contaminants remain a threat and requires more attention (U.S. EPA and Environment Canada 2007). Contamination of near-shore waters and recreational waters with microorganisms is increasing, and water quality is deteriorating with associated beach closures and waterborne outbreaks (U.S. EPA and Environment Canada 2007).

In 1987, the United States and Canada identified 43 areas of concern (AOC) with the greatest pollution in the Great Lakes basin, which would require an effort to clean up and restore. Michigan has 11/26 AOCs within the United States (White, Deer, Torch and Muskegon Lakes, Saginaw River and Saginaw Bay, and the Kalamazoo, Manistique, St. Mary's, Raisin, Rouge, and Clinton Rivers) and two shared AOCs with Canada (Detroit and St. Clair rivers). The AOCs are severely degraded geographic areas within the Great Lakes Basin that fail to meet the general or specific objectives of the Great Lakes Water Quality Agreement (1987). Beach closings are one of the beneficial use impairments at nine of these AOCs. These beach closings signify that the areas have high fecal bacterial indicator loading and are unfit for recreational use.

In Michigan, a total of 33,856 perennial river miles have been assessed for recreational use impairment, and are graded from pristine to degraded or impaired (Edly and Wuycheck 2006). The impairment status includes poor fish habitats as well as poor water quality for recreational use. A water quality assessment by the Michigan Department of Environmental Quality (MDEQ) reported that between 1999 and 2004, 597 of Michigan's 33,856 perennial river miles were not supporting the total body contact recreation designated use, and 19 of those 597 perennial river miles were also not supporting the partial body contact recreation designated use (Edly and Wuycheck 2006). The primary sources of microbial contaminants to these non-attaining water bodies included combined sewer overflows (CSOs), sanitary sewer overflows (SSOs), urban runoff, and waste water effluent (Edly and Wuycheck 2006).

The surface water in the Great Lakes and Michigan, are an important resource for recreational activities that involve full body contact with water, such as swimming, water-skiing, and sail-boarding. Recreational beaches in the Great Lakes are an important income source for businesses in the coastal region and economic losses caused by beach closures can have huge impacts on local economy. Across the USA, beach closures have caused billions of dollars of economic loss. In 2003, there were more than 18,000 days of closings and advisories on coastal beaches. In the Great Lakes region in 2005, 19% of monitored beaches (of the total of 1085) were posted or closed more than 10% of swimming season mainly because of high levels of fecal indicator organisms (U.S. EPA and Environment Canada 2007). In Michigan alone in 2005, water quality standards were exceeded from 77 of 406 monitored public beaches, resulting in 80 beach closures (474 beach closing days). Based on the benefit transfer policy analysis by Rabinovici et al.

(2004), each closure would cost would-be swimmers between $1274 and $37030 per day, and the net economic loss in Michigan in 2005 may total anywhere between six and seventeen million dollars (Rabinovici et al. 2004).

Conditions that may contribute to microbiological contamination of surface water and groundwater in the Great Lakes region are from both point and non-point sources. Some examples of non-point sources for waterborne pathogens are combined sewer overflows (CSO), urban runoff, septic tanks, boat dumping, and concentrated animal feeding operations (CAFO) (Jiang et al. 2001; Sharma et al. 2003). In cities with combined sewer systems (CSSs), CSOs are major concerns for surface water quality.

Combined sewer overflows are a problem nationwide. Combined sewer systems are sewer systems that are designed to collect sanitary sewage and other waste water (snowmelt, storm water and urban runoff, and industrial wastewater) in the same pipe to be transported to and treated by wastewater treatment plants (WWTPs). Currently, there are approximately 772 communities in the United States with combined sewer systems (MDEQ 2008). CSSs are mainly located in older cities in the Northeast and the Great Lakes regions. In Michigan, CSOs were identified among the major sources for beach closings and other water quality impairments. Combined sewer overflows after heavy precipitation result in discharges of raw or partially treated sewage from sewer systems that are designed to carry both domestic sewage and storm water to wastewater treatment facilities (Whitman et al. 1995). The action to regulate and phase out combined sewer systems in Michigan began in 1988, as a result of a citizen and public interest group outcry following a large CSO event in Grand Rapids that impaired the water quality as downstream as Grand Haven (MDEQ 2008). In 1994, the federal government developed

a nationwide CSO Control Policy that required CSO communities to implement interim measures to improve the quality of combined sewer discharges by January 1, 1997, and to develop CSO Long-Term Control Plans (LTCPs). As of 2007, 75% of 613 untreated CSO outfalls that existed in 1988 were eliminated and the remaining 25% are scheduled for correction under LTCPs. In addition, though the total CSO volume depends largely on the annual precipitation volume, the portion of partially treated discharge increased.

In the United States, including the Great Lakes, bacterial indicators (total coliform, fecal coliform, *E. coli*, enterococcus) have been used for judging microbial water quality to measure fecal contamination and public health risks. Although bacteria indicator testing is relatively simple and inexpensive, there are several drawbacks associated with its application. Bacterial indicators are useful as first level indicators of fecal contamination in watersheds. However, for wastewater effluents, the indicators do not reflect the human virus load because of different survivability through waste water treatments. They lack the ability to predict human health risk and the ability to differentiate between human and animal contamination for natural waters because they are not host specific (Colford et al. 2007). The source of fecal coliform bacteria and other traditional indicators (e.g., enterococci) are not limited to humans, they are excreted by other warm blooded animals and have been reported to occur naturally in soils (Byappanahalli and Fujioka. 1998; Solo-Gabriele et al. 2000). A study has shown that *E. coli* and enterococci accumulate on sand and algae mats at Great Lakes beaches during the summer and are able to survive for over six months on sun-dried algae mats stored at 4 °C (Whitman et al. 2003). In tropical climates, they have been shown to regrow in the environment after excretion by their hosts (Springthorpe et al. 1993). Complicating

matters, studies have shown that bacterial indicators do not correlate with waterborne viral and protozoal pathogens in water, traditional bacterial indicators generally die off quickly in coastal and marine waters when compared to viruses and protozoa (Bordalo 2002; Solic 1992).

In order to improve swimming conditions and reduce beach closures, the United States, in the BEACH Act of 2000, has set a goal for 90% of monitored high-priority beaches around the Great Lakes to meet bacterial indicator standards (*E. coli* and fecal coliform) for more than 95 % of the swimming season by 2010 (U.S. EPA 2002). However, the actual benefit of beach closure decision based on bacterial indicators is not clear because bacterial indicators do not reflect the risk from many important waterborne pathogens, such as viruses, stressed pathogenic bacteria (viable but non-culturable), and protozoa (Borrego et al. 1987). Infectious enteric viruses have been isolated from aquatic environments as mentioned previously that are in compliance with bacterial indicator standards in recreational waters (Fong et al. 2005, Jiang et al. 2001).

The Occurrence of Viruses in Waters of the Great Lakes

Pathogenic viruses have consistently been isolated from tap water, surface water, groundwater, and wastewater globally (Deetz et al. 1984; Shieh 1997; Vantarakis and Papapetropoulou 1998; Jiang et al. 2001; Borchardt et al. 2003; Haramoto et al. 2004). Viruses have not been monitored extensively in the Great Lakes area but a few studies have shown that viruses are present in groundwater as well as surface water in the region (Borchardt et al. 2003; Borchardt et al. 2004; Jenkins et al. 2005; Xagoraraki et al. 2007). In 2003, a microbiological water quality survey of nine major rivers in the Lower

Peninsula of Michigan found that three of five sites that exceeded the *E. coli* standard for recreational waters for the State of Michigan also tested positive for viable enteric viruses (Jenkins et al. 2005). A water quality survey of two beaches in Indiana and Michigan showed that human adenoviruses were present in the range of $7(\pm 2) \times 10^0$ and $3.8(\pm 0.3) \times 10^3$ viruses/L (Xagoraraki et al. 2007). The presence of enteric viruses and elevated levels of microbial indicators suggests that these waters may pose a threat to the health of individuals using them for recreational activities. Threats to drinking water are generally found during outbreaks. An investigation of a waterborne outbreak at Put-In-Bay, Ohio, including the assessment of virus concentrations and transport behavior in surface water in the Great Lakes region, was undertaken.

Case Study: Investigation of a Waterborne Outbreak and Virus Occurrence in the Great Lakes Region

A possible viral outbreak on an island, South Bass Island, in Lake Erie, was investigated in 2004 (Fong et al. 2007). This allowed for the development of methods and tools for virus testing and highlighted the concern for sewage contamination and viral loading to drinking and recreational waters. The investigation of the Put-In Bay outbreak also showed the importance of a multi-disciplinary approach in linking contamination sources to transport and exposure.

A groundwater-associated outbreak of gastrointestinal disease that affected approximately 1450 people was reported on South Bass Island, OH, between July and September 2004. In support of the Ohio Department of Environment, water samples were collected on the island as a follow-up to the outbreak approximately one month after the

peak of the cases. Wastewater was suspected to be the primary source of contamination and viruses were suspected as one of the major etiologic agents of the outbreak.

Between September 15 and September 21, 2004, 16 groundwater wells (PB1-PB19) that provide potable water on South Bass island, OH in Lake Erie, were tested for fecal indicator bacteria (total coliform, *E. coli*, *C. perfringens* and enterococci), viruses (coliphages, human enteroviruses (HEV), adenoviruses (HAdV) and norovirus (NV)) and parasites (*Cryptosporidium* and *Giardia*) (Figure 1.1).

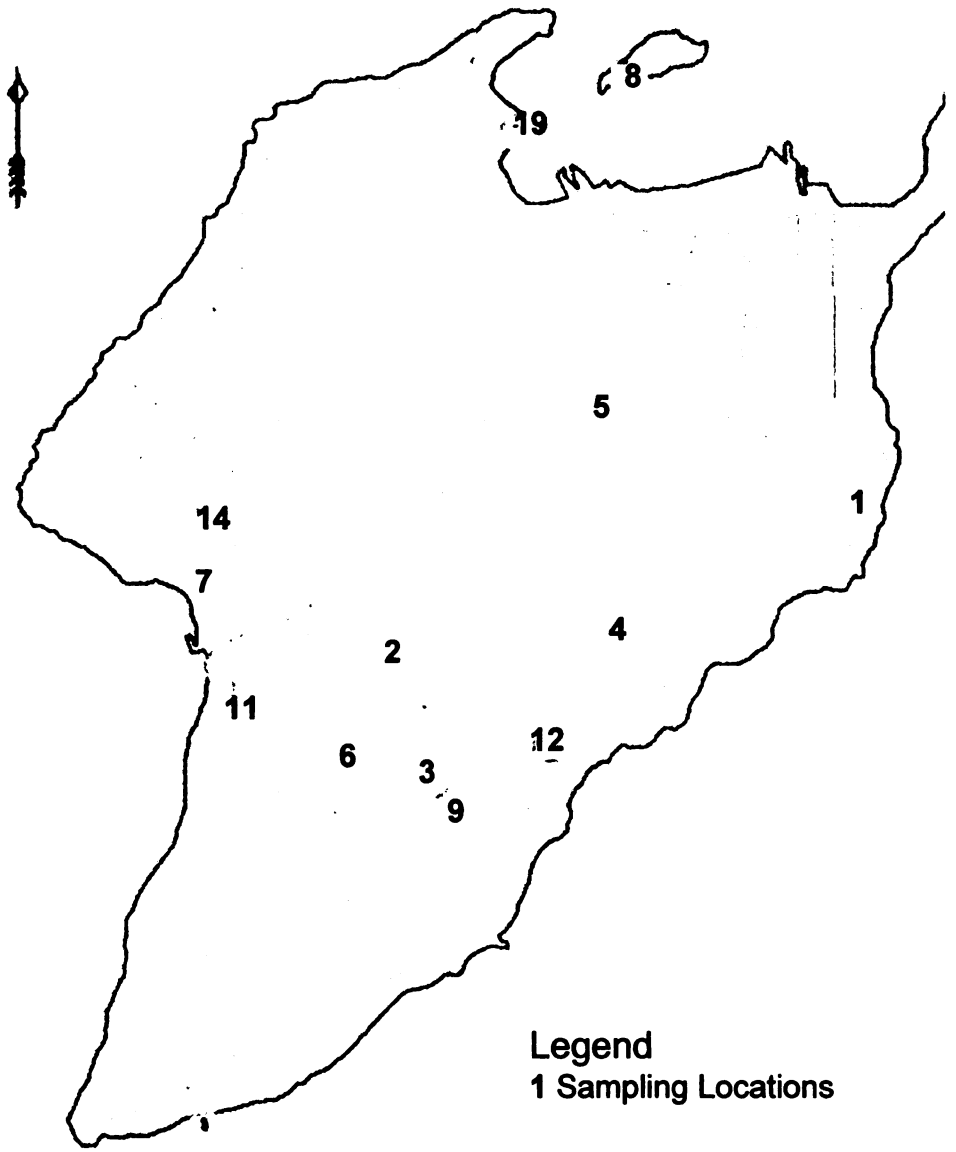

Figure 1.1 Wells sampled on South Bass Island, Lake Erie, Ohio (labeled PB-1 through -19 from 9/15-9/21/2004 (map reproduced from Ohio EPA 2005).

Eleven and eight wells were positive for total coliforms (range: 2.2 CFU/100 ml, 90 CFU/100 ml; mean: 12.55 CFU/100 ml) and *E. coli* (range: 0.1 CFU/100 ml and 4 CFU/100 ml; mean: 0.64 CFU/100 ml), respectively, by the membrane filtration method.

All wells were positive for both total coliform and *E. coli* by the Colilert® Presence/Absence test kit (which has been known to recover chlorine injured bacteria). Seven wells tested positive for enterococci, with counts ranging from 0.1 CFU/100 ml to 6.6 CFU/100 ml (mean: 1.38 CFU/100 ml). *C. perfringens* was not detected in any well. Overall, five wells (PB-3, PB-9, PB-6B, PB-7A, and PB-12) were positive for three bacterial indicators and PB-3 had the highest counts for all three indicators (total coliform, *E. coli* and enterococci). Three wells were positive for both somatic and F^+-specific coliphages with *E. coli* C3000 host and four wells were positive for F^+-specific coliphage with *E. coli* F amp host. *Arcobacter spp.*, an emerging bacterial pathogen, was present in seven wells tested. None of the wells were positive for the protozoa. Overall, three wells were positive for all three bacterial indicators, coliphages, and *Arcobacter* spp.

For the detection of viruses from groundwater, large volumes of water (1000 liters) were filtered through 1 MDS filter. Samples were then concentrated to approximately 30 ml by organic flocculation as described by the U.S. EPA ICR Microbial Laboratory Manual (Fout et al. 1996). Water samples were tested for the presence of viable viruses by inoculating on Buffalo Green Monkey (BGM) cells. These samples were also tested for the presence of human adenovirus DNA, and human enterovirus and norovirus RNA through nested polymerase chain reaction (PCR).

Primers in PCR assays were selected from highly conserved regions of the HAdV, HEV and NV genomes, which allowed for detection of multiple members from each group of viruses. Primers used for the detection of viral DNAs/RNAs are shown in Table 1.4. For HAdV, the nested-primer set designed by Allard et al. (Allard et al. 1992) was

used to amplify HAdV in this study. The primers are able to identify 47 HAdV serotypes, including the more common enteric HAdV types 2, 40, and 41 (Allard et al. 1992; Puig et al. 1994). For HEV detection, a reverse transcriptase (RT)-nested PCR was used with the pan-enterovirus primer set (ENT-up-2 and ENT-down-1) by De Leon et al. (De Leon 1990) and HEV primer set from Fong et al. (2005). HEV primer sets amplified 5' untranslated region of HEV genomes and were able to pick up at least 25 different HEV; Echovirus 22 is not detected with these primers (De Leon 1990; Donaldson et al. 2002).

No viable virus was detected in any of the 16 samples cultured on BGM cells. By using PCR, adenoviral DNA was detected in two of three most contaminated wells (PB-9 and PB-12) by bacterial indicator standard. Sampled groundwater wells, in the order of contamination (from the highest to the lowest bacteria indicator and virus counts) are listed in Table 1.5. All samples were negative for *C. perfringen. Cryptosporidium spp.*, *Giardia spp.*, noroviruses and human enteroviruses.

Table 1.4 Primer sets for virus detection. The equivalent original volume of water analyzed for each sample ranged between 4.58 L and 6.46 L for NV, between 1.37 L and 1.94 L for HAdV and between 2.29 L and 3.23 L for HEV.

Virus group	Primers	Sequence (5' to 3')	Amplicon (bp)	References
HAdV	AV-A1	GCCGCAGTGGTCTTACATGCACATC		
	AV-A2	CAGCACGCCGCGGATGTCAAAGT	300	Allard et al. 1992
	AV-B1[a]	GCCACCGAGACGTACTTCAGCCTG		
	AV-B2[a]	TTGTACGAGTACGCGGTATCCTCGCGGTC	143	Allard et al. 1992
HEV	ENT-up-1	GTAGATCAGGTCGATGAGTC		Fong et al. 2005
	ENT-down-1	AC(T/C)GG(A/G)TGGCCAATC	330	De Leon et al. 1990
	ENT-up-2[a]	CCTCCGGCCCCTGAATG		De Leon et al. 1990
	ENT-down-2[a]	ATTGTCACCATAAGCAGCC	154	Fong et al. 2005
NV	NVp110	AC(A/T/G)AT(C/T)TCATCATCACCATA	398	Le Guyader et al. 1996
	NVp36	ATAAAAGTTGGCATGAACA		

[a] Primers used for the second round of PCR.

Table 1.5 Samples are arranged in the order of contamination (from the highest to the lowest bacteria indicator and virus counts).

	Bacteria (CFU /100 ml)						Coliphages (Enrichment/ L)		Enteric Viruses
Sample ID	Total Coliform (MF)	Total Coliform (Colilert)	*E. coli* (MF)	*E. coli* (Colilert)	Enterococci	*Acrobacter*	Total	F-specific	HAdV *
PB-9	7.8	+	1.3	+	1.9	+	<1	+	+
PB-12	7.7	+	0.3	+	0.6	+	+	<1	+
PB-6B	38	+	0.4	+	0.1	+	+	+	-
PB-5	3.4	+	< 0.1	+	2	+	+	<1	-
PB-3	90	+	4	+	6.6	+	<1	<1	-
PB–6C	26	+	0.1	+	< 0.1	+	<1	+	
PB–6A	5.9	+	< 0.1	+	< 0.1	+	<1	+	-
PB–19	12.8	+	< 0.1	+	5.9	< 0.1	<1	<1	-
PB-7A	3.7	+	0.7	+	5	< 0.1	<1	<1	-

Table 1.5 cont'd

PB–11	2.2	+	0.9	+	< 0.1	< 0.1	<1	<1	-
PB-8	3.3	+	2.6	+	< 0.1	< 0.1	<1	<1	-
PB-14	< 0.1	+	< 0.1	+	< 0.1	< 0.1	<1	<1	-
PB-2	< 0.1	+	< 0.1	+	< 0.1	< 0.1	<1	<1	-
PB-4	< 0.1	+	< 0.1	+	< 0.1	< 0.1	<1	<1	-
PB-1	< 0.1	+	< 0.1	+	< 0.1	< 0.1	<1	<1	-
PB-7B	< 0.1	+	< 0.1	+	< 0.1	< 0.1	<1	<1	-

* HAdV: human adenoviruses

The isolation of these pathogens from the wells showed that groundwater on the island was contaminated by sewage. Moreover, the isolation of human adenovirus DNA showed that groundwater on South Bass Island was susceptible to virus contamination. Failure to detect viable enteric viruses may have been due to chlorination of the wells prior to sampling and inefficient virus concentration and detection method. The possible sources of microbial contamination included sewage disposal to lake, onsite septic systems and land application of septage on the island. Waterborne enteric viruses, because of their small sizes (generally range between 20-200 nm) were able to infiltrate easily through the limestone-based aquifer on the island. The presence of adenoviruses confirmed the susceptibility of groundwater on the island to fecal contamination.

In order to link fecal contamination of groundwater to its sources as well as surface water-ground water interaction, the hydrogeology and hydrodynamics of the island were examined. Because the bedrock on South Bass Island consists of dolomite that is between 30 to 65 feet deep across the island (ODH 2005), the porous limestone aquifer on the island provided little to no natural filtration of microorganisms from sewage that might normally occur during water movement through the soil into the groundwater. Transport of bacteria and viruses throughout the subsurface through fractures in the limestone aquifer is also highly possible. Viruses have been shown to persist for extended period (for months) in groundwater (Yahya et al. 1993). In addition, because groundwater on the island is recharged mainly by precipitation and surface water, heavy precipitation in May and June 2004 (200% and 120% of 50-year average, respectively), might have contributed to increase transportation of the pathogens from surface water into ground water.

Daily averaged, vertically integrated modeled currents in the South Bass Island area were plotted for eight days prior, during and after the outbreak, were examined for a relationship between current speed and direction, and outbreak cases (Figure 1.2). On July 24, 2004, directly prior to the beginning of the largest peak in cases during the outbreak, the current pattern shows clockwise circulation around the island with current speeds exceeding 20 cm/sec on the south shore. Water movement was almost stagnant on August 22, 2004, around the time of the sudden decrease in cases of the disease (the boil order was not initiated until August 26, 2004). The current pattern on September 9, 2004, prior to the virus sampling showed the strongest (more than 20 cm/sec) currents that can occur during fall storms (Fong et al. 2006).

In conclusion, we were able to show that ground water contamination on South Bass Island was the cause of the outbreak and sewage was the source of the contamination. The isolation of adenovirus DNA indicated that the ground water on South Bass Island is susceptible to virus contamination, and other enteric viruses could have been presence in the ground water during the time of the outbreak. In addition, the result of this study also showed that adenoviruses may be more persistent in water than other enteric viruses, such as norovirus (which was identified in several patient's samples but not in the water), thus making them good candidates as index viruses. Also, while some of the cases documented could have been caused by adenoviruses, adenoviruses were not adequately addressed as one of the etiological agents, suggesting that a more sensitive method for the detection and quantification of adenoviruses (i.e. quantitative PCR) should be applied for future outbreak and water quality evaluations

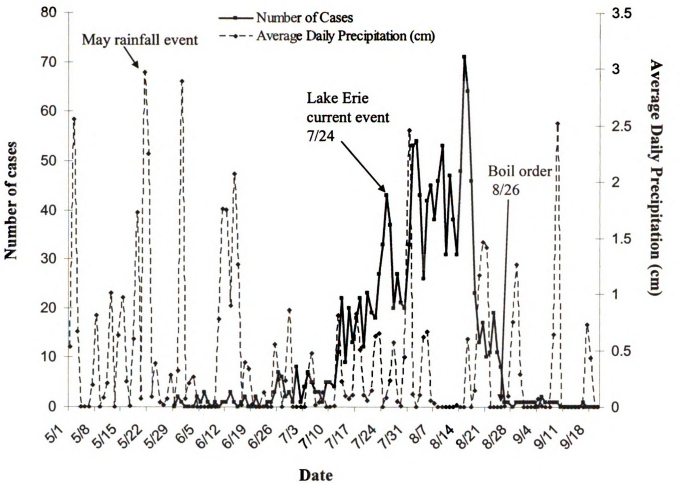

Figure 1.2 Gastroenteritis cases by date, average daily rainfall (cm) and key events over the duration of the outbreak. Index cases were reported on May 30, 2004. (N= 1,450) (estimated number of cases was obtained from O'Reilly et al. 2007

Quantitative Microbial Risk Assessment (QMRA)

Exposure to varying levels of viruses through drinking water and recreational water is likely occurring in the population in the Great Lakes region. The level of disease that may be associated with this exposure can not be measured with current epidemiological and health surveillance methods. In order to accurately estimate health risk related to direct contact with contaminated surface water in the Great Lakes, it is necessary to measure the concentration of viruses and study the survival and transport behavior of viruses in an actual water system, and then incorporate these data into a quantitative risk assessment model. With information on enteric virus occurrence and concentration in aquatic environment, health risk related to exposure to these viruses in the environment can be estimated using a risk assessment framework.

Risk assessment is the process of integrating scientific and assessment data regarding an environmental hazard into a framework to address the risk of exposure and the potential health impacts (Rose and Grimes 2001). Risk assessment frameworks have been used extensively to examine human health risks associated with exposure to toxic chemicals in the environment (e.g. residential area, workplace, heavy-metal contaminated site), health care costs and benefits of developing better management policies and prevention controls. Risk assessment is the first step of risk analysis, results from risk assessment can be used to direct risk management and risk communication. For example, policy makers may refer to risk assessment results to decide if additional treatment of a contaminated source is necessary to significantly reduce effect on public health. Quantitative microbial risk assessment (QMRA) has been used to assess health risks associated with waterborne pathogens in drinking water and treatment requirements

under the Surface Water Treatment Rule (Gerba et al. 1996; Haas et al. 1996; Rose et al. 1991).

Risk assessment models of several microbial pathogens in drinking water and recreational water have been developed (Haas et al. 1993; Haas et al. 1999; Regli et al. 1991; Rose et al. 1991; van Heerden et al. 2005). Data gaps in previous studies included the lack of actual virus monitoring and survival data. Most virus risk assessments use virus concentration data from the literature and rarely include actual virus monitoring data. A basic QMRA framework suggested by the National Research Council (NRC) includes four steps:

1. Hazard identification (identify types of pathogens and description of illnesses caused by the pathogens, hospitalization, and mortality rate associated with diseases caused).

2. Dose-response assessment (quantitative relationship between dose and outcome described as a probability).

3. Exposure assessment (prevalence, concentrations, distribution of a particular pathogen in time and space in contaminated water or food consumed).

4. Risk characterization (the quantitative likelihood of potential adverse health outcome based on the above).

Dose-response of viral pathogens in human subjects has not been widely studied because of high cost associated with these experiments, ethical issues as well as difficulty in culturing and administering desired dose (most enteric viruses are non cultivable). Viruses with a published dose response relation in either human or animals include adenovirus, coxsackievirus, echovirus, poliovirus, and rotavirus (Gerba et al. 1996;

Crabtree et al. 1997; Haas et al. 1999; Mena et al. 2003). Dose-response for rotavirus is the most widely-used model for assessing health risk through the ingestion route because of the high quality human data set and its high potency. Dose-response for adenovirus is the only model for virus exposure through inhalation of contaminated aerosol. These dose-response models have been used to calculate probability of infection via various exposure pathways including recreational exposure (Mena 2002).

Hypotheses and Objectives

Adenoviruses may be one of the best viral pathogens for monitoring fecal contamination of water and evaluating public health risk because of their high concentrations and survival in recreational water mentioned previously. Adenoviruses have been detected in Great Lakes waters and an adenovirus probability of infection model has been developed (Crabtree et al. 1997; Fong et al. 2007; Xagoraraki et al. 2007). To date, however, no systematic examination of adenovirus and the public health risks from polluted waters have been undertaken in freshwater systems. Thus there is a need for development of sensitive and specific methods for detecting adenoviruses in water, and addressing the levels of adenovirus contamination in Great Lakes waters for the interpretation of the risk of waterborne diseases.

The overall objective of this research was to develop an understanding of the human health risks posed to people in waterways and beaches in Michigan associated with the presence of viral pathogens, particularly adenoviruses, from sewage inputs. Through field-based experiments and transport models, a risk assessment was developed for recreating at Great Lake beaches. In this study, dose-response probability of infection

model for rotavirus (representing high risk) and dose-response for echovirus 12 (representing low risk) coupled with actual virus monitoring data from sewage, CSO and surface water were used to compare health risks associated with contamination sources for the Great Lakes beaches.

The main hypotheses were: 1) Human adenoviruses as important waterborne pathogens, are present consistently in sewage and rivers receiving sewage effluents in Michigan; 2) Wastewater treatment does not efficiently remove adenoviruses (or their DNA); 3) virus transport and attenuation in a river can be assessed by running a tracer study using a bacteriophage (i.e. P22) as a surrogate for waterborne viruses; 4) the risks for adenovirus exposure at Great Lakes beaches are elevated to above an acceptable risk for recreational waters during the swimming season.

The four specific objectives of this research were 1) to evaluate wastewater treatment efficiency for removing adenoviruses; 2) to study the prevalence of adenovirus species in wastewater and fecally-contaminated surface water; 3) to use bacteriophage P22 as a biological tracer and viral surrogate, and to study the transport of this viral surrogate in surface water; 4) to develop a risk assessment model for recreational use of the lower Grand River and beaches at Lake Michigan based on the virus prevalence data and results from the tracer study.

To meet these specific goals, in this research:

a) adenovirus distribution and loading in sewage contaminated water (i.e. influent and effluent of a wastewater treatment plant, river and beaches influenced by wastewater discharge) using real-time PCR was determined [Chapter 2].

b) the transport behavior of viral contaminants in the lower Grand River in Grand Rapids, MI to Lake Michigan by using a viral tracer (bacteriophage P22) compared to a fluorescent tracer was evaluated [Chapter 3].

c) adenovirus loading data, virus inactivation and attenuation data from the tracer study was used in a risk assessment model to estimate public health risks associated with recreational activities, such as wading, fishing, boating and swimming in the lower Grand River and beaches on Lake Michigan [Chapter 4].

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CHAPTER 2

MONITORING OF HUMAN VIRUSES IN SURFACE WATER AND WASTEWATER IN MICHIGAN

Abstract

Enteric viruses are important pathogens of surface water and have frequently been detected from waters of the Great Lakes. Human adenoviruses have been suggested as index viruses because of their high prevalence and persistence in aquatic environments. In this study, we aim to quantify adenoviruses in different aquatic environments: waste water, surface water and combined sewer overflow discharges. Raw sewage (n=13), primary-treated effluent (n=13), secondary-treated effluent (n=10) and chlorinated effluent (n=10) were collected from an East Lansing waste water treatment plant between August 2005 and August 2006. Surface water samples (n=26) were collected from seven sampling sites at the lower Grand River in Grand Rapids, MI. Combined sewer overflow samples (n=6) were collected from CSO retention basin in Grand Rapids, MI. Conventional PCR and real-time PCR assay were used for virus detection and quantification. Adenoviruses were detected in 100% of waste water and CSO discharge samples. Average adenovirus DNA concentration in raw sewage and primary sewage were 1.15×10^6 viruses /L and 1.12×10^6 viruses /L, respectively. Analysis of variance (ANOVA) shows that virus concentrations in CSO samples (average: 5.35×10^5 viruses /L) were not significantly different from virus concentration in raw sewage and primary-treated effluent (p-value: 0.39). In addition, adenovirus removal was less than 2 $\log_{10}$ (99%) at the East Lansing wastewater treatment plant. Adenovirus type 41 (60%), type

12 (29%), type 40 (3%), type 2 (3%) and type 3 (3%) were predominant in raw sewage and primary effluents (n=28). Multiple adenovirus serotypes were detected in six samples. Six of twenty surface water samples analyzed by real-time PCR and showed virus concentration above detection limit (average: 7.8 $x10^3$ viruses /L). This research demonstrated that wastewater effluents and surface water in Michigan contain high levels of viruses and may not be suitable for full-body recreational activities. High concentration of adenoviruses in these waters may be due to inefficient removal during wastewater treatment and high persistence of viruses in the environment.

Introduction

Enteric viruses are important waterborne pathogens. They are frequently isolated from fecal-contaminated water and have been linked to numerous waterborne outbreaks (Craun 1991; Tani 1995; Jiang et al. 2001; Lee and Kim 2002). This group of pathogens includes adenoviruses, rotavirus, hepatitis A virus, noroviruses and enteroviruses. In the Great Lakes region, enteric viruses were isolated from recreational beaches and ground water for municipal usage, indicating elevated public health risk when consuming or coming into contact with these waters (Fong et al. 2007; Xagoraraki et al. 2007) Although recent developments in molecular detection assays substantially increases detection of viruses from waters, from a management standpoint, it is impractical to test for all viruses when determining microbial quality of water. Here, we proposed using adenoviruses as an index virus for monitoring water quality.

Adenovirus, which has a high prevalence in water, has been suggested as a preferred candidate as an index organism for viral pathogens because they fit some of the

criteria of an ideal indicator (Pina et al. 1998; Griffin et al. 2001). Human adenoviruses (HAdVs) are present in higher concentrations in sewage than other enteric virus; and it is estimated that more than 90% of the population are seropositive for adenovirus. An infected patient may excrete up to 10^{11} viral particles per gram of feces (D'Ambrosio et al. 1982; Haramoto et al. 2005; Jiang et al. 2005; Pina et al. 1998; Wadell et al. 1987).

Adenoviruses were first isolated from humans and identified as the causative agent of epidemic febrile respiratory disease among military recruits in the 1950s (Hilleman 1954; Rowe 1953). Human adenoviruses are the second most important viral pathogen of infantile gastroenteritis after rotavirus (Basu et al. 2003; Cruz et al. 1990; Topkaya et al. 2006; Uhnoo et al. 1984). Serotypes of adenoviruses have different tissue tropisms (i.e. cells and tissues of a host which support growth of a particular virus) and have been found to cause symptomatic infections in several organ systems, including the respiratory system (pharyngitis, acute respiratory disease and pneumonia), eye (conjunctivitis), gastrointestinal tract (gastroenteritis), central nervous system (meningoencephalitis) and genitalia (urethritis and cervicitis) (Crabtree et al. 1997; Kapikian 1992). Human adenovirus types 40 and 41 have been associated with gastroenteritis in children, while human adenovirus type 4 is linked to persistent epidemics of acute respiratory disease in the United States (Cruz et al. 1990; McNeil 1999). It was once estimated that 2-7% of all lower respiratory tract illnesses in children may be caused by adenoviruses (Brandt et al. 1969; Foy et al. 1973).

Transmission includes the fecal-oral route and inhalation of aerosols. Adenoviruses have been associated with outbreaks in different settings, including military camps (Kolavicâ-Gray et al. 2002; Chmielewicz et al. 2005; Kajon et al. 2007),

hospitals (Chaberny et al. 2003; Hatherill et al. 2004; Jalal et al. 2005), and daycare centers (Akihara et al. 2005; Shimizua et al. 2007) and schools (Harley et al. 2001). Adenoviruses waterborne outbreaks have involved swimming in swimming pools (Turner et al. 1987; Papapetropoulou et al. 1998).

The aim of this part of the research was to evaluate the presence of adenoviruses, from sewage inputs. Raw sewage, wastewater effluent, CSO discharges and surface water in the lower Grand River, Michigan were surveyed for the occurrence and concentration of human adenoviruses. Real-time PCR was used for quantification of adenoviruses. Predominant adenovirus genotypes in sewage were determined in order to evaluate the role of surface water contamination as a possible vehicle for the transmission of adenovirus, and the relevance of adenoviruses as an additional tool in water quality assessment.

Materials and Methods

Sample collection and concentration. Grab water samples were collected from the East Lansing wastewater treatment plant approximately once a month between August 2005 and August 2006. Wastewater treatment processes in the East Lansing wastewater treatment plant consist of primary treatment (sedimentation), secondary treatment (aeration, activated sludge and secondary sedimentation) and tertiary treatment (chlorination, rapid gravity sand filters, dechlorination and post-filtration aeration).

Surface water samples were collected from the lower Grand River in Kent and Ottawa counties during a one-week intensive study in June 2005 and intermittently between June 2005 and August 2007. A total of 16 surface water samples were collected from different sites at the lower Grand River during the intensive study (Figure 2.1).

Following the intensive study, several sites were chosen for continued monitoring because of high bacterial indicator concentrations (data not shown) and detection of HAdV DNA from two of those sites (Deer Creek and Sixth Street Park) (Figure 2.1). Our sample collection locations included three beach sites (Rosy Mound, North Shore and North Beach Park) and four park sites (Riverside Park, Deer Creek Park, Grand River Park and Sixth Street Park).

Between February and June 2008, six combined sewer samples were collected from CSS retention basin in Grand Rapids, MI.

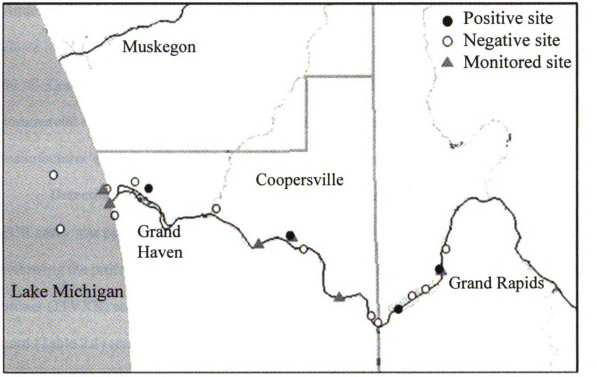

Figure 2.1 Sampling sites along the lower Grand River during the intensive study in June 2005, sites that was positive for adenoviruses (●), sites that were negative for adenovirus (○) and sites selected for continued monitoring between 2005 and 2007 (▲).

The procedures of Haramoto et al. (2004) were used for water filtration and concentration for viruses. Briefly, between 500 ml and two liters of sewage or surface water were filtered through a 90-mm, type HA, negatively charged membrane (Millipore, Billerica, Mass.) with 0.45-μm pore size. A volume of 100 ml of 0.5 mM H_2SO_4 was then passed through the membrane, and viral particles were eluted with 10 ml of 1 mM NaOH. Eluates were stored in a tube containing 0.1 ml of 50 mM H_2SO_4 and 0.1 ml of 100x Tris-EDTA (TE) buffer for neutralization before further concentration. All 10-ml eluates were stored at - 20°C.

For further purification and concentration, eluates were thawed and centrifuged in Amicon Ultra 100K concentrator columns (Millipore). The final volume of concentrated eluate recovered for each sample was approximately 200 μl. Concentrates were stored at -80 °C. Concentrated samples were extracted for viral nucleic acids and purified through commercial spin columns from Qiagen (QIAGEN, Valencia, CA) following the manufacturer's protocol. Purified viral DNA was eluted in 60 μl of RNase-free water.

Detection and quantification of human adenoviruses. A real-time TaqMan PCR assay was performed for the quantification of HAdV DNA in water samples following the protocol by Xagoraraki et al. (2007). The forward primer (JTVXF), reverse primer (JTVXR) and TaqMan probe (JTVXP) designed by Jothikumar et al. (2005) were used (Table 2.1) and targeted the hexon gene of HAdV. PCR reaction mixtures contained 10 μM forward primer, 10 μM reverse primer, 10 μM probe, 5 μl DNA template, and PCR-grade water for a total volume of 20 μl. The PCR conditions were as follows: hot-start denaturation step at 95 °C for 15 min, followed by 50 cycles with a 95 °C denaturation for 10 s, 55 °C annealing for 30 s and 72 °C elongation for 15 s. All

amplification reactions were carried out in duplicate. PCR products were selected randomly for visualization by gel electrophoresis on a 2 % average strength Omnipur agarose gel (EM Science, Darmstadt, Germany). The gel was stained with gelStar nucleic acid stain and viewed under UV light. Both real-time PCR assays were performed in a Roche LightCycler® 2.0 Instrument (Roche Applied Sciences, Indianapolis, IN). The samples (i.e., viral DNA extracts) and standards were each run at least in triplicate. All PCR runs included a negative control reaction (PCR-grade H_2O without template) and a positive control reaction. The crossing point (Cp) of each PCR reaction was automatically determined by the LightCycler® Software 4.0 and used to calculate the hexon gene concentration. The concentrations of HAdVs in the river water samples from the studied recreational sites was normalized from hexon gene copies per liter to viruses per liter using a ratio of one (i.e., each HAdV viral particle consists of one copy of hexon gene as previously reported). The detection limit of this real-time PCR assay was determined to be 10 copies/PCR reaction through serial dilution of cloned PCR amplicon (Xagoraraki et al. 2007).

Nested PCR and adenovirus DNA sequencing. Adenovirus species present in water samples were identified by amplifying and sequencing of the hypervariable region 1-6 of adenovirus hexon gene. Samples that were positive by the real-time PCR assay were amplified using a conventional PCR assay prior to sequencing. Primers used and PCR conditions were as described by Lu and Erdman 2006 (Table 2.1); primers AdhexF1 and AdhexR1 yield amplicons ranging in size from 764 to 896 bp. If insufficient DNA was amplified from the first PCR, a second PCR was performed using internal primers AdhexF2 and AdhexR2, which yields amplicons between 688 and 821 bp (Lu and

Erdman 2006). The sensitivity of this conventional nested-PCR assay was determined by limited dilution experiments of pure adenovirus stock in cell culture lysates (~10^8 viral particles ml $^{-1}$). Virus stocks were extracted for DNA, serially diluted to 10^0 virus DNA copies/reaction (as quantified by real-time PCR) and assayed by conventional PCR. The detection limit of this conventional PCR was between 10 and 100 virus DNA copies/reaction.

Amplicons were visualized on an agarose gel and purified using a QiaQuick DNA purification kit (Qiagen, Valencia, CA, USA) prior to sequencing. All HAdV positive samples were identified by sequencing of at least two independent PCR products in both directions, i.e., each nucleotide was determined at least four times. Sequencing was performed by the Research Technology Support Facility at Michigan State University. Sequences were determined on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems).

Sequence analysis. Sequences were aligned with hexon protein sequences of selected adenovirus prototype strains available from Genbank using web-based ClustalW (http://www.ebi.ac.uk/clustalw/) with the following default settings: gap opening (10), gap extension (0.2) and hydrophilic residue gap penalties enabled. For analysis of serotypes, approximately 600 bp of sequences of the PCR products were used. Amino acid alignment of the sequences was performed in ClustalW. A phylogenetic tree was then constructed using the neighbor-joining method. The branching confidence was estimated by bootstrapping with 1000 resamplings in MEGA 4.1 (Beta). The GenBank accession numbers of adenovirus prototypes used in alignment and phylogenetic analysis are: species A: human adenovirus type 12 (AB330093), type 18 (DQ149610), and type

31 (AB330112); species B1: human adenovirus type 3 (EF494650), and type 7 (AC000018); species B2: adenovirus type 35 (AC000019); species C: adenovirus type 1 (AC000017), and type 2 (EU867481); species D: adenovirus type 8 (AB361058), type 19 (AB330133), and type 51 (AB330132); species E: adenovirus type 4 (AB330085); and species F: adenovirus type 40 (AB330121) and type 41 (EF429128).

Table 2.1 Primers and probes used in the detection of adenovirus DNA in water samples.

Assay	Primer/Probe	Name (polarity)	Gene amplified	Sequence (5-3)	T_m(°C)	Amplicon size (bp)	Reference
HAdV TaqMan PCR	Primer	JTVXF (+)	Hexon	GGACGCCTCGGAGTACCTGAG	68	95	Jothikumar et al. 2005
		JTVXR (-)		ACIGTGGGGTTTCTGAACTTGTT	63		
	Probe	JTVXP (+)		CTGGTGCAGTTCGCCCGTGCCA	78		
HAdV nested PCR	Primer	AdhexF1(+)	Hexon	TICTTTGACATICGIGGIGTICTIGA	46	764-896	Lu and Erdman 2006
		AdhexR1(-)		CTGTCIACIGCCTGRTTCCACA	53		
	Nested primer	AdhexF2(+)	Hexon	GGYCCYAGYTTYAARCCCTAYTC	45	688-821	
		AdhexR2(-)		GGTTCTGTCICCCAGAGARTCIAGCA	59		

Virus recovery efficiency. The virus recovery efficiency of the virus adsorption-elution method by Haramoto et al. (2005) was evaluated in two seeded studies. In the first experiment, the efficiency of the method in recovering naturally occurring adenovirus in sewage was tested. Two different volumes (3.8 liters and 700 ml) of MilliQ water were inoculated with raw sewage (collected from East Lansing wastewater treatment plant) in the ratio of 100,000:1, 10,000:1, 1,000:1, 100:1 and 10:1. Inoculated water was mixed at room temperature for at least an hour before processing. Seeded samples were concentrated, extracted and detected by real-time PCR following protocols described previously. For each concentration, three to five replicates were tested. For the sewage-seeded experiment, viruses were eluted directly from the membrane with 1mM NaOH.

In the second experiment, the efficiency of the method in recovering bacteriophage P22 from environmental water was evaluated. Bacteriophage P22 was inoculated into one-liter of surface water samples and CSO discharge samples in the ratio range of 10:1 and 5000:1. Both eluate and filtrate were assayed for infectious phage using phage assay (Adams 1959). Retention ratio, $R_{filtration}$, by the filter was calculated as follow:

$$R_{filtration} = \frac{(Total_{seeded} - Total_{filtered})}{Total_{seeded}}$$

where $Total_{seeded}$ and $Total_{filtered}$ are the total number of virus seeded and total number of virus after filtration, respectively.

Bacteriophage P22 was eluted from the membrane using two methods: 1) 20-ml of 1mM NaOH was dispensed onto the membrane and the eluent was collected in a 50ml-tube with neutralizing buffer; 2) membrane was transferred into a 50-ml tube containing

20ml 1mM NaOH, the 50-ml tube was then pulse-vortexed for 10 s to elute the viruses, membrane was removed after vortexing and buffers (50mM H_2SO_4 and 10X TE buffer) were added to neutralize the NaOH. Percent recovery was calculated using the formula below:

$$\%\ \text{recovery} = \frac{\text{number of bacteriophage P22 eluted}}{\text{number of bacteriophage P22 seeded}} * 100\,\%$$

Results and Discussion

Waste water. Between August 2005 and August 2006, 46 waste water samples (13 raw sewage, 13 primary-treated, 10 secondary-treated and 10 tertiary-treated samples) were collected. Wastewater samples were assayed by real-time PCR for determining the concentration of adenoviral DNA present (Figure 2.2). Adenoviruses were consistently isolated in significantly higher concentrations from raw sewage and primary effluent compare to secondary and tertiary effluents (p-value: <0.001). The concentrations of HAdVs ranged between 2.63 x 10^5 and 2.82 x10^6 viruses/L (mean: 1.15 x10^6 viruses/L) in raw sewage, 53.7 and 4094 viruses/ml (mean: 1.12 x 10^6 viruses/ L) in primary effluent, 1.05 x 10^3 and 4.42 x 10^4 viruses/ L (mean: 2.0 x 10^4 viruses/L) in secondary effluent and 1.35 x 10^4 and 4.28 x 10^5 viruses/ L (mean: 8.3 x 10^4 viruses/ L) in tertiary effluent. A seasonal trend was not observed for the concentration of adenoviruses DNA in wastewater samples. Figure 2.3 and Table 2.2 shows average adenovirus concentration and $\log_{10}$ removal ratio of human adenovirus at the East Lansing wastewater treatment plant. The ratio removal was calculated by using the average virus concentration in a particular treatment process over the virus concentration in the raw sewage. The overall adenovirus $\log_{10}$ removal from raw sewage to tertiary

effluent was approximately 1.14 $\log_{10}$. Tukey's honestly significant difference test showed that virus concentrations were not significantly different between raw sewage and primary effluent (p-value: 0.74), and between secondary effluent and tertiary effluent (p-value: 0.11).

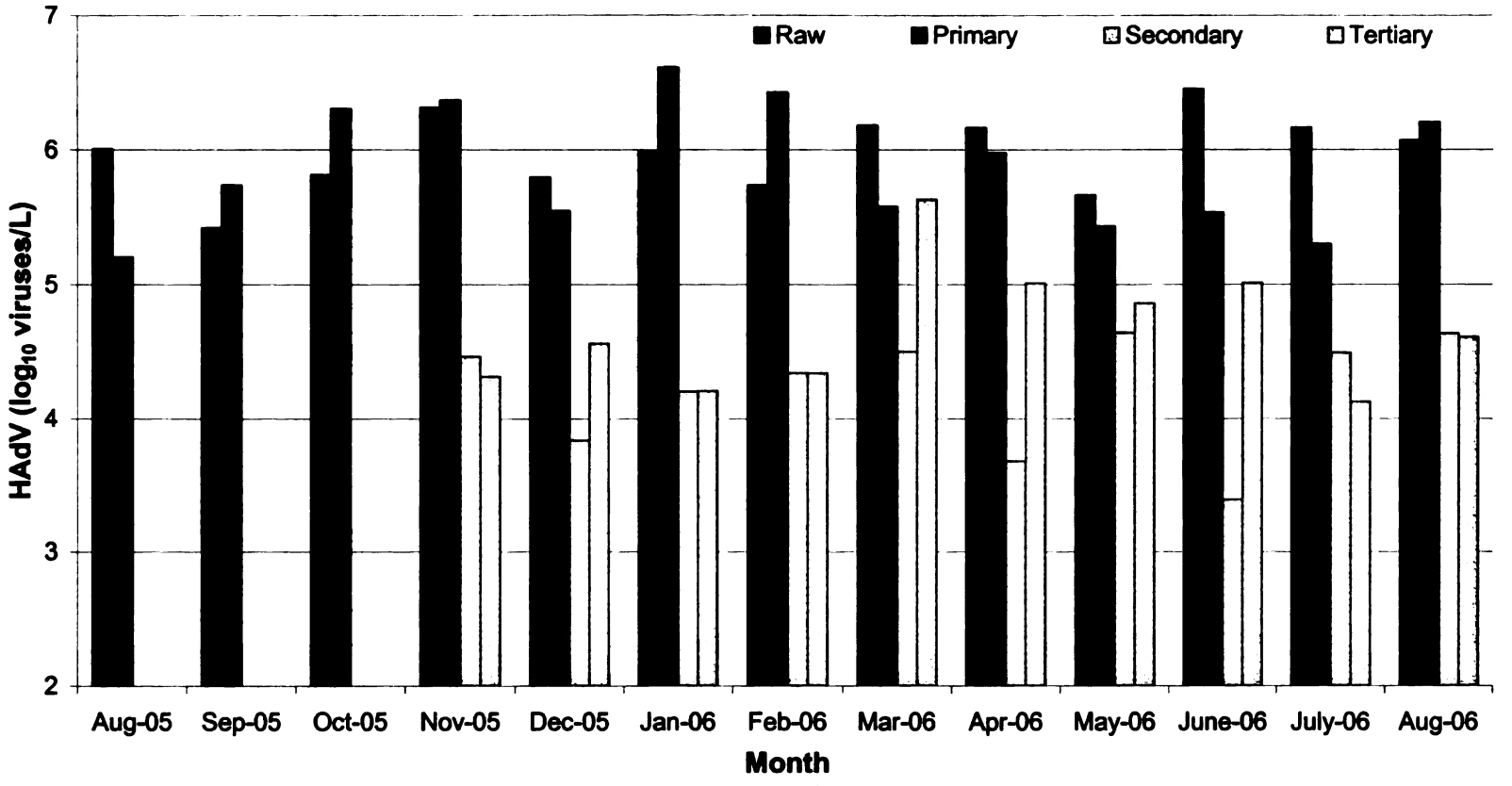

Figure 2.2 Concentrations of HAdV (log_{10} viruses/L) in waste water collected from an advanced wastewater treatment plant in East Lansing, Michigan between August 2005 and August 2006.

Table 2.2 Arithmetic mean and range of adenoviruses detected from each treatment step and average $\log_{10}$ removal compared to virus concentration in raw sewage.

Sample Type	Number of samples (n)	Mean (10^3 viruses /L)	Range (10^3 viruses/L)	Log Removal
Raw	13	1152*	263-2817	
Primary	13	1123*	53.7-4094	0.01
Secondary	10	20**	1.05-44.2	1.77
Tertiary	10	83**	13.5-428	1.14

* virus concentrations were not significantly different between raw sewage and primary effluent (p-value: 0.74).

** virus concentrations were not significantly different between secondary effluent and tertiary effluent (p-value: 0.11).

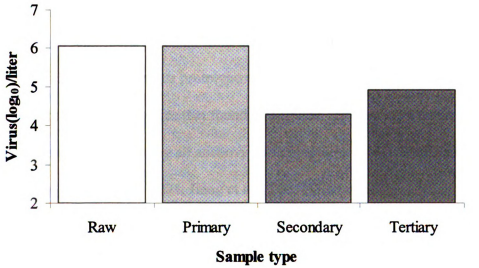

Figure 2.3 Mean adenovirus concentrations ($\log_{10}$ virueses/L) in raw sewage, primary effluent, secondary-treated effluent and tertiary-treated effluent.

A total of 28 wastewater samples (resulted in 35 isolates) were amplified by nested PCR for genotyping (Table 2.3). Samples that produced a high enough concentration of amplified adenoviral DNA were sequenced. Sequences were cropped to approximately 600 bp in length and aligned in ClustalX 2.0.10, with reference sequences from GenBank. Blast analysis of all amplicon sequences identified 92 to 100 % sequence identity with reference prototypes listed below, number of insertion/deletion between amplicons and closest prototypes ranged between 0 and 6 (Table 2.3). Five adenovirus prototypes (adenovirus type 2, 3, 12, 40 and 41) were dominant in raw sewage and primary effluent from wastewater in East Lansing, MI. Adenovirus type 41 (21/35; 60%) was the most frequently isolated adenovirus, followed by adenovirus 12 (11/35; 28.5%), adenovirus 40 (1/35; 2.9%); adenovirus 2 (1/35; 2.9%) and adenovirus 3 (1/35; 2.9%) (Fig. 2.4). A neighbor-joining tree was generated from alignment of the nucleotide sequence of amplicons obtained from this study and hexon gene sequences on GenBank corresponding to adenovirus prototype species A to F (Fig. 2.5). This finding is in agreement with other studies that found that enteric serotypes (adenovirus type 40 and 41) are predominant among all adenovirus serotypes in aquatic environments, especially in sewage (Santos et al. 2004; Jiang et al. 2005; Haramoto et al. 2007).

Table 2.3 Comparison between human adenovirus isolates detected in raw sewage and primary effluent of East Lansing wastewater treatment plant and their closest prototypes on GenBank. Adenovirus prototypes used in the comparison are: species A: human adenovirus type 12 (AB330093), type 18 (DQ149610), type 31 (AB330112); species B1: human adenovirus type 3 (EF494650), type 7 (AC000018); species B2: adenovirus type 35 (AC000019); species C: adenovirus type 1 (AC000017), type 2 (EU867481); species D: adenovirus type 8 (AB361058), type 19 (AB330133), type 51 (AB330132); species E: adenovirus type 4 (AB330085); and species F: adenovirus type 40 (AB330121) and type 41 (EF429128).

Date	Sample Type	Highest score prototype		No. of insertion/deletion
		type	% nt identity	
Aug-05	raw	41**	100	0
Sep-05	raw	12*	93	3
		41**	100	0
Oct-05	raw	12*	93	3
		41**	100	0
Nov-05	raw	41	98	0
Dec-05	raw	12	99	1
		2	93	6
		41	95	2
Jan-06	raw	41**	100	0
Feb-06	raw	3	100	0
Mar-06	raw	41**	100	0
Apr-06	raw	12*	93	3
		41**	100	0
May-06	raw	12*	93	3
June-06	raw	12*	93	3
July-06	raw	41**	100	0
Aug-06	raw	12	92	4
		41***	99	0
June-07	raw	41**	100	0
July-07	raw	12	98	0
Aug-05	primary	41**	100	0

Table 2.3 cont'd

Sep-05	primary	41**	100	0
Oct-05	primary	41***	99	0
Nov-05	primary	12*	93	3
		41	99	1
Dec-05	primary	41**	100	0
Jan-06	primary	41**	100	0
Feb-06	primary	41**	100	0
Mar-06	primary	40	99	0
Apr-06	primary	12	93	3
May-06	primary	41**	100	0
June-06	primary	41**	100	0
Aug-06	primary	41**	100	0
July-07	primary	12	96	0

* These isolates were 100% homologous to each other and were most closely related to prototype HAdV 12.

** These isolates were 100% homologous to each other and prototype HAdV 41.

*** These two isolates were 100 % homologous to each other and were most closely related to prototype HAdV 41.

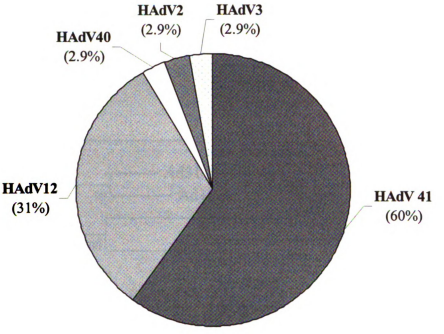

Figure 2.4 Adenovirus serotypes isolated from raw sewage and primary effluent samples collected from East Lansing waste water treatment plant (n=35).

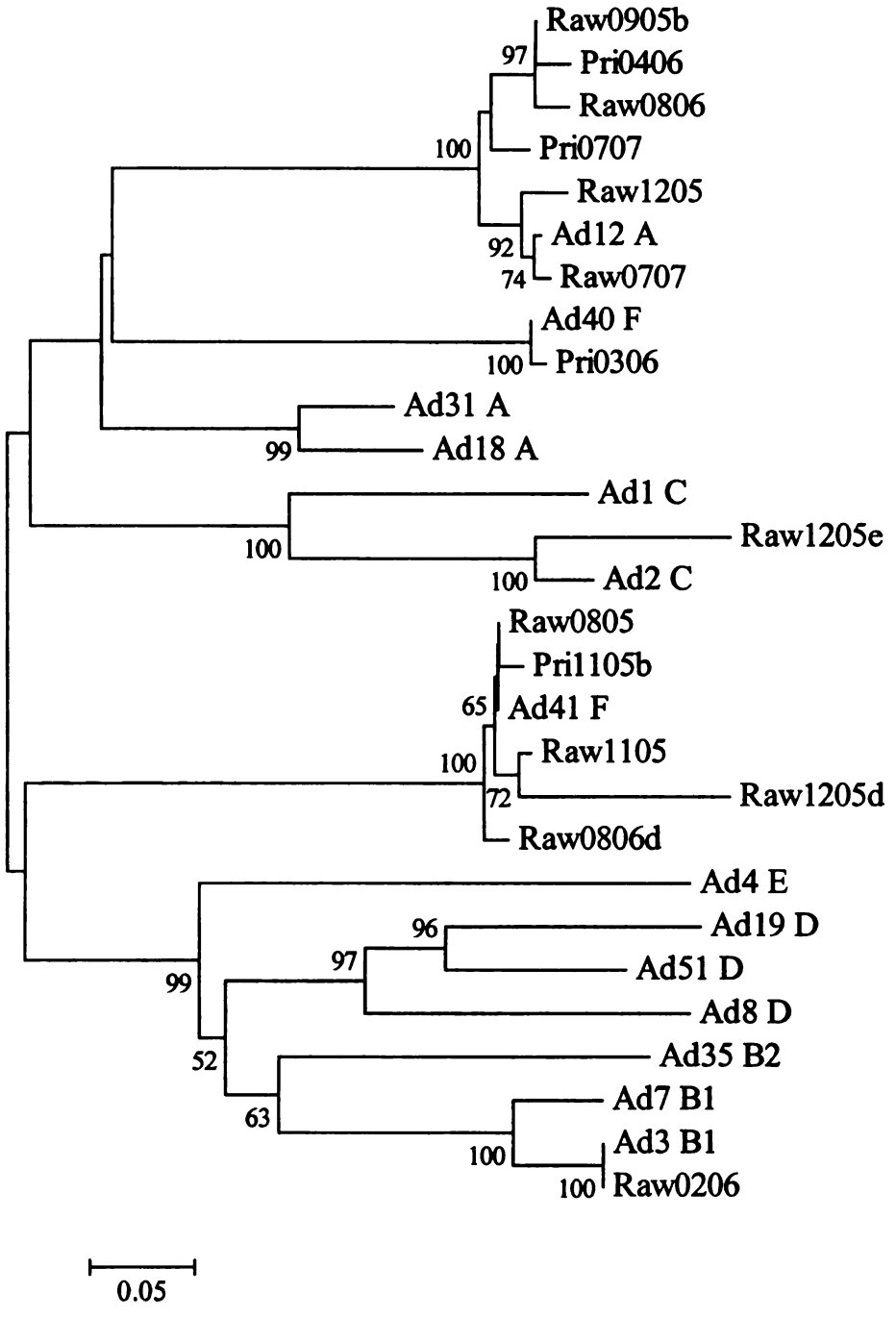

Figure 2.5 A neighbor-joining tree of adenovirus hexon amplicons detected in waste water. The scale indicates nucleotide substitutions per position. Numbers above the branches indicate boostrap percentage (>50) based on 1000 replicates. Reference strains of human adenovirus were selected from GenBank under the accession number indicated in the text.

In this study, adenovirus 41 was identified as the predominant serotype in sewage, followed by adenovirus type 12, 40, 2 and 3. This trend is agreeable with results from other environmental studies (Santos et al. 2004; van Heerden et al. 2005). Santos et al. (2004) isolated adenoviruses 40 and 41 from 62 out of 69 sewage and surface water samples collected in San Paulo city, Brazil, over a three-year period. In South Africa, adenovirus types 2, 40, 41 and species D HAdVs were isolated from treated drinking water and river water (van Heerden et al. 2005). However, though enteric adenovirus (serotype 40 and 41) were most frequently identified in river water, treated drinking water was predominated by species D human adenovirus. In addition, Gray et al. (2007) reported the most prevalent serotypes among civilian (n=1608) were types 3 (34.6%), 2 (24.3%), 1 (17.7%) and 5 (5.3%). Among specimen collected from gastrointestinal tract, only 26 of 234 (11.1%) were adenovirus species F (i.e. adenovirus 41). This suggests that adenovirus 41 may have greater persistence in natural environments than other adenovirus serotypes.

Adenoviruses species F (types 40 and 41) have been identified as one of the most prevalent viruses in the etiology of childhood gastroenteritis in developed countries (Topkaya et al. 2006). Several researchers claimed that adenovirus type 41, the most prevalent serotype isolated in the current study, had gradually replaced serotype 40 as the predominant serotype isolated from gastroenteritis patients globally starting in the 1980s and was currently identified as the most prevalent serotype in wastewater (Grimwood et al. 1995; Logan et al. 2006; Shinozaki et al. 1991; Yamashita et al. 1995). These findings were consistent with the fact that in the current study adenovirus 40 was detected in only one out of 35 isolates. Adenovirus type 12, the second most prevalent adenovirus isolated

in this study (11/35), was often associated with meningoencephalitis and no waterborne outbreak related to it has been identified to date. However, it was recently identified as the etiologic agent of a diarrhea outbreak in a hematology ward in London (Jalal et al. 2005).

Adenoviruses type 2 and 3 were both isolated from one waste water sample. They are generally associated with pneumonia and childhood respiratory diseases (Horwitz 2001). Adenovirus type 3 has also been identified as the etiologic agent for pharyngoconjunctival fever and conjunctivitis in several outbreaks associated with contaminated recreational water (Foy et al. 1968; Martone et al. 1980; McMillan et al. 1992). Gray et al (2007) identified adenovirus type 3 and type 2 as the two most prevalent adenovirus types in civilian specimens (34.6% and 24.3%, respectively). Adenovirus type 3 is also the second most prevalent (2.6%) in specimens collected from military trainees (Gray et al. 2007).

The slightly higher concentration of adenovirus in tertiary effluent (after tertiary treatment) may be the artifact of real-time PCR caused by the presence of a higher level of inhibitory substances in samples after secondary treatment. This is in concert with the findings by He and Jiang (2005), in which higher concentrations of adenovirus DNA was detected in secondary effluent.

Surface Water. Surface water samples were collected from the lower Grand River during a one-week intensive study in June 2005 and periodically over a two-year period between 2005 and 2007. During the intensive study, a total of 16 surface water samples were collected from different sites at the lower Grand River and human adenovirus was detected by conventional PCR in four samples (16%) (Table 2.4).

Following the intensive study, several sites were chosen for continued monitoring because of high bacterial indicators concentration (data not shown) and detection of HAdV DNA from two of 16 intensive sites (Deer Creek and Sixth Street Park). Over the two-year period, 26 samples were collected among the seven sampling sites (North Beach Park, North Shore, Riverside Park, Rosy Mound, Deer Creek park, Grand River Park and Sixth Street Park) (Table 2.5). Adenovirus DNA was detected in four of six samples collected in 2005 through conventional PCR. For the 20 remaining samples collected in 2006 and 2007, adenovirus concentration ranged between < 0.01 and 66 viruses /ml (mean: 7.76 viruses/ml) was quantified in these samples by real-time PCR. Six of these twenty samples with adenovirus DNA concentration were above the detection limit.

Table 2.4 Surface water samples positive for adenovirus DNA by nested PCR collected during one-week intensive sampling of the lower Grand River, Michigan, June 19-June 24, 2005. Samples were arranged in the order of sampling sites, from Lake Michigan to the most inland site on Grand River, Riverside Park, Grand Rapids, MI. Refer to Figure 2.1 for location of sampling sites.

Site	Nested PCR results
Lake Michigan 1	-
Lake Michigan 2	-
Rosy Mound	-
N. Beach Park	-
Petty's Bayou	+
Spring Lake	-
Deer Creek Park	+
Lamont	-
Crockery Creek	-
Johnson Park 1	-
Johnson Park 2	-
Kent county 1	+
Kent county 2	-
Kent county 3	-
Sixth Street Park	+
Riverside Park	-

Table 2.5 Nested PCR detection of HAdV (viruses /ml) from surface water samples from the lower Grand River, MI, between 11/2/05 and 8/9/07.

Site name	Conventional/real-time PCR results Sampling dates								
	11/02/05	11/16/05	12/14/05	01/10/06	03/21/06	06/12/06	07/11/06	07/26/07	08/09/07
Rosy Mound	-								
Sixth Street Park		+	+		<0.02	<0.04		**65.82**	<0.02
Deer Creek Park		+	+		<0.04	<0.05	<0.01	<0.04	**1.89**
North Beach Park				**0.08**	<0.03	<0.02	<0.02		
Riverside Park	-			<0.03	<0.04	<0.03			
North Shore								**59.75**	<0.03
Grand River Park								**27.27**	**0.09**

CSO samples. Six CSO samples were collected from a wastewater retention basin in Grand Rapids, between February and June 2008. Adenovirus DNA was detected from 100 % of CSO samples with concentrations ranged between 6.02×10^4 viruses/L and 1.32×10^6 viruses/L (average: 5.35×10^5 viruses/L; standard deviation: 500.81) (Fig. 2.6). The concentration of adenovirus DNA in the CSO discharge was not significantly different from adenovirus DNA concentration in raw sewage and primary-treated samples (p-value: 0.39). This suggests that in the case where virus concentration in CSO samples is unknown, virus concentrations in raw sewage or primary-treated samples may be used as substitutes for estimating total virus discharge during a CSO event.

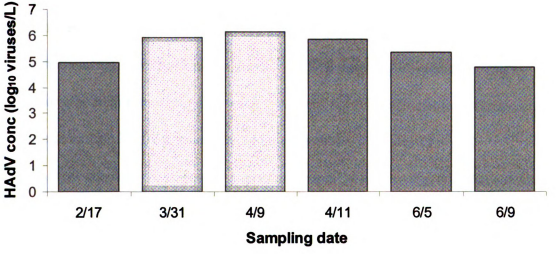

Figure 2.6 Human adenovirus concentrations ($\log_{10}$ viruses /L) in CSO discharge samples collected from Market St retention basin in Grand Rapids, Michigan.

Virus recovery efficiency. The virus recovery efficiency of the virus adsorption-elution method by Haramoto et al. (2005) was evaluated in two seeded studies, a sewage seeded study and a bacteriophage P22 seeded study. For the sewage seeded study, detection limit is also affected by sample volumes. Conventional nested-PCR was able to detect adenovirus DNA in 1:10000 dilution in the larger volume tested (0.38ml of sewage seeded in 3.8 L) but only up to 1:1000 dilution in the smaller volume tested (0.7ml of sewage in 700 ml MilliQ water).

In the second experiment, bacteriophage P22 was seeded into river water. Bacteriophage P22 was inoculated into one-liter of surface water samples in the virus to volume ratio range of 10:1 and 5000:1. The P22 recovery was determined by plaque assay. Preliminary results by plaque assay showed that elution of viruses by transferring the membrane into elution buffer (NaOH) followed by pulse-vortexing is one log more efficient than eluting directly from the membrane. The recovery efficiency of eluting directly from the membrane range between 0.001 and 0.11% while pulse-vortexing the membrane in elution buffer gives recovery range between 0.11 and 19.94%.

The low recovery from seeded experiments may be due to loss of viruses during elution step, viruses may not be completely eluted from membrane as the membrane was folded into quarters before vortexing or inactivated during elution. Bacteriophage may not be representative of animal virus recovery because of the sensitivity of low pH during the elution step (McAlister et al. 2004).

Conclusions

In this part of the research there were two general hypotheses I was addressing: 1) human adenoviruses as important waterborne pathogens, are present consistently in sewage and were occasionally detected in rivers receiving sewage effluents in Michigan; 2) wastewater treatment does not efficiently remove adenoviruses (or at least their DNA). The real-time PCR assay used in the current study was not able to evaluate viability of viruses detected after disinfection step, but virus concentration in tertiary treated effluent showed little physical removal.

In general, there is not a consistent seasonal trend for the presence adenoviruses in wastewater and surface water in Michigan but their isolation from surface water can be used to indicate fecal contamination and lack of efficient treatment. In general, waste water treatment reduces level of adenoviruses by 95 % from untreated to treated sewage. Real-time PCR is quantitative and more sensitive than conventional PCR. Quantitative detection of adenoviruses in surface water could give a better understanding of the risk associated with recreation in contaminated water.

The presence of adenoviruses in surface waters does represent to some extent a public health risk. It has been shown that there is a connection between environmental and clinical virus isolates in a given year within specific geographic areas (Sedmak et al. 2003). Sedmak et al. (2003) compared clinical and sewage isolates of enteroviruses from Milwaukee, Wisconsin collected between 1994 and 2002, and found that the predominant clinical serotype was most often the predominant sewage serotype for that year. In this study, typing of isolates from sewage and environmental water may help to alert the community health professionals to predominant and new serotypes that are in circulation

in the populations, providing new insights to vaccine development and other prevention strategies.

Concentrations of human adenoviruses obtained from this study using real-time PCR were the basis of the exposure and were then incorporated into the quantitative risk assessment model for evaluating human health risk from recreation in the lower Grand River after a CSO event (Chapter 4).

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CHAPTER 3

THE USE OF BACTERIOPHAGE P22 AS A TRACER FOR STUDYING VIRAL TRANSPORT AND SURVIVAL IN THE LOWER GRAND RIVER, MICHIGAN, USA

Abstract

More than 50% of water-related acute gastroenteritis cases may be attributable to viruses. Viruses are highly persistent in aquatic environments and their transport in surface water is not fully elucidated. There has been a strong interest in using bacteriophages in tracer studies because they highly resemble pathogenic viruses and more adequately describe colloidal virus transport than dissolved chemical tracers. The objective of this research was to characterize factors affecting virus transport in surface water. In the first part of the study, the transport behavior of bacteriophage P22, an extensively-studied index virus, was evaluated in a dual-tracer study performed on a 40km reach of the lower Grand River in Grand Rapids, Michigan. We examined the performance of bacteriophage P22 in relative to Rhodamine WT (RWT), a conventional chemical tracer. Our analysis indicated that bacteriophage P22 can be successfully used as a tracer in complex surface water environments such as the lower Grand River. Bacteriophage P22 reacts somewhat differently than RWT to surficial geology and transient storage zones indicating different processes contributed to virus attenuation within the same "reach". Estimated bacteriophage P22 attenuation rates were found to be in the range of 0.27 to 0.57 per day. The highest attenuation rate was found in a "reach" with high suspended solids concentration, relatively low dissolved organic carbon content and sediment with high clay content. Virus attenuation estimates, along with other river

parameters (i.e. river discharge, river width, CSO discharge etc.) related to virus transport were then incorporated into an analytical hydrology model for predicting virus arrival time and concentration at a location downstream after a CSO event.

Introduction

Enteric viruses are important etiologic agents for waterborne acute gastroenteritis. More than 100 types of viruses have been isolated from sewage contaminated water and virus related waterborne outbreaks have been reported in marine water, chlorinated-recreational waters, pond, groundwater, and rivers (Bosch 1998; Griffin et al. 2003). Factors that influence their transport in complex surface water systems are not fully elucidated; the majority of studies examining virus transport either were smaller scale studies or were performed in laboratories. Because viruses are present in water as colloidal particles, they may be transported more conservatively than dissolved chemical tracers, such as lithium, bromine, fluorescein dye, and Rhodamine WT (RWT) (Rossi et al. 1998).

Bacteriophages are viruses that infect and replicate in host bacterial cells and have no adverse effect on humans or animals. Bacteriophages have been suggested and used as an index virus for indicating human virus fecal contamination because of their morphological similarity to pathogenic viruses, such as enteroviruses, adenoviruses and noroviruses. In addition, bacteriophages are very host-specific, they only infect bacteria with compatible receptors. Laboratory-strain bacteriophages are increasingly being used as tracers in hydrology studies because they are not present in natural waters, they have a low detection limit, as well as have inexpensive culture and enumeration methods.

Bacteriophages were first used in studying hydrology of aquifers in the early 1970s (Martin and Thomas 1974). Bacteriophages offer several advantages over colored dyes as tracers for water movement: they can easily be obtained in high titer in a relatively small volume (e.g. 10^{14} pfu in 1-10 liters versus 30-40 liters for Rhodamine WT dye) and are less costly than chemical tracers. The phage assay employed examines viable viruses and this allows for examination of both virus transport and virus inactivation in water. Bacteriophages are not visible to the eye, even in high concentrations and this may allow for their application in testing water movements in inhabited urban areas. In addition, because of the host specificity of the bacteriophages, different bacteriophages can be injected at the same time or at different time points in the same water system (Rossi et al. 1998).

Large varieties of bacteriophages have been recovered from diverse geographic areas, such as coliphages (viruses that infect *E. coli*), and bacteriophages P22, PRD1, H/l40, and T2 (Ackermann and DuBow 1987). Bacteriophages range in sizes from ten to several hundred nanometers and differ morphologically (Rossi et al. 1998). In general, bacteriophages have a capsid that encapsulates the genetic material, a neck that connects its capsid to its tail, a tail and tail fibers (for attachment and adsorption into host cells). Bacteriophages are categorized into six families based on their morphology and nucleic acid type (i.e. DNA versus RNA, enveloped capsid versus nonenveloped capsid, tailed versus tailless and contractibility of the tail). Bacteriophages, such as bacteriophage P22 (previously confused with bacteriophage PRD1 in some studies), bacteriophage ΦX174 and bacteriophage MS2 have been used as biological tracers to study water movements and transports of microbiological water pollutants in surface water, groundwater, aquifers

and soil (Dowd et al. 1998; Rossi et al. 1998; John and Rose 2005; Collins et al. 2006). Factors affecting attenuation of bacteriophages/viruses in surface water are complex, and may depend upon interactions of several factors. In general, temperature and sunlight irradiation are considered two most influential factors for virus survival in surface water (Ferguson et al. 2003).

Recovery rate of bacteriophage from water may also be dependent upon water composition. Bacteriophages are readily adsorbed to suspended particulate matter and settle to the bottom of the water column through aggregation. Some previous tracer studies reported inconsistent virus recovery and this may have deterred hydrologists from using phage to monitor wastewater and surface movement. In one of the earliest tracer studies, reported recoveries of phage tracers from waste water were 25% and 0.023% for bacteriophage F52 and bacteriophage T7, respectively (Kinnunen 1978). However, in a more recent surface water tracer study, recovery of bacteriophage type H40/1 was higher, 61%, compared to fluorescein dye recovery of 49% (Rossi et al. 1998).

In Michigan, 33% of nine major rivers tested positive for the presence of viable enteric viruses and they are suspected to be the chief cause of swimming-associated diseases in recreational waters (Jenkins et al. 2005). In older cities in Michigan, such as Detroit, Lansing and Grand Rapids, discharge of untreated/partially treated waste water from the combined sewer systems during heavy precipitation events is one of the main sources of surface water contamination. Though chemical tracers have been used to study contributing factors to flow path of chemical contaminants in surface water, the reliability

of these tracers in predicting microorganism flow paths has not been evaluated. In this study, the performance of a biological tracer, bacteriophage P22 in a complex surface water system (the lower Grand River) was evaluated relative to RWT and was used to determine the relative importance of environmental factors that influence the attenuation of viruses. Site-specific parameters and inactivation rates were obtained from this tracer study to estimate virus arrival times and concentrations at recreational sites downstream from CSO discharge outlets at Grand Rapids area to beaches at Lake Michigan. In addition, analysis based on a transient storage (TS) model by Shen et al. (2008) was compared with parameters obtained from analytical solutions used in this study.

Material and Methods

Phage Identification and Isolation

Bacteriophage P22, which was once confused with bacteriophage PRD1, has been extensively used in groundwater studies as a biological tracer for tracing subsurface flow and shown as a reliable tool for studying the transport of contaminants (Enriquez et al. 2003; Harvey and Ryan 2004; John and Rose 2005). Recently, in an attempt to develop a molecular detection assay for the bacteriophage tracer "PRD1" in our laboratory (Michigan State University (MSU) Water Quality and Health Laboratory), it was shown that the bacteriophage genetically characterized as bacteriophage P22 (MSU strain P22 is available on Genbank with accession number AB362338). The same isolate has been used in many hydrology studies and was misclassified as bacteriophage PRD1. Some of the affected studies are bacteriophage PRD1 publications from laboratories of Dr C. P. Gerba, Dr J. B. Rose (John and Rose 2005; Nicosia et al. 2001; Paul et al. 1995; Paul et

al. 1997) and Dr S. Farrah (Lukasik et al. 2000; Lukasik et al. 2003; Scott et al. 2002), Dr R. Harvey (Abudalo et al. 2005; Bales et al. 1995; Blanford et al. 2005; Harvey 1997; Harvey and Ryan 2004; Loveland et al. 1996; Pieper et al. 1997; Ryan et al. 1999; Ryan et al. 2002; Van Cuyk et al. 2004) and Dr M. Abbazadegan (Drees et al. 2003; Gerba et al. 2003). The bacteriophage research involving bacteriophage P22 are shown in Table 3.1.

Table 3.1 Identity of bacteriophage "PRD1" stocks received from Dr Charles Gerba, University of Arizona, between 1993 and 2002.

Dates of Publication	Direct /Indirect Evidence	Investigator / Laboratory	Identity of Stock	References
1995-2005	Direct sequencing	Rose/ USF	P22	Paul et al. 1995; Paul et al. 1997; Nicosia et al. 2001; John & Rose 2005
1995-2005	Direct sequencing	Harvey/ USGS	P22	Bales et al. 1995; Loveland et al. 1996; Harvey 1997; Pieper et al. 1997; Ryan et al. 1999; Ryan et al. 2002; Harvey and Ryan 2004; Van Cuyk et al. 2004; Abudalo et al. 2005; Blanford et al. 2005
1999-2002	Direct Sequencing	Schijven/NL	PRD-1	Schijven et al. 1999; Schijven and Hassanizadeh 2000; Schijven et al. 2000; Schijven et al. 2002a; Schijven et al. 2002b; Schijven and Simunek 2002
2000-2003	Direct sequencing	Farrah/ UFL	P22	Lukasik et al. 2000; Scott et al. 2002; Lukasik et al. 2003
2003	Direct sequencing	Abbaszadegan/ ASU/UA	P22	Drees et al. 2003; Gerba et al. 2003
2004	Direct Sequencing	Brion/UKY	PRD1	Brion et al. 2004

Bacteriophage P22 was isolated by Zinder and Lederberg in the 1950s and has since been used as a generalized transducing agent for *S. enterica* serovar typhimurium LT2 (Zinder and Lederberg 1952). It was the first generalized transducing phage to be discovered. Bacteriophage P22 contains double stranded DNA (dsDNA) that is approximately 43,400 bp and has a very short tail. Bacteriophage P22 infects smooth strain of *Salmonella typhimurium* (those that carry O-antigen surface polysaccharide) (Steinbacher et al. 1997). Bacteriophage P22 is relatively more stable than other bacteriophages without dsDNA, such as MS2 (single stranded RNA (ssRNA)) and ΦX174 (ssDNA) because it is able to repair damages to its DNA using host cell repair mechanism. Both bacteriophages PRD1 and P22 have isoelectric point of less than pH 4.0 and attach poorly to soil particles under field conditions (usually pH 6 to 8) (Ryan et al. 1999; Schijven and Hassanizadeh 2000). Bacteriophages P22/PRD1 are favorable surrogates for ground water studies because they can tolerate a large range of temperature and have low inactivation rates of 0.02-0.05 $\log_{10}$ per day between 10 and 23 °C (Harden et al. 2003; Harvey and Ryan 2004; Paul et al. 1995). In addition, both bacteriophages are structurally and morphologically similar to human adenovirus, an important waterborne pathogen; thus bacteriophages P22/PRD1 are good model viruses for understanding the behavior of relevant pathogenic viruses, e.g. adenoviruses (Belnap and Steven. 2000). The comparisons between bacteriophage PRD1, bacteriophage P22 and human adenoviruses are shown in Table 3.2.

Table 3.2 Comparison of bacteriophage PRD1, bacteriophage P22 and human adenovirus, an important human pathogen.

Virus	PRD1	P22	Human Adenoviruses
Family	Tectiviridae	Podoviridae	Adenoviridae-Mastadenovirus
Size	63 nm	50-60 nm	70-90 nm
Envelope	No	No	No
Structure	Icosahedral, inner hydrophobic lipid layer, tails (conditional)	Icosahedral, short tails	Icosahedral, nucleocapsid with surface projection
Genome	double-stranded DNA, linear, 14 kbp	double-stranded DNA, circular, 41 kbp	Double-stranded DNA linear, 30-42 kbp
Host	Gram-neg bacteria with N, P & W plasmid incompatibility groups	*Salmonella enterica* serovar typhimurium	Different human cell receptors, mainly respiratory & gastrointestinal

Tracer Study

Study Site. This dual tracer study was conducted on May 8, 2006 on a 40-km stretch of the lower Grand River, from the city of Grand Rapids to Coopersville, Michigan. The Grand River is the longest river in the lower peninsula of Michigan, running 420 km, originating from the city of Jackson, through Lansing, Grand Rapids and discharges at Grand Haven, Lake Michigan. Currently, there are eight CSO outlets that discharge approximately 6100 m^3 partially and untreated sewage into the lower Grand River in 2007. The Ann Street Bridge, which located near CSO outlets at downtown Grand Rapids, was selected as the injection point. Sample collection was carried out at three downstream bridges (site 1: Wealthy St. Bridge; site 2: 28th Street and site 3: Lake Michigan Drive) based on feasibility for sample collection (Figure 3.1). Distances of site 1, 2 and 3 from the injection point are 4.56, 13.69 and 28.38 km, respectively. Distances of each site from injection point are shown in Table 3.3.

Because this river is significantly wider in comparison with river width reported in many previous tracer studies, sampling was done at three locations (left, right and center) at site 1 and site 2 and two locations at site 3 (left, right) to account for lateral variability (variability in flow rate across the river). A United States Geological Survey (USGS) river discharge gaging station (#04119000) is located 1.05 km upstream from the first sampling site. The river discharge on the study dates (7 am on May 8th to 7:30 am May 9th) gradually declined from 3230 to 3010 cubic feet per second (cfs) (114,066 to 106297 m^3/ s). In addition, an RD Instrument 1200 kHz Rio Grande Acoustic Doppler Current Profiler (ADCP) was used to measure discharges at all the sampling locations.

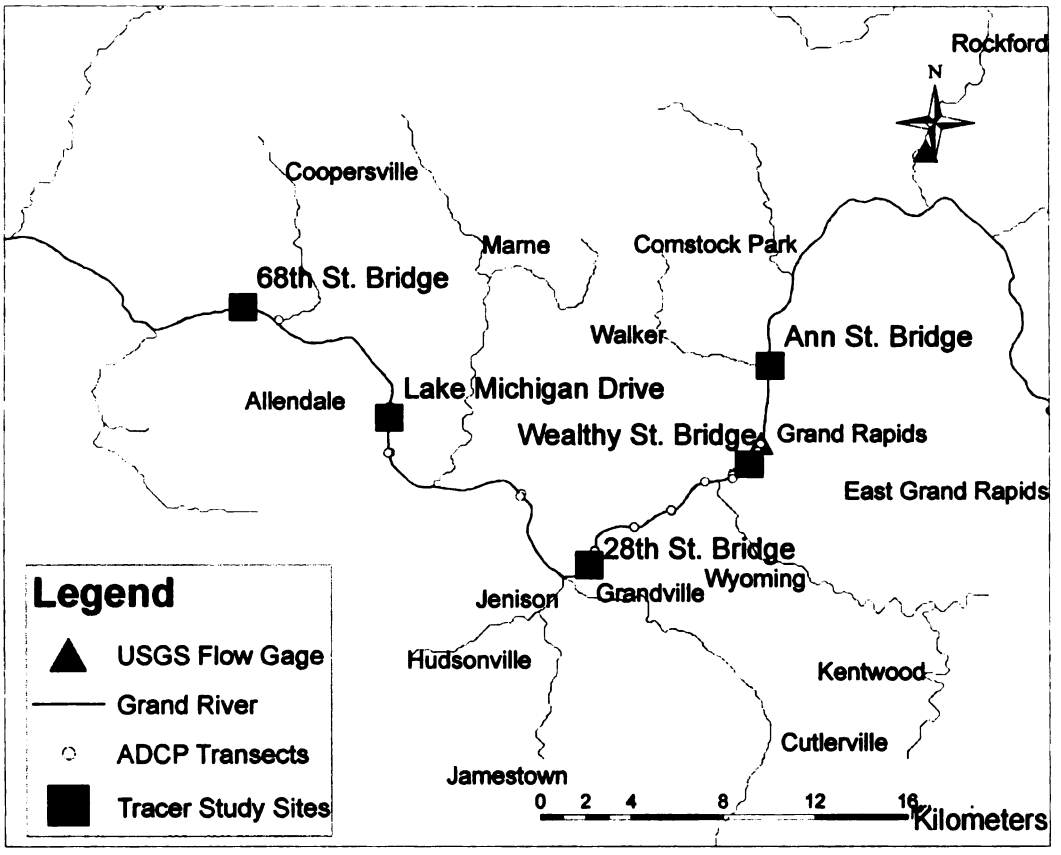

Figure 3.1 Map of the Grand River showing the spatial distribution of land use in the watershed and the three sampling locations.

Rodamine WT and bacteriophage P22 preparation. Rodamine WT 20% (w/w) solution was used in the study. Rhodamine WT tracer was prepared based on a peak concentration limit of 10 μg L^{-1} at the last sampling station, which is 37.43 km downstream from the injection site (only RWT samples were collected from this site). The bacteriophage P22 stock used in this study was obtained from Samuel Farrah, University of Florida, Gainsville, FL, and was maintained on the host *Salmonella typhimurium* LT-2 (ATCC 19585). Bacteriophage P22 stock were grown by inoculating 100-ml log-phase *S. typhimurium* host with one milliliter of bacteriophage P22 stock

(~ 10^{11} pfu/ml) and incubated at 37 °C for approximately 3-5 hours. After incubation, 0.01 g of lysozyme and three milliliters of 0.2 M sterile EDTA were added to the flask and mixed well. The culture was then centrifuged at 4000 rpm for 10-15 min and the supernatant was filter sterilized through a 0.45 μm membrane. Bacteriophage P22 stock was stored at 4 °C until used.

Tracers release and sampling conditions. Rhodamine WT and bacteriophage P22 solutions were released into the river (slug release) from the Ann Street Bridge on May 8, 2006 at 7:00 am. A total of 8770 g of RWT and 16 liters of bacteriophage P22 (4 x 10^{11} pfu/ml) were released. At each station, 100-ml grab samples were collected manually from just below water surface. Two samples were taken at the same time. One was stored in a dark cooler for RWT analysis, and 5X trypticase soy broth (TSB) was added to the other sample to stabilize the bacteriophage for bacteriophage P22 analysis. All samples were kept on ice and transported to the laboratory within 12 hours after collection and analyzed within 48 hours. Meanwhile, water temperature, pH, suspended solids and weather data (i.e., ambient temperature, rainfall, wind, etc.) were noted during sampling. A Turner Designs 10-AU field fluorometer (Turner Designs, Inc, Sunnyvale, California), was used to initially detect the dye at the first three sites. In order to capture a complete breakthrough curve, the sampling interval for both tracers was decreased after receiving a RWT signal. Rhodamine WT samples were analyzed in the laboratory using the same 10-AU unit. Total suspended solids concentration was determined according to Standard Method 2540-D-Total Suspended Solids Dried at 103-105 °C (Greenberg et al. 1992).

Bacteriophage P22 detection and enumeration. Water samples were assayed for bacteriophage P22 following the double layer agar plate assay originally described by Adams (1959). Samples from site 1 were diluted to 10^{-3} concentration, and between 1 ml and 2 ml of each sample in at least duplicate were assayed for the phage presence on tryptic soy agar (TSA). The plates were incubated for 24 h at 37°C. The detection limit of this method is less than one plaque-forming unit per ml of river water sample.

Tracer data analysis. Estimation of the average velocity (m/s) and average recovery rate (%) of bacteriophage P22 at each sampling station were computed by fitting a sine curve to the observed bacteriophage P22 data. Velocity and recovery rates were compared with calculations computed by Shen et al (2008). Initial virus concentration was estimated using the discharge data obtained from the USGS gauging station. The initial concentration of bacteriophage P22 in the river after tracer release, C_0, was estimated using the formula below:

$$C_0 = \frac{C_{tracer} \cdot Q_{tracer}}{(Q_{tracer} + Q_R)} \quad (1)$$

where C_{tracer} denotes average concentration of bacteriophage P22 in the discharge (pfu/ml); Q_{tracer} is the flow rate of tracer (m^3/s); Q_R is the discharge of the river (m^3/s).

Average velocity (m/s), V_{ave}, at each site was calculated by dividing time of peak concentration on bacteriophage P22 breakthrough curves, t_{peak}, by distance traveled, x.

$$V_{ave} = \frac{t_{peak}}{x} \quad (2)$$

Predicting Virus Concentration after a CSO Event

For prediction of virus concentration at Lake Michigan beaches after a CSO event, an analytical virus transport model was developed from the formula below (O'Loughlin and Bowmer 1975):

$$C(x,t)=\frac{C0}{2}\left\{\begin{array}{l} e^{\frac{Ux}{2E}(1-\Gamma)}\left[\operatorname{erfc}\left(\frac{x-Ut\Gamma}{2\sqrt{Et}}\right)-\operatorname{erfc}\left(\frac{x-U(t-\tau)\Gamma}{2\sqrt{E(t-\tau)}}\right)\right]+ \\ e^{\frac{Ux}{2E}(1+\Gamma)}\left[\operatorname{erfc}\left(\frac{x+Ut\Gamma}{2\sqrt{Et}}\right)-\operatorname{erfc}\left(\frac{x+U(t-\tau)\Gamma}{2\sqrt{E(t-\tau)}}\right)\right] \end{array}\right\}$$

where $\Gamma=\sqrt{1+4\eta}$ and $\eta=\frac{kE}{U^2}$ (3)

in which, $C_{(x,t)}$ denotes tracer concentration as a function of distance traveled, x (m), and elapsed time since tracer release, t (s); τ represents spill duration (s); C_0 is the initial tracer concentration at discharge point (virus/ml^{-1}); U is average velocity (ms^{-1}); E is longitudinal dispersion coefficient (m^{2}s^{-1}); k is first order decay rate constant (s^{-1}).

For uniformity in presentation, virus inactivation estimates were obtained from analysis performed by Shen et al. (2008). Shen et al. (2008) used a watershed hydrologic model based on the Soil and Water Assessment Tool (SWAT) to verify the estimated lateral inflow and includes contributions from the lateral inflow, and solute exchange between the main channels and storage zones in solute transportation estimation. Solute transport in the longitudinal direction was then described using the transient storage (TS)

formulation (Runkel 1998). Briefly, the model solves two separate equations – one for solute concentration in the main channel and another in the storage zones. Solute exchange between the bulk flow and the storage zones was described using a first-order exchange coefficient α. The governing equations appear as shown below (Runkel 1998):

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C}{\partial x}\right) + \frac{q_L}{A}(C_L - C) + \alpha(C_s - C) - kC \quad (4)$$

$$\frac{\partial C_s}{\partial t} = \alpha\frac{A}{A_s}(C - C_s) - kC_s \quad (5)$$

in which Q denotes the discharge, A and A_S the cross-sectional areas, C and C_S the concentrations in the main channel and the storage zones respectively, D the dispersion coefficient, q_L the lateral inflow, α the mass exchange coefficient between the main channel and the storage zones and k the first order inactivation/decay rate (for bacteriophage P22). Concentration associated with lateral inflow, C_L, was assumed zero in this case.

Equations (4) and (5) were applied on a reach basis and parameters were estimated separately for RWT and bacteriophage P22.

For evaluating the relative performance of the two tracers, Shen et al. (2008) used equation 6, which estimates the fractional recovery of tracer mass by integrating the tracer breakthrough data assuming complete mixing:

$$f = \frac{1}{M_0}\int_0^\infty C(x,t)Q(x,t)dt \quad (6)$$

Here f is the fractional recovery, $C(x,t)$ is the tracer concentration, $Q(x,t)$ is the discharge, and M_0 is the mass released. Because the actual river discharge was not available at all spatial locations (x), river discharge from the USGS gauging station was used.

Sensitivity Analysis

Site specific data were collected and analyzed for seasonal and monthly trends. Historical river discharge data (between 1930-2008) for the lower Grand River at Grand Rapids (gage no.: USGS 04119000) were obtained from the USGS website (http://waterdata.usgs.gov/nwis/dv?referred_module=sw&site_no=04119000). CSO discharge data in Grand Rapids were obtained from CSO and SSO annual reports published by the Michigan Department of Environmental Quality (www.deq.state.mi.us/csosso). Distributions of these parameters were evaluated and fitted using EasyFit software (MathWave Technologies). Seasonal and monthly means for river discharge, CSO discharge volume, CSO discharge duration and frequency were then computed using SAS software (SAS, Cary, NC). Analysis of variance (ANOVA) was conducted to evaluate differences in seasonal and monthly means of these parameters. For parameter that showed significant seasonal/monthly variations in ANOVA, Tukey's honestly significant difference procedure was conducted to determine groups that were significantly different from each other.

Output distributions of viral concentrations were obtained using Monte Carlo simulation techniques that randomly sample each parameter according to its distribution. A minimum of 10,000 iterations was performed for each simulation. All distribution functions were assumed to be statistically independent. Monte Carlo simulation was carried out with Risk solver (Frontline Systems Inc., Incline Village, NV), an add-on to Microsoft Excel software.

Univariate sensitivity analyses were performed to determine the influence of input parameters on the shape of virus breakthrough curve and virus concentration downstream from discharge point.

Results and Discussion

Recovery estimation of bacteriophage P22 using sine curve fitting method was very close to recovery estimation by Shen et al. (2008) using eq. (6) (Table 3.3). By using the sine curve fitting method, recovery of bacteriophage P22 at site 1, site 2 and site 3 was 32.93%, 28.56% and 16.52%, respectively, compare to 31.04%, 27.20% and 17.38% computed by Shen et al (2008). Because breakthrough was incomplete at site 3 (tracer concentration in the river water did not drop to baseline level when sampling ended), virus recovery at the site may be underestimated.

Recoveries of bacteriophage P22 were lower than the recoveries of RWT at all three sites (113.95%, 108.01% and 64.27%, respectively) (Shen et al. 2008). The low recovery of bacteriophage P22 may be attributable to the turbulence generated by a dam in downtown Grand Rapids (before site 1) that inactivated the bacteriophage as well as to potential losses associated with aerosolization of bacteriophage P22 (during release from the bridge) resulting in overestimation of the initial mass released. These levels of recovery were also reported in previous literature and may reflect the actual microbial pathogen survival rate after discharge into a water body (Hodgson et al. 2004). Bacteriophage P22 concentrations at each sampling site are shown in Figure 3.2. Bacteriophage P22 concentrations recovered from all sample sites are shown in Figure 3.3.

Table 3.3 Distance to the sampling sites from the injection point, comparison of average velocity and average recovery of bacteriophage P22 using a hydrological model (Shen et al. 2008) and sine curve fitting.

Site	Distance (km)	Avg. velocity, m/s*	Avg. velocity, m/s**	Avg. % recovery *	Avg. % recovery **
Site 1 (Wealthy St)	4.56	0.42	0.42	31.04	32.93
Site 2 (68th St)	13.69	0.51	0.51	27.20	28.56
Site 3 (Lk MI Dr)	28.38	0.55	0.50	17.38	16.52

* average velocity and average percent recovery computed by Shen et al. (2008)

** average velocity and average percent recovery estimated by sine curve fitting method.

Examination of the reach-averaged velocities for bacteriophage P22 and RWT seems to indicate that bacteriophage P22 arrived early compared to RWT though arrival and peak times for both tracers were fairly close (within 15 minutes of each other) at all three sites. This may be attributed to the lower detection limit of bacteriophage P22 compare to RWT (this was also noted in Rossi et al. (1998) for comparison between recoveries of bacteriophage H40/1 and uranine). Other differences in the transport of the two tracers can be explained by difference in adsorptivity to riparian vegetations, high organic matter content and suspended solid content as well as the effects of land use characteristics. Bacteriophage P22 arrived at site 1 approximately 2:38 h after dye release, at site 2 at 6:47 h and it took 13:45 h to arrive at site 3, which is approximately 28 km from the injection site. Peak bacteriophage concentrations at site 1, site 2 and site 3 were 1477 pfu/ml, 388 pfu/ml and 74 pfu/ml, respectively.

Table 3.4 Bacteriophage P22 and Rhodamine WT arrival and peak time at each sampling site.

Sampling Sites	P22			RWT		
	T_{ARR}	T_{MAX}	Peak (pfu/ml)	T_{ARR}	T_{MAX}	Peak (mg/ml)
1. Wealthy St. Bridge	2h 38min	2h 59min	1477	2h 41min	3h 4min	69
2. 68th St. Bridge	6h 18min	7h 28min	388	6h 22min	7h 32min	17.69
3. Lake Michigan Dr. (L)	13h 50min	16h 32min	74	13h 35min	16h 16min	7.38
4. Lake Michigan Dr. (R)	13h 42min	15h 29 min	113	13h 50min	15h 12min	8.66

T_{ARR}: time of the first positive sample T_{MAX}: time of the maximum tracer

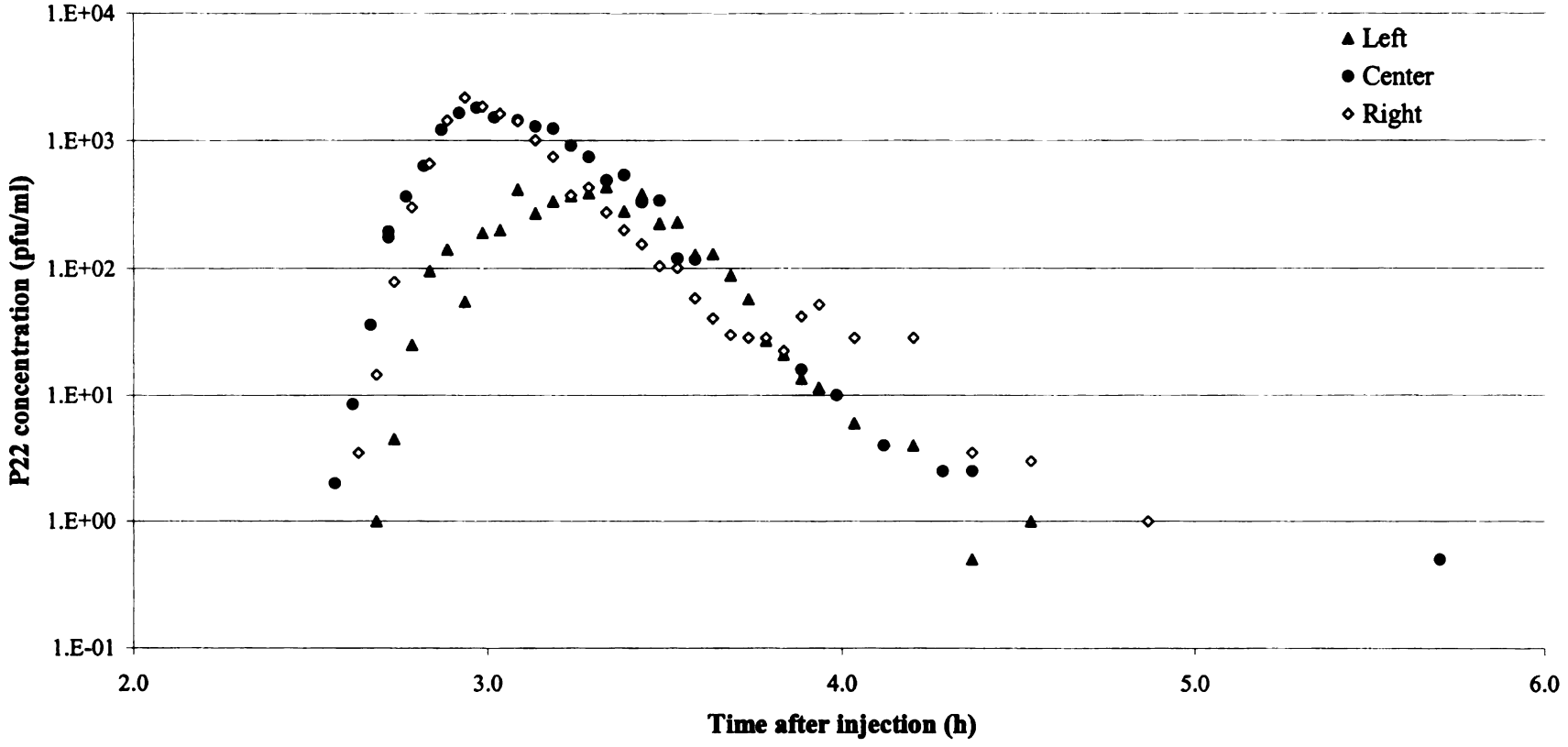
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1.E-01
P22 concentration (pfu/ml)
2.0
3.0
4.0
5.0
6.0
Time after injection (h)
Left
Center
Right

Figure 3.2 a

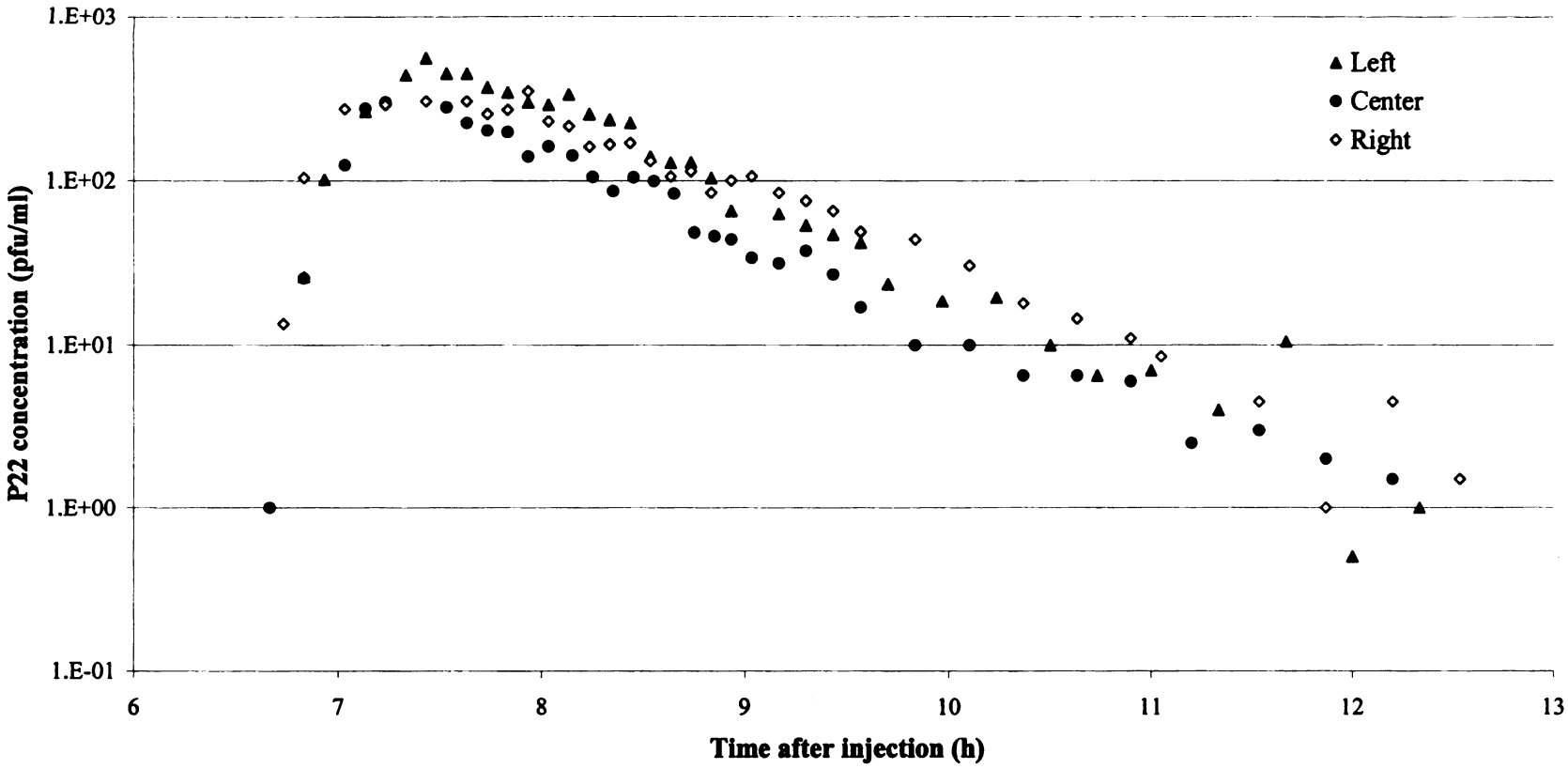
1.E+03
1.E+02
1.E+01
1.E+00
1.E-01
P22 concentration (pfu/ml)
6
7
8
9
10
11
12
13
Time after injection (h)
Left
Center
Right

Figure 3.2 b

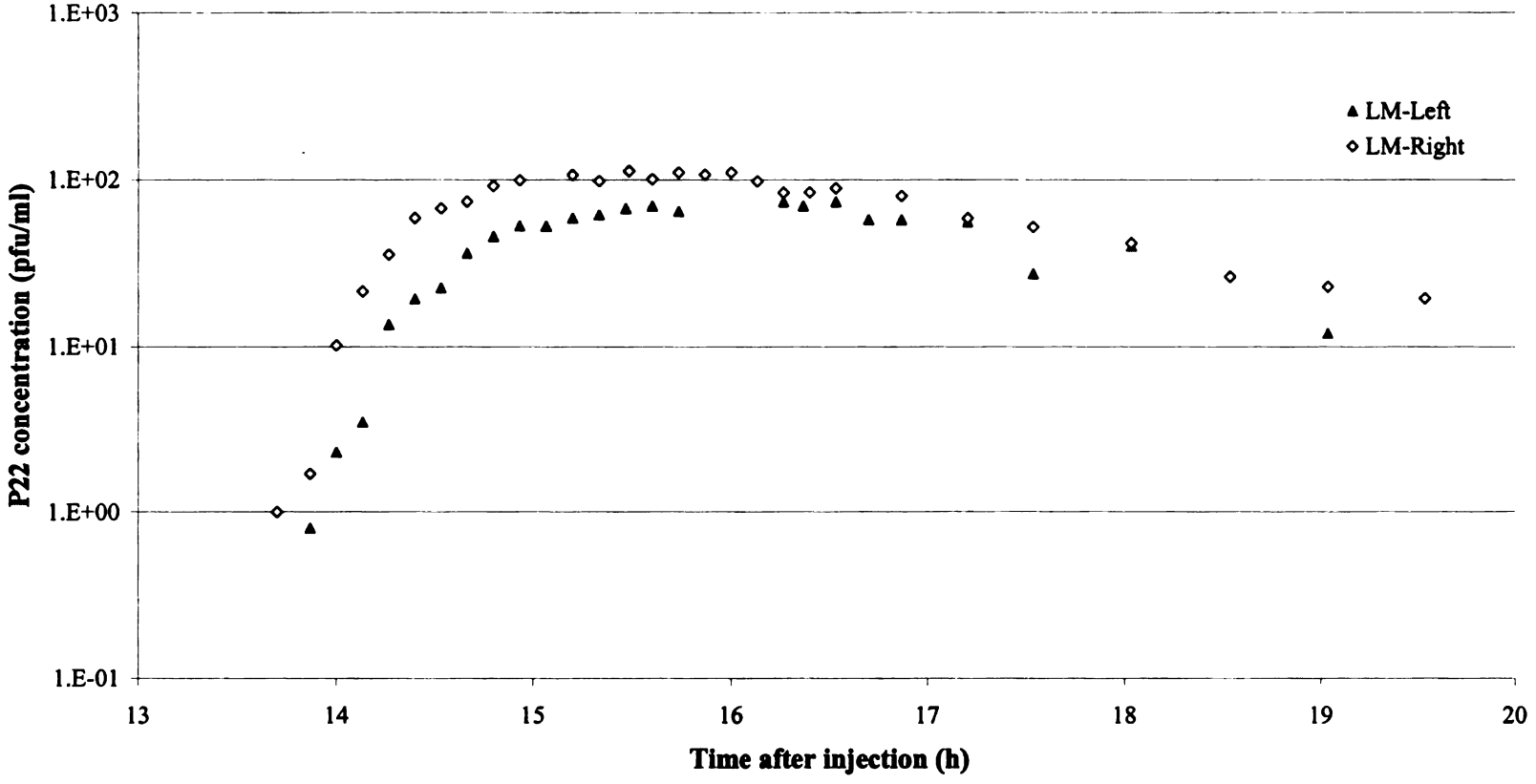

Figure 3.2c

Figure 3.2 Bacteriophage P22 concentrations (pfu/ml) at sampling sites versus time after dye injection (h). (a) At site 1, Wealthy St bridge, average peak bacteriophage concentration was 1477 pfu/ml; (b) At site 2, 28th St bridge, average peak bacteriophage concentration was 388 pfu/ml; (c) At site 3, Lake Michigan Drive bridge, average peak bacteriophage concentration was 74 pfu/ml.

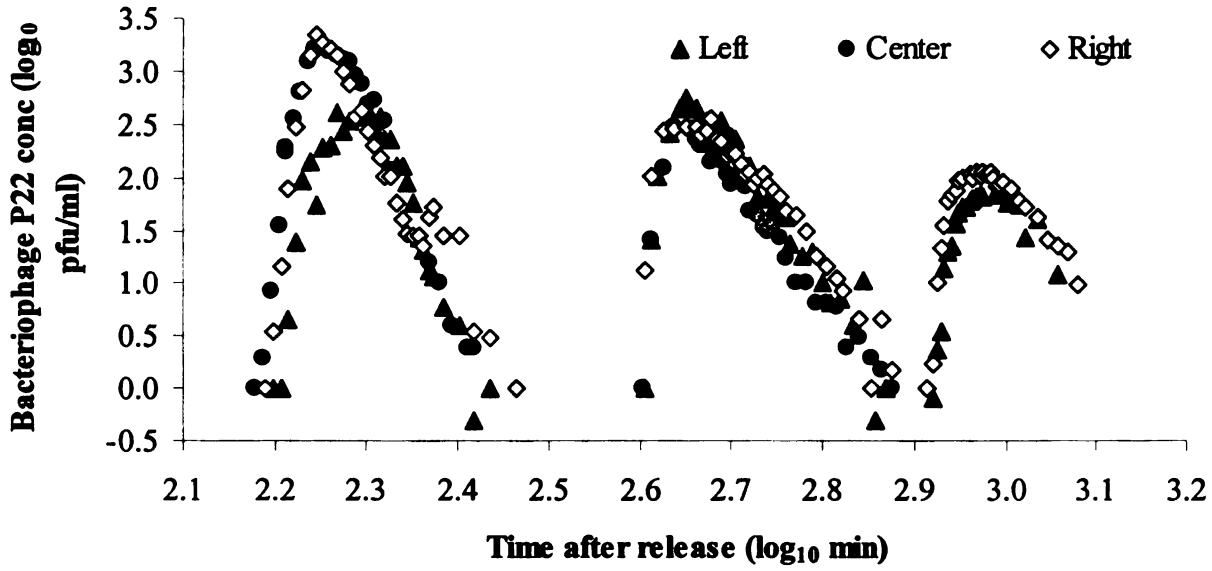

Figure 3.3 Bacteriophage P22 concentrations (log_{10} pfu/ml) at all three sampling sites versus time after release (log_{10} min).

Shen et al. (2008) determined the average first-order inactivation rate in reach 2 and reach 3 using the formula:

$$k = k_0 + k_I I(t) \quad (7)$$

where, k_I is the rate of inactivation caused by solar radiation (d^{-1} kW^{-1}); $I(t)$ is the net shortwave radiation (kW) as a function of time (and geographical location); k_0 denotes virus loss caused by other factors such as temperature and sedimentation. It was reported that average first-order inactivation rates in reach 2 and reach 3 was 0.27 and 0.57 per day, respectively; similar solar inactivation values obtained at both reaches suggest comparable effects of solar irradiation. The inactivation rates of bacteriophage P22 in the water column in this study are similar to the rates reported in other studies (Schijven et al. 1999). Schijven et al. (1999) reported an inactivation rate of 0.3 per day for bacteriophage PRD1 in a constructed wetland with significant surface flow. The higher inactivation rate for reach 3 compared to reach 2 may be attributed to a higher total suspended solid concentration (TSS) in reach 3 and inactivation caused by solar radiation as bacteriophage P22 traveled downstream and dilutions. Bacteriophages are known to adsorb to suspended particulate matter, and due to their colloidal nature, can aggregate into clumps large enough to settle out of the water column although the factors that influence these processes are complex (Ferguson et al. 2003). This information, coupled with the fact that reach 3 had the highest TSS, higher percentage clay, lower dissolved organic carbon (DOC) and the lowest cross-sectional average velocities, suggests that adsorption and sedimentation was the main cause of bacteriophage P22 attenuation at reach 3.

In order to better characterize virus attenuation, Shen et al. (2008) also examined the influence of transient storage sizes and land use characteristics (urban versus agricultural and forested land uses) in their tracer analysis. They concluded that the estimated transient storage zone sizes correlated negatively with urban land use and positively with agricultural and forested land uses for both tracers. Because of the complexity of transient storage size and land use characteristics effects, these factors were not included in the analytical solution for estimating virus concentration after a CSO event.

Sensitivity Analysis

Sensitivity analysis was performed to determine the influence of site specific parameters (i.e. area of channel, river discharge, CSO discharge volume and CSO discharge duration) and virus inactivation factors (i.e. sunlight intensity, virus inactivation by factors other than sunlight irradiation) on final virus concentration. Distributions of some of these parameters, i.e. river discharge, CSO discharge volume and CSO discharge duration, were first evaluated and fitted using EasyFit software. Seasonal and monthly means for river discharge, CSO discharge volume, CSO discharge duration and frequency were subsequently computed using SAS software. Analysis of variance (ANOVA) was conducted to evaluate differences in seasonal and monthly means of these parameters. For parameter that shows significant seasonal variations in ANOVA, Tukey's honestly significant difference procedure was conducted to determine groups that are significantly different from each other (in order to determine the most representative set to be used in risk assessment).

Annual, seasonal and monthly means for these site specific parameters were then evaluated for selection of sets of values that most represent important climatological events in the lower Michigan. For river discharge, historical daily discharge data for the hydrology station on the lower Grand River in Grand Rapids, from 1930 to 2008, were collected from USGS website. Annual, seasonal and monthly means were computed in SAS for analysis of trend. Figure 3.4 shows that annual means for river discharge between year 1930 and 2008 were relatively consistent with perhaps a slight trend upward. Therefore, discharge data from all years were used for computing seasonal and monthly averages.

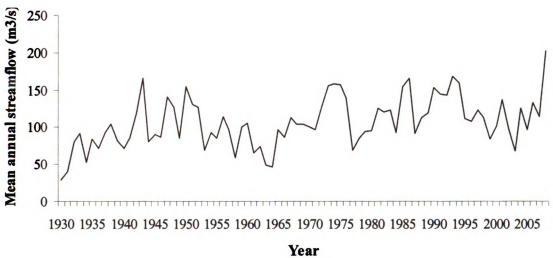

Figure 3.4 Mean annual river discharge for the lower Grand River at Grand Rapids, MI, between 1930 and 2008.

CSO monitoring data in Grand Rapids between summer 2000 and 2008, were obtained from CSO and SSO annual reports published by the Michigan Department of Environmental Quality. Total CSO discharge volume between year 2000 and 2008 are

shown in Figure 3.5. Though the number of CSO outlets in Grand Rapids has decreased from 26 (untreated) in 1988 to 8 (7 untreated, 1 partially treated) in 2008, total CSO volume fluctuated from year to year, influenced heavily by annual precipitation (MDEQ 2008). The highest total CSO discharge volume was observed in 2008 (8.22×10^5 m^3), followed by 2004 (7.45×10^5 m^3), and 2001 (2.38×10^5 m^3), respectively. Total and mean CSO discharge followed similar trends except in January, in which an elevated mean in CSO discharge was observed (Figure 3.6). This was probably caused by an unusually high single event discharge volume during snowmelt in January.

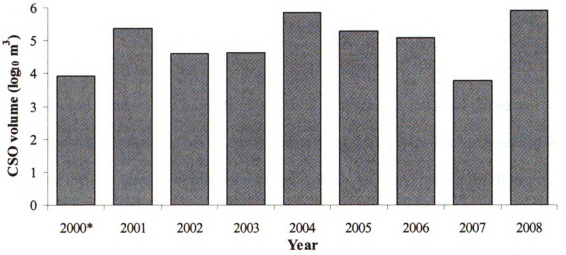

Figure 3.5 Annual CSO volume discharging into the lower Grand river in Grand Rapids area, MI, between year 2000 and 2008.

* Partial year data were used for year 2000 because CSO reporting in Grand Rapids started in July 2000.

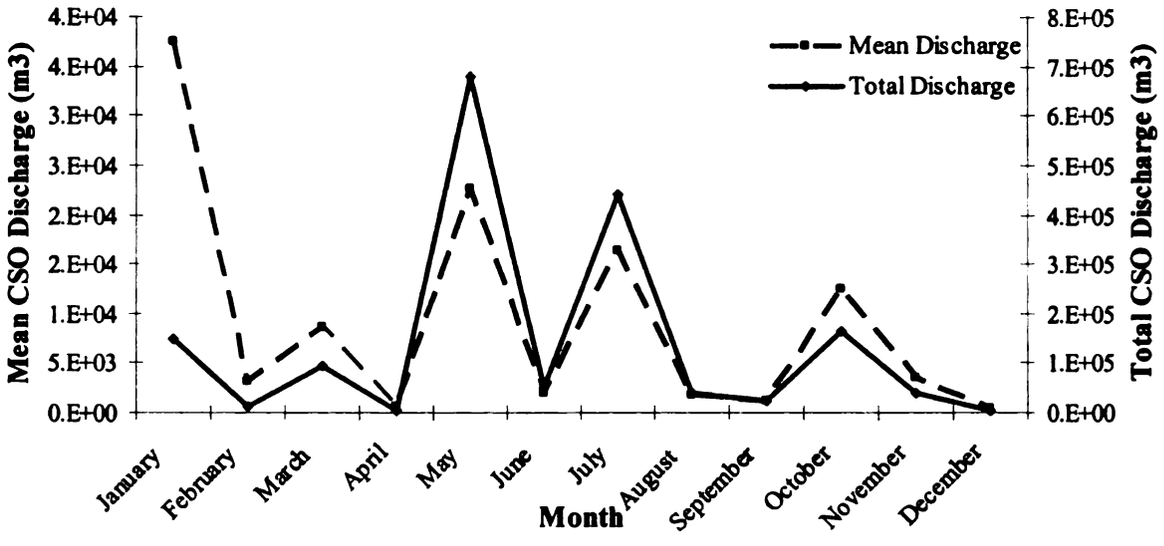

Figure 3.6 Mean and total CSO discharge volume into the lower Grand river from CSO outlets in Grand Rapids, MI, by month. Mean and total CSO volume generally followed the same trend except in January, when mean CSO volume was the highest.

Comparison between monthly means for river discharge and CSO discharge volume showed that they did not follow the same trend. Mean river discharge peaked in March and then declined until it hits the lowest point in August (Figure 3.7). However, monthly average CSO discharge showed several peaks over a year, suggesting that it had little correlation with river discharge. The highest peak for CSO discharge occurred in January, followed by four smaller peaks in May, July, October and March. This phenomenon can be explained by climatological events such as snow melt during end of Winter (between January and March) and high precipitation in Summer (between May and July). Correlation analysis shows that mean river discharge and mean CSO volume were not significantly related to each other.

In addition, monthly average CSO discharge duration did not show any apparent trend over the year (Figure 3.8). Average CSO discharge durations ranged between 0.66 and 5.17 h, with an overall average of 3.88 h. Analysis of variance did not show significant difference between monthly average for river discharge, CSO volume and CSO duration. This suggested that monthly data fluctuated regularly and were not ideal for predicting final virus concentration in relative to climatological events.

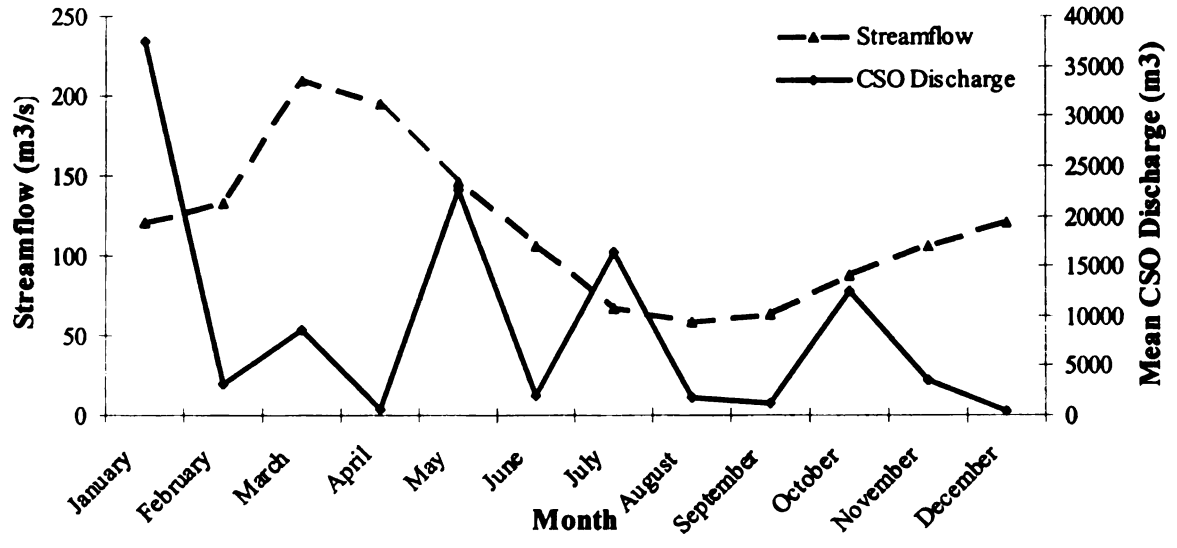

Figure 3.7 Mean river discharge (m^3/s) versus mean CSO discharge volume (m^3).

Correlation analysis did not show any significant correlation between river discharge and CSO discharge.

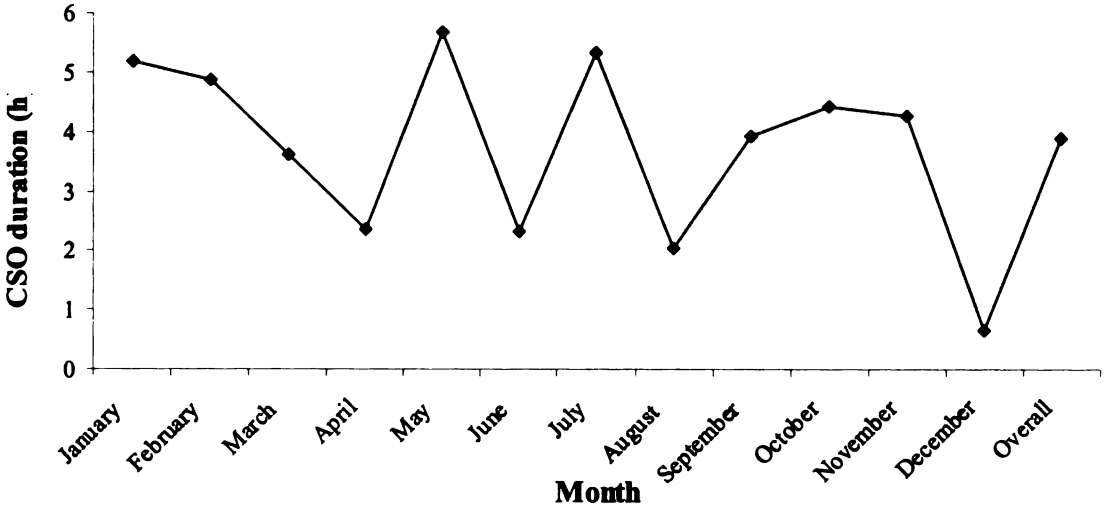

Figure 3.8 Average CSO discharge duration (h) by month, no apparent trend was observed for CSO discharge duration by month.

Analysis of seasonal means of both river discharge and CSO discharge volume gave a more apparent trend. Analysis of variance (ANOVA) showed that there was a significant difference in seasonal river discharge (p-value:<0.001) (Table 3.5). According to Tukey's honestly significant difference procedure, river discharge in Spring and Winter were significantly higher than river discharge in Summer and Fall.

It can be observed from Figure 3.8 that average CSO discharge volume in Spring was higher than other seasons; however, ANOVA concluded that average CSO discharge volume was not significantly different seasonally (p-value: 0.53). The lack of significant difference in average seasonal CSO discharge volume may be highly attributable to large variances (range of CSO discharge volume within a season). In addition, seasonal CSO discharge durations had a relatively smaller range (between 3.3 and 4.6 h), and were not significantly different from each other (p-value: 0.57) (Figure 3.9).

Table 3.5 Overall and seasonal mean, and standard deviation for river discharge, CSO volume and CSO discharge duration. p-values for ANOVA results were listed in column four. Significant differences in means were observed in seasonal river discharge.

Variable	Mean	Standard deviation	p-value
River discharge (m^3/s)	106.64	33.50	<0.01
Spring	180.42	123.19	
Summer	*66.87	50.31	
Fall	*67.93	56.01	
Winter	110.39	85.01	
CSO volume (m^3)	9332.14	38042.53	0.53
Spring	15576.51	55716.48	
Summer	6991.80	31986.31	
Fall	5210.13	21862.10	
Winter	12631.63	28564.44	
CSO duration (h)	3.88	5.47	0.57
Spring	4.63	6.79	
Summer	3.31	4.71	
Fall	4.17	5.37	
Winter	3.34	4.28	

* average river discharge for Summer and Fall were significantly different from river discharge for Winter and Spring.

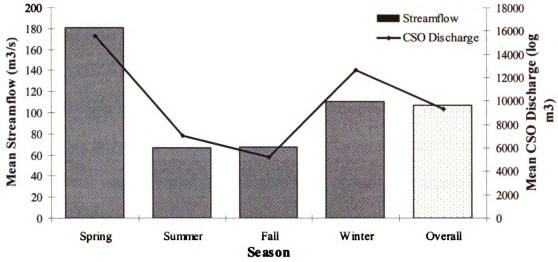

Figure 3.9 Mean river discharge (m^3/s) and mean CSO volume (m^3) by season.

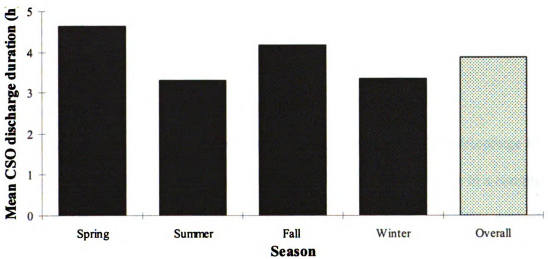

Figure 3.10 Mean CSO discharge duration (h) by season.

In order to determine the importance of site specific parameters on output distribution of virus concentrations, parameters such as virus concentration in CSO discharge, CSO discharge volume, CSO discharge duration, river discharge, river width etc with their respective mean, standard deviation and distribution type were entered into eq. (3) (p. 103), and randomly sampled using Monte Carlo simulation. A minimum of 10,000 iterations was performed for each simulation. Monte Carlo simulation showed that in general, CSO discharge volume, virus concentration in CSO discharge and river discharge were the three most influential factors in deciding final virus concentration at a location. Total uncertainty attributable to each factor is distance, and time related. For example, at 50 km from CSO discharge points, CSO volume contributes to approximately 20 % of total uncertainty in final virus concentration, while initial virus concentration and river discharge each contributed to approximately 8 % and 2 % of total uncertainty. Virus inactivation and sunlight irradiation had minimal effect on final virus concentration (contribute to less than 1 % of total uncertainty).

Conclusions

In conclusion, this study found that the transport behavior of bacteriophage P22 correlated well with RWT, a conventional tracer and was a suitable tracer in a complex fresh water system as indicated by the travel times, reach-averaged velocities, percent mass recovery, and response to other environmental factors (residence times, storage zone characteristics and dependence on land use patterns were examined by Shen et al. 2008). The fact that bacteriophage P22 has a lower recovery rate than RWT does not

affect its candidacy as an ideal tracer for microbial pathogens because its behavior may be more representative of colloid substances, i.e. microorganisms, in surface water.

Bacteriophage P22 has inactivation rates between 0.27 and 0.57 per day and these values were similar to the values reported in other studies (Schijven et al. 1999). Parameters obtained or sorted in this study (i.e. virus inactivation rates, CSO discharge volume, CSO discharge duration, river discharge etc) were evaluated and incorporated into a risk assessment model to estimate risk of recreation in the lower Grand River in the aftermath of a sewage overflow (Chapter 4).

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CHAPTER 4

RISK OF GASTROINTESTINAL DISEASE ASSOCIATED WITH EXPOSURE TO ENTERIC VIRUSES AT RECREATIONAL BEACHES DOWNSTREAM OF CSO SITES ON THE LOWER GRAND RIVER, GRAND RAPIDS, MICHIGAN

Abstract

In cities with combined sewer systems, intentional discharge of untreated waste water directly into surface water during heavy precipitation events has been identified as one of the main culprits for surface water quality degradation. The objective of this study was to evaluate the risk of viral gastrointestinal disease associated with combined sewer overflow (CSO) discharges into the lower Grand River in Grand Rapids, Michigan. Concentrations of human adenoviruses in surface water (n=20), raw sewage (n=13) and CSO discharge (n=6) of Grand River were measured and used in exposure assessment. Probability of enteric virus infection from swimming and wading in the contaminated surface water after a CSO event were estimated. Estimation for virus attenuation in the river was obtained from a virus tracer study. Parameters used in the model included CSO discharge duration, CSO discharge volume, virus concentration in CSO discharge, hydrogeometry of the river segment (i.e. river discharge, depth, width, cross-sectional area and slope etc.) and sunlight intensity. Risk estimations were performed on five exposure scenarios: (i) swimming in a recreational park 25 km downstream from a CSO outlet, (ii) swimming in Lake Michigan beaches 50 km downstream from a CSO outlet, (iii) wading/angling in a recreational park 25 km downstream from a CSO outlet, (iv) wading/angling in Lake Michigan beaches 50 km downstream from a CSO outlet, (v)

swimming in the recreational parks or Lake Michigan beaches without a CSO event. Dose-response relationships for rotavirus and echovirus 12 were used in the study for comparing risk differences between a highly infectious and a moderately infectious virus. A point risk estimate was conducted using the means of input variables. The point risk estimate produced mean probability of infection using the rotavirus model of $2.9x10^{-1}$ and $2.2x10^{-1}$ for swimming and wading, respectively, with a duration of elevated risk (when illness risk is greater than EPA recreational freshwater level of 8×10^{-3}) that began at 12h after discharge and ended at 33.5 h after discharge 25 km from discharge point. At the same location, the echovirus 12 model estimated a risk of $5.4x10^{-2}$ for swimming and $1.5x10^{-2}$ for wading, with duration of elevated risk between 14.5 h and 27.5 h after discharge. An interval risk estimate was performed using Monte Carlo techniques to characterize uncertainty in input variables (i.e. CSO discharge duration, CSO discharge volume etc). Overall, point risk estimates gave higher mean risk values than interval risk estimates. Using river discharge and CSO parameters for summer, interval risk estimate gave durations of elevated risk that lasted up to 200 and 350 hours at 25 km and 50 km downstream from the discharge point, respectively. Sensitivity analysis showed that both probability of infection and duration of elevated risk (duration after CSO discharges when risk of illness > 0.008) at a location were highly dependent upon river discharge. With the use of this risk analysis model, effects of CSO discharge on surface water quality in other watersheds can be estimated as long as site specific parameters are available. Long-term virus survey data are essential for better estimation of seasonal risk.

Introduction

Enteric viruses (i.e. adenoviruses, enteroviruses, rotaviruses) are frequently detected in surface water contaminated by fecal sources, and are known to cause not only gastroenteritis, but also a wide spectrum of other diseases, such as conjunctivitis, respiratory infections, myocarditis, encephalitis, and diabetes, in infected individuals. The presence of these enteric viruses in surface water designated for recreational purpose elevates public health risk associated with ingestion of contaminated water during recreational activities.

In the United States, major sources for enteric viruses in the surface water are urban runoff, agricultural runoff, discharges from wastewater treatment plants, and in cities with older sewer systems, discharges from combined sewer systems (CSSs) and sanitary sewer systems (SSSs). Currently, there are approximately 770 CSS communities, mainly concentrated in the Northeast and the Great Lakes regions, serving a total of 40 million people in the country. The Federal Government's effort to control CSOs started in 1994, when the U. S. EPA published CSO Control Policy as the national framework. In Michigan, the first CSO policy was drafted by the Department of Environmental Quality in 1983. However, the first non-contested permit requiring a long term CSO correction program was issued to the Grand Rapids waste water treatment plant (WWTP) only in Fall 1988, following a large CSO event in the city that affected water quality downstream in Grand Haven (MDEQ 2008). To date, Michigan communities have eliminated 75% of the 613 untreated CSO outfalls that existed in the year 1988 and the remaining 25% are scheduled for correction/elimination through implementation of long term control plans (LTCPs). However, water quality after CSO or any sewage spill remains a public health

concern to the individuals recreating at recreational parks and beaches downstream of discharge sites. High concentrations of waterborne pathogens, especially viruses, in CSOs, may pose adverse risk to human health.

Risk assessment is a valuable tool for estimating adverse effects associated with certain hazards and has been used to estimate health risk from microorganisms and chemicals. It is often an initial step prior to risk management, for example, in the case of wastewater discharge, providing important information for setting treatment criteria. Quantitative risk assessment gives a probability and describes the magnitude of a hazardous event based on exposure patterns and dose-response data. In the field of water quality, quantitative risk assessment has been used for the interpretation of microbial water quality data to estimate the public health effects of low levels of waterborne pathogens in the water (Donovan et al. 2008; Haas et al. 1993; Soller et al. 2003; van Heerden et al. 2005). Previously, the greatest limitation and uncertainty for risk assessments has been the lack of information about survival, transport, occurrence of and exposures to key specific infectious agents of concern.

The objective of this analysis was to characterize and quantify virus-related gastrointestinal disease risk for users of recreational parks and beaches located downstream of CSO discharge sites in Grand Rapids following a CSO event. Grand Rapids, the second largest city in the state of Michigan, is the first city in the state of Michigan that implemented a long term CSO control plan, partly because of the recreational activities taking place in the lower Grand River. Grand Rapids is located approximately 50 km upstream from the mouth of the lower Grand River that discharges into Lake Michigan. In 2007, the number of CSO outfalls in the city of Grand Rapids was

reduced from 26 to eight (one partially treated and seven untreated), and released an annual discharge of 6100 m^3 partially and untreated sewage into the river (MDEQ 2008). The landuse directly downstream of the CSSs is mainly urban (52.2%) and slowly transforms to agricultural (69.2%) when approaching the mouth of the river. In this analysis, representative concentrations of viruses in the river during baseflow and after CSO events were calculated from existing virus monitoring data (Chapter 2). Estimation for virus attenuation while being transporting down the river were obtained from a tracer study, in which bacteriophage P22 was used as a biotracer (Chapter 3). Probabilities of gastrointestinal infection and illness from recreating at several recreational parks and beaches of the lower Grand river in Grand Rapids and Grand Haven, MI, following CSO events were estimated based on established dose-response relationships for several waterborne enteric viruses (i.e. human rotavirus (high risk), human echovirus 12 (moderate risk)) representing different risk levels (Haas et al. 1993).

Materials and Methods

Hazard identification. Human adenoviruses were chosen as the index viruses for this study because of their frequent isolation and higher persistence compared to other enteric viruses in aquatic environments. In addition, the use of quantitative PCR assay allowed easy enumeration of this virus in the environment. Outbreaks of adenoviruses associated with swimming in contaminated swimming pool water has been reported (Turner et al. 1987; Papapetropoulou and Vantarakis 1998). Route of transmission for adenoviruses include inhalation, ingestion and dermal contact and for recreational water, ingestion of fecal contaminated water is considered a primary route. This is supported by

the monitoring data and literature with enteric adenoviruses, including adenovirus 41 as predominant viruses detected (refer to Chapter 2). In this analysis, adenovirus was chosen as the worst case scenario representative because of their high occurrence and persistence in environmental water. An end point of gastroenteritis with a fecal-oral transmission route was used as the health hazard as AGI is the most commonly reported virus attributable illness associated with recreational water. Literature on waterborne adenovirus outbreaks report attack rates around 50% (Gray et al. 2007).

Dose response determination. A number of human and animal studies had been conducted to determine the infectivity of viruses on their hosts (Couch 1966; Katz and Plotkin 1967; Supter 1963). The risk of infection based on dose-response models for several enteric viruses, representing different potencies are listed on Table 4.1. Dose-response models for these viruses generally were obtained from human feeding studies, with the exception of Coxsackievirus dose response study (Suptel 1963).

The exponential and beta-Poisson models have been found to provide the best fit to the experimental data. Each model is set up according to several assumptions. Exponential model assumes that one microorganism is capable of initiating an infection and that host-microorganism interactions are constant. In exponential model, "r" represents the proportion of ingested microorganisms that survive to cause an infection. In Table 4.1, adenovirus 4 and poliovirus I are the two viruses that have exponential dose response models. Exponential models are represented by the equation below:

$$P_i(N) = 1 - \exp(-rN) \quad (1)$$

where:

P_i : probability of infection;

N: number of organisms ingested or inhaled

r = proportion of ingested microorganism that survive to cause an infection

A beta-Poisson model, on the other hand, assumes heterogeneity for either the infectivity of individual microorganisms or host-microorganism interactions. A beta-Poisson model differs from an exponential model in that it is characterized by two parameters, β and α. As α increases, the model becomes closer to an exponential model (Haas et al. 1993). The equation for a beta-Poisson model is:

$$P_i(N) = 1 - (1 + \frac{N}{\beta})^{-\alpha} \quad (2)$$

where:

N: number of viruses ingested
α, β: parameters define the dose-response curve

The most potent virus group, which had the lowest infectious level in Table 4.1, is an adenovirus. The exponential dose response relationship for adenoviruses was developed from a study evaluating human inhalation of adenovirus 4 particles by Couch et al. (1966) (Haas et al. 1993). However, because the route of exposure for the adenovirus 4 study was inhalation, it is not ideal model to represent risk through ingestion and for enteric viruses. The second most infectious virus in Table 4.1 is poliovirus III. This dose response study conducted by Katz and Plotkin (1967) is inappropriate and may over estimate risk in our case, because it was performed by direct

delivery of attenuated poliovirus into stomach of premature infants, a very susceptible population group. Consumption of one coxsackievirus B4 virus gave a probability of infection of 8.0×10^{-3}, however, it may not be suitable for studying human health risk as this dose response relationship for coxsackievirus B4 was extracted from a mouse inhalation study and mortality was used as the end point of analysis (Suptel 1963). Overall, the rotavirus model seemed to be the most suitable one for estimating risk caused by a highly infectious enteric virus because it is the most potent virus in which study was conducted on adult human subjects through a fecal oral route. Echovirus 12, which is approximately 100 times less potent than rotavirus when a single particle is consumed, was chosen to represent moderately infectious viruses. Echovirus 12 model resemble the rotavirus model in which it is also conducted on adult human subjects and doses were administered orally. Table 4.1 provides the point estimates for parameters in several virus dose response models and comparison of probability of infection when consuming or inhaling one viral particle.

Table 4.1 Dose response models for several enteric viruses representing different potency.

Virus	Dose-response model	Model parameters	Transmission Route	Model type	Reference*	Risk assuming virus no. =1	Risk ranking
Adenovirus 4	Exponential	r = 0.4172	inhalation	human	Couch 1966	0.3411	1
Poliovirus III	Beta-poisson	$\alpha = 0.409$; $\beta = 0.788$	fecal-oral	human (infant)	Katz and Plotkin 1967	0.2847	2
Rotavirus	**Beta-poisson**	**$\alpha = 0.265$; $\beta = 0.42$**	**fecal-oral**	**human**	**Ward et al. 1986**	**0.2759**	**3**
Poliovirus I	Exponential	r = 0.009102	fecal-oral	human (infant)	Minor et al. 1981	0.0091	4
Coxsackievirus B4	Exponential	r = 0.007752	inhalation	mice	Suptel 1963	0.0077	5
Echovirus 12	**Beta-poisson**	**$\alpha = 0.374$; $\beta = 186.69$**	**fecal-oral**	**human**	**Schiff 1984**	**0.0020**	**6**
Poliovirus I	Beta-poisson	$\alpha = 0.1097$; $\beta = 1524$	fecal-oral	human (infant)	Lepow et al. 1962	0.0001	7

*original study referenced. Models were obtained from Haas et al. (1993) and Haas et al. (1999).

Exposure assessment. The third step of risk assessment is exposure assessment, which was determined by estimating the number of virus ingested during recreational activities. Exposures were determined by the magnitude of a contamination event (virus concentration in CSO discharge, CSO discharge volume and duration of discharge), distance from overflow outlet and travel time, transport and survival properties of viruses as well as volume consumed during recreational activities. In this study, several exposure scenarios were set up to elucidate the influence of different factors on the magnitude of risk: (i) swimming in a recreational park 25 km downstream from a CSO outlet, (ii) swimming in Lake Michigan beaches 50 km downstream from a CSO outlet, (iii) wading/angling in a recreational park 25 km downstream from a CSO outlet, (iv) wading/angling in Lake Michigan beaches 50 km downstream from a CSO outlet, (v) risk of swimming in the recreational parks or Lake Michigan beaches without a CSO event.

Site-specific parameters such as river discharge, CSO discharge volume, CSO discharge duration, area of channel and slope were either extracted from historical data or estimated from field measurements. Information regarding CSO discharge volume and discharge duration from CSO outlets in Grand Rapids between 2000 and 2008 were obtained from Michigan Department of Environmental Quality (http://www.deq.state.mi.us/csosso/). The State of Michigan required municipalities to report CSO events starting in summer 2000. Historical river discharge data from 1930 to 2007 for the USGS gage at Grand Rapids (hydrological station: USGS#04119000) were obtained from the USGS website (http://waterdata.usgs.gov/nwis/dv?referred_module=sw&site_no=04119000). Inactivation caused by sunlight irradiation on a specific day was determined by

computing the mean sunlight intensity of the day using SolarCalc 1.0 from USDA-ARS web page (http://www.ars.usda.gov/services/software/download.htm?softwareid=62). Mean attenuation caused by factors other than sunlight was estimated using data obtained from the tracer study (Chapter 3).

Information regarding the occurrence of enteric viruses was essential for risk assessment. In this study, virus concentration data were obtained from a monitoring study of the Grand river. Concentration of viruses in waste water, surface water and CSO discharge of the Grand River were quantified using real-time PCR, detailed protocols and results are described in Chapter 2. Based on the consideration that DNA in viruses with damaged viral capsids are reported to degrade within a short period of time, one copy of virus DNA was used to represent one viable virus in this risk estimate (Wetz et al. 2004).

Distribution of all exposure parameters included river discharge, CSO discharge volume, CSO discharge duration, virus concentration in CSO and volume consumed during a recreational event, and were determined using EasyFit software (MathWave Technologies, Spokane, WA).

Mean, standard deviation and range of virus concentrations in surface water and CSO discharges are presented in Table 4.2. The initial concentration of viruses in the river after CSO discharge, C_0, was estimated using the formula below:

$$C_0 = \frac{C_{CSO} \cdot Q_{CSO}}{(Q_{CSO} + Q_R)} \qquad (3)$$

where C_{CSO} denotes average concentration of viruses in CSO discharge (pfu/ml); Q_{CSO} is the flow rate of CSO discharge (m^3/s); Q_R is the flow rate of the river (m^3/s).

The changes in concentration of viruses as they were transported downstream were computed using an analytical formula developed by O'Loughlin and Bowmer (1975):

$$C(x,t) = \frac{C0}{2}\left\{ \begin{array}{l} e^{\frac{Ux}{2E}(1-\Gamma)}\left[\text{erfc}\left(\frac{x-Ut\Gamma}{2\sqrt{Et}}\right) - \text{erfc}\left(\frac{x-U(t-\tau)\Gamma}{2\sqrt{E(t-\tau)}}\right)\right] + \\ e^{\frac{Ux}{2E}(1+\Gamma)}\left[\text{erfc}\left(\frac{x+Ut\Gamma}{2\sqrt{Et}}\right) - \text{erfc}\left(\frac{x+U(t-\tau)\Gamma}{2\sqrt{E(t-\tau)}}\right)\right] \end{array} \right\}$$

where $\Gamma = \sqrt{1+4\eta}$ and $\eta = \frac{kE}{U^2}$ (4)

where, $C_{(x,t)}$ denotes tracer concentration as a function of distance traveled, x (m), and elapsed time since tracer release, t (s); τ represents spill duration (s); C_0 is the initial virus concentration at discharge point (virus/ml^{-1}); U is average velocity (ms^{-1}); E is longitudinal dispersion coefficient (m^{2}s^{-1}); k is first order decay rate constant (s^{-1}).

Number of viruses ingested, N, were calculated based on the formula:

$$N = C_{(x,t)} * V \quad (5)$$

where, V denotes volume of contaminated water consumed (Table 4.3).

Table 4.2 Mean, standard deviation and range of adenovirus concentrations in wastewater, river water and CSO discharges.

Sampling location	Sample type	NO. of positive sample/NO. of sample collected	Concentration (viruses/ml)			
			Mean	Standard Deviation	Minimum	Maximum
East Lansing WWTP	Raw sewage	13/13	1152	713.3	263	2817
	Effluent	13/13	82.6	119	13.5	428
Grand Rapids WWTP	Raw sewage	2/2	666	156	556	777
Lower Grand River	Surface water	6/20	7.76	19.8	<0.01	65.8
Sixth Street Park		1/4	16.5		<0.02	65.8
Deer creek Park		1/5	0.39		<0.01	1.89
Riverside Park		0/3	<0.02		<0.03	<0.04
Grand River Park		2/2	13.68		0.09	27.27
North Beach Park		1/4	0.03		<0.02	0.08
North Shore		1/2	29.9		<0.03	59.75
Market St. CSO	Untreated wastewater	6/6	535.4	500.8	60.23	1322.2

Risk characterization. The final step in a risk assessment is to combine information from the previous three steps: hazard identification, dose response assessment and exposure assessment to characterize health risk associated with a specified hazard. In this study, risk estimation was based on two estimation models, comparing both point and interval estimations. A point estimate model consists of a single numerical value for each parameter resulting in a value for probability of infection, usually using the mean, median or on occasion, 95% confident limit. In contrast, an interval estimate model presents either a confidence interval or a full probability distribution of risk, taking into account distributions and uncertainties associated with input parameters.

Point risk estimate

A point risk estimate of exposure to viruses when swimming in contaminated water 25 km and 50 km downstream of the discharge point was computed based on mean values for river discharge (summer), CSO discharge volume (summer) and CSO discharge duration (overall). River discharge and CSO discharge volume values for summer were used because they were significantly different from those values computed for winter and spring (Chapter 3) and summer represents a key exposure period for recreational activities, such as swimming and wading. In addition, summer was identified as a high risk season considering a higher frequency of CSO events. In order to show influences of virus concentration in CSO discharge on the risk estimate, the highest and the lowest virus concentrations determined from monitoring CSO discharges were used. Risk estimates were determined using the beta-Poisson dose response models for rotavirus and echovirus 12, each representing highly and moderately infectious viruses.

Consumption volumes of 50 ml and 10 ml were used for swimming and wading/angling, respectively (see Table 4.3; Dufour et al. 2006; Donovan et al. 2008). Point estimates were used for infection, P_i, morbidity, $P_{morbidity}$ and mortality rate, $P_{mortality}$ with formula as described below.

The daily risk of morbidity ($P_{morbidity}$) was calculated using a morbidity rate of 0.5 (Haas et al. 1993):

$$P_{morbidity} = P_i * 0.5 \quad (6)$$

The risk of mortality was calculated using a mortality rate of 0.001 (Bennett et al. 1987):

$$P_{mortality} = P_{morbidity} * 0.001 \quad (7)$$

The annual risk of infection, morbidity and mortality, P_d, were estimated based on the formula:

$$P_d = 1 - (1 - r)^d \quad (8)$$

where, r is the single event risk and d is the total number of days exposed.

The annual risk for swimming was estimated based on the number of CSO events in a regular swim season in Michigan, which normally begins on Memorial day (May 31) and ends by Labor day (September 1), a maximum of 12 events per swimming season was used (assuming one swim event per CSO day). The annual risk for wading angler was based on the number of CSO events during a typical fishing season in the lower Grand River, which generally starts in mid May and ends by late September, a maximum of 20 events per angling season was computed based on historical data. Mean sunlight intensity ($I(t)$) of 348.22 (simulated value for 07/15/2008 by SolarCalc 1.0 software)

was used in simulations. A summary of parameters and their values used in the point estimate are listed in Table 4.3.

Table 4.3 Parameters and their associated values used in point risk estimation.

Variable	Symbol	Unit	Parameters	Notes
River Discharge	Q_R	m^3/s	107	
CSO discharge volume	V_{CSO}	m^3	9332	
CSO discharge duration	D_{CSO}	h	4	$Q_{CSO}= V_{CSO}/D_{CSO;}$ CSO was assumed to discharge at a constant rate
Virus concentration in CSO discharge	C_{CSO}	viruses/ml	low conc: 60; high conc: 1322	Chapter 2
Water ingestion rate				
Swimming	V	ml/event	50	Dufour et al. 2006
Wading	V	ml/event	10	Donovan et al. 2008
Exposure frequency				
Swimmer	d	Days/yr	12	Based on maximum no. of CSO event during a swim season
Wader/angler	d	Days/yr	20	Based on maximum no. of CSO event during an angling season
Mean sunlight intensity	$I(t)$	kW	348.22	Value for 7/15/2008 was used for all simulation
Inactivation due to sunlight irradiation	k_I	$d^{-1}kW^{-1}$	0.4	
Inactivation due to all other factors	k_0	d^{-1}	0.3	

Interval risk estimate

Recently, interval risk estimates have been preferred by researchers because they provide a probability range of the resulting risk, giving a better sense of precision and distribution of the estimation.

As a first step in performing an interval risk estimate, the distribution of each parameter was defined either based on actual data fitting or the best estimates. Distribution type, mean and standard deviation of input parameters used in this study are summarized in Table 4.4. Parameters used for characterizing virus transport included river discharge, CSO discharge volume, CSO duration, and virus concentration in CSO discharge. For estimating single event risk, accidental consumption volume for adult and non-adult were used as determined from experimental data from Dufour et al. (2006). Annual risk for swimming and wading referred to swimming and angling season in the lower Michigan. Due to the lack of actual data, triangular distribution was used to describe annual occurrence and frequency of swimming and angling events. Monte Carlo techniques were used to characterize the uncertainty associated with parameters. Monte Carlo techniques are computational algorithms that rely on repeated random sampling to compute results. It has been widely used in risk assessment to generate a distribution of derived risk caused by uncertainty and variability of data.

The exposure and risk estimation models were developed in both Excel and Matlab (Mathworks, Inc., Natick, MA). Output distributions of viral concentrations and risk of infections were obtained using Monte Carlo simulation techniques. A minimum of 10,000 iterations was conducted for each scenario. All distribution functions were

assumed to be statistically independent. Monte Carlo simulation was run with Risk solver (Frontline Systems, Inc, Incline Village, NV), an add-on to Microsoft Excel.

Table 4.4 Parameters and their associated distribution type, mean and standard deviation used in interval risk estimation.

Parameters	Symbol	Unit	Distribution	Values	Notes
River discharge *	Q_R	m^3/s	Lognormal	Overall: mean=106.64, SD**=33.50; Summer: mean=66.87, SD=50.31	
CSO discharge volume***	V_{CSO}	m^3	Lognormal	Overall: mean=9332,SD=38042; Summer: mean=6992, SD=31986	
CSO discharge duration**	D_{CSO}	h	Lognormal	Overall: mean=3.88,SD=5.47	
Virus concentration in CSO discharge	C_{CSO}	Viruses/ml	Triangular	10th=60, 50th=535, 90th=1322	n=6
Water ingestion					
Swimming (adult)	V	ml/event	Lognormal	mean=19, SD=19	n=12; Dufour et al. 2006
Swimming (non-adult)	V	ml/event	Lognormal	mean=38, SD=31	n=41; Dufour et al. 2006
Angling	V	ml/day		10	Donovan et al. 2008
Exposure frequency					
Swimmer	d	Days/yr	Triangular	Minimum=5, mode=9, maximum=12	

Table 4.4 cont'd

Angler	d	Days/yr	Triangular	Minimum=8, mode=15, maximum=20	
Mean sunlight intensity	$I(t)$	kW		348.22	Value for 7/15/2008 was used for all simulation
Inactivation due to sunlight irradiation	k_I	$d^{-1}kW^{-1}$	Uniform	0.38-0.47	Shen et al. 2008
Inactivation due to all other factors	k_0	d^{-1}	Uniform	0.002-0.46	Shen et al. 2008

* River discharge data (9/30/1930-4/22/2008) were obtained from hydrological USGS hydrological station (USGS#04119000) at Grand Rapids, Michigan. (Source: USGS National Water Information System, http://waterdata.usgs.gov/mi).

** SD: standard deviation

*** Kent county, MI, CSO discharge data from 2000-2008 were obtained from Michigan Department of Environmental Quality. Sanitary and Combined Sewer Overflow database (http://www.michigan.gov)

Recreational Risk on Days without a CSO Event

Both point and interval risk estimates were performed for swimming and wading in the area on days without a CSO event in order to examine the risk difference when distributions of parameters are included in analyses. Dose response models for rotavirus and echovirus 12 were used for risk comparisons between highly infectious viruses and moderately infectious viruses.

In point estimate, ingestion rates of 50 ml and 10 ml were used for swimming and wading, respectively. An average virus concentration of 7.76 viruses /ml was used. The annual risk for swimming was estimated based on a maximum of 94 days and the annual risk for wading was based on an annual exposure of 140 days. Partial Monte Carlo analysis was performed using average ingestion volumes for swimming (50 ml) and wading (10 ml), in conjunction with lognormal distribution of virus concentration in surface water (mean: 7.76 viruses/ml, standard deviation: 19.8) . Distributions for maximum exposure days for swimming and wading activities were also included in the partial Monte Carlo analysis. The annual risk for swimming was estimated based on a regular swim season in Michigan, which normally begins on Memorial day (May 31) and ends by Labor day (September 1), with a triangular distribution, with a maximum of 94 days (min:1, mode:12, max:94). The annual risk for wading was based on a typical fishing season in the lower Grand River, which generally starts in mid May and ends by late September (triangular distribution: min:1, mode:27, max:140). In the full Monte Carlo analysis, distributions of water ingestion rate during swimming for adults (mean: 19 ml, standard deviation: 19 ml) and non-adults (mean: 38 ml; standard deviation: 31 ml) were added. All parameters used are listed in Table 4.5.

Table 4.5 Parameters and their associated distribution type, mean and standard deviation used in risk estimation for recreation in surface water without a CSO event.

Parameters	Symbol	Unit	Distribution	Values	Notes
Point estimate					
Water ingestion					
Swimming	V	ml/event		50	
Wading	V	ml/event		10	Donovan et al. 2008
Virus concentration	C_0	viruses/ml		mean=7.76	n=20; non-detects were substituted with detection limit*0.50
Exposure frequency					
Swimming	d	Days/yr		94	
Wading	d	Days/yr		140	
Partial Monte Carlo					
Virus concentration	C_0	virus/ml	Lognormal	mean=7.76, SD=19.8	n=20; non-detects were substituted with detection limit*0.50
Exposure frequency					
Swimmer	d	Days/yr	Triangular	Min=1, mode=12, max=94	
Angler	d	Days/yr	Triangular	Min=1, mode=27, max=140	
Full Monte Carlo					
Water ingestion					
Swimming (adult)	V	ml/event	Lognormal	mean=19, SD=19	n=12; Dufour et al. 2006
Swimming (non-adult)	V	ml/event	Lognormal	mean=38, SD=31	n=41; Dufour et al. 2006

Sensitivity Analysis. The influence of input parameter values (i.e. mean, standard deviation and distribution) on risk estimation were determined by sensitivity analyses. Individual parameters were plotted against probability of infection outputs to examine if an important trend occurred.

Results

Public health risks associated with recreation in viral contaminated surface water were estimated for days followed a CSO event and on days without such an event. Results of risk estimation through point risk and interval risk estimation methods were compared. Sensitivity analyses were carried out to determine the most influential factors contributing to health risks.

Point risk estimate

Point risk estimate of swimming at recreational sites located 25 km and 50 km downstream of discharge point were calculated based on the mean summer river discharge, mean summer CSO discharge volume, mean CSO discharge duration and two virus concentrations (i.e. 60 viruses/ml and 1322 viruses/ml). Incidental consumption volume of 50 ml/event was used. Annual risk of infection was calculated based on a maximum of 12 swim days per year. All parameters used are summarized in Table 4.3 (p. 143).

In general, risk of rotavirus infection is 0.7 to 1.6 $\log_{10}$ higher than risk of echovirus 12 infection, based partly on the potencies of the virus models chosen for moderately and highly infectious viruses. In the scenario in which CSO virus concentration was 60 viruses/ml, the mean risk for rotavirus infection when swimming

were 1.4×10^{-1} and 8.6×10^{-2} for the 25-km and 50-km locations, respectively; the mean risk of echovirus 12 infection for these two locations were 3.9×10^{-3} and 2.1×10^{-3}. For the scenario in which CSO virus concentration was 1322 viruses/ml, mean risks of rotavirus infection were 2.8×10^{-1} and 1.9×10^{-1}, while the mean risks for echovirus 12 infections were 5.4×10^{-2} and 3.2×10^{-2}, for the 25-km and 50-km locations, respectively. The peak infection risk happens approximately 20 hours after the CSO event for the 25-km location and 40 hours after the CSO event for the 50-km location (Fig. 4.1). The probability of infection curves for both virus groups are shown in Fig. 4.1a (25-km location) and Fig 4.1b (50-km location).

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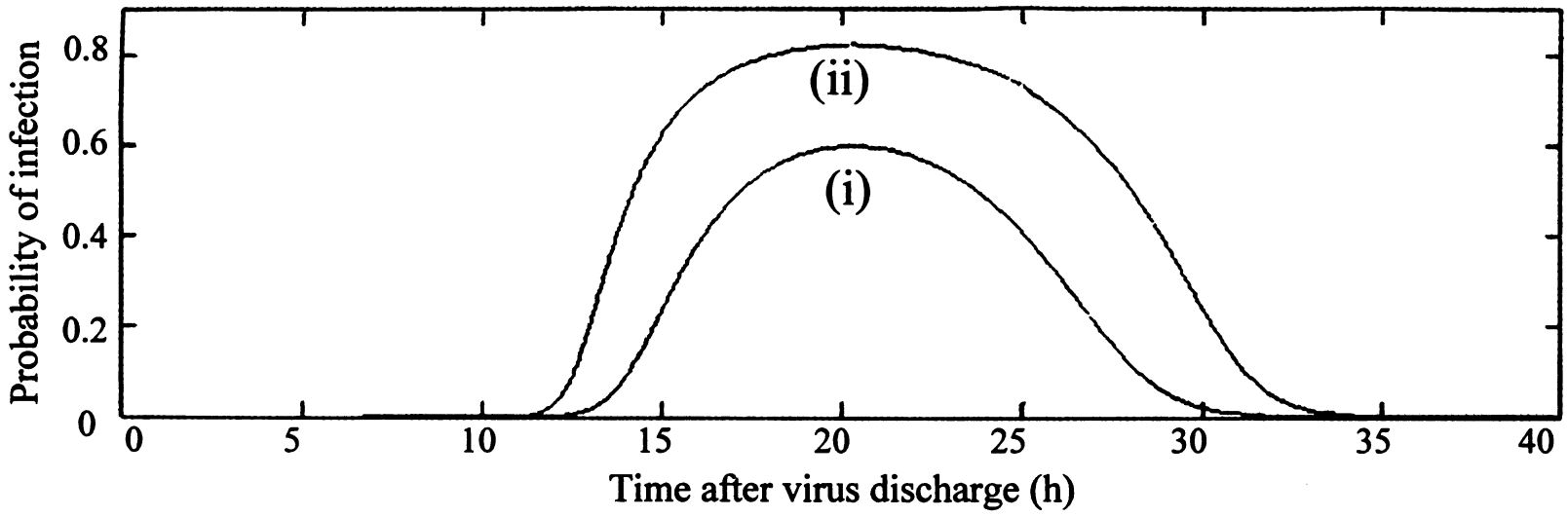

Figure 4.1a Probability of rotavirus infection versus time after CSO discharge 25 km downstream of discharge point. For (i), mean virus concentration is 0.03 viruses/ml, mean probability of infection is 1.4×10^{-1} with peak probability of infection of 5.7×10^{-1}. For (ii), mean virus concentration is 0.64 viruses/ml, mean probability of infection is 2.8×10^{-1} with peak probability of infection of 8.1×10^{-1}.

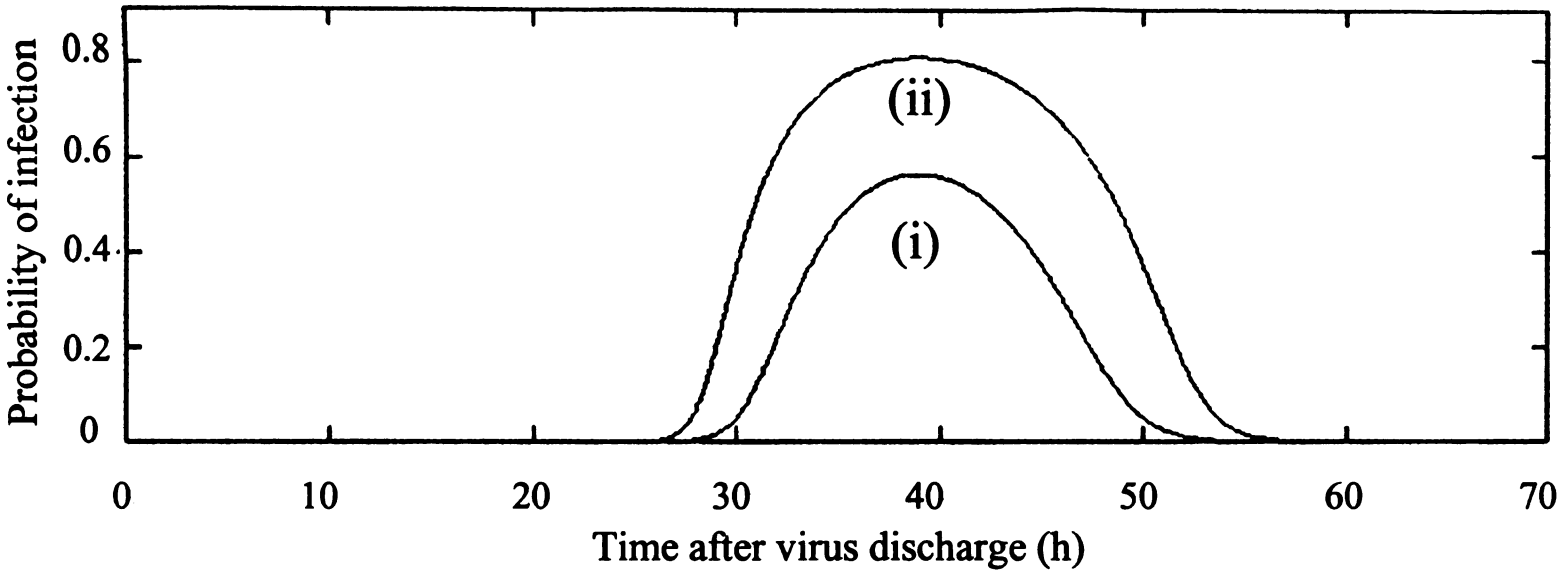

Figure 4.1b Probability of rotavirus infection versus time after CSO discharge 50 km downstream from discharge point. For (i), mean virus concentration is 0.01 viruses/ml; mean probability of infection is 8.6×10^{-2} with peak probability of infection of 4.9×10^{-1}. For (ii), mean virus concentration is 0.25 viruses/ml; mean probability of infection is 1.9×10^{-1} with peak probability of infection of 7.7×10^{-1}.

Figure 4.1 Probability of rotavirus infection versus time after CSO discharge 25 km (Fig 4.1 a) and 50 km (Fig 4.1 b) downstream of discharge point. CSO virus concentrations of (i) 60 viruses/ml and (ii) 1322 viruses/ml were compared.

Mean risk of infection decreased as distance from discharge point increased, the mean virus infection risk from swimming decreased approximately 0.22 $\log_{10}$ (range: 0.16-0.27 $\log_{10}$), over 25 km, this decrease is associated with the attenuation of viruses during transport.

The duration of elevated risk correlated directly with virus infectivity, travel distance, and tolerable risk levels (Table 4.6 and Table 4.7). The US EPA tolerable risk level for recreational freshwater is eight illness per 1000 recreational events (i.e. $P_{morbidity}$ of 0.008, which is equal to Pi of 0.016 (eq. 6)). The rotavirus model, which represents highly infectious viruses, gives a longer duration of elevated risk than the echovirus 12 model, which represents moderately infectious viruses. At the 25-km location, duration of elevated risk based on US EPA recreational standard for freshwater using rotavirus model began at approximately 12 h after virus release and ended at 33.5 h, compared to a "duration of elevated risk" that began at 14.5 h and ends at 27.5 h after virus release for echovirus 12 model. At the 50-km location, durations of elevated risk were 27-55 h, and 31- 48 h after virus release, for rotavirus and echovirus 12 models, respectively. The difference between duration of risk obtained from the different virus dose response models showed that the selection of an appropriate dose response model is crucial in decision making regarding duration of a beach closure to protect public health.

In cases where a more stringent standard is necessary, the US EPA tolerable risk level for drinking water could be used as a guideline. Here, duration of elevated risk based on tolerable risk level for drinking water (infection risk, P_i of less than 1.0×10^{-4} per annum) were determined and compared. At the 25-km location, estimated

duration of elevated risk associated with rotavirus infection began at approximately 9.5 h after virus discharge and lasted through 39 h after virus release, compare to duration of elevated risk that began at 12 h and ended at 33.5 h when US EPA recreational risk standard was applied (Table 4.6 and Table 4.7).

Risk estimates for wading are lower than risk estimates for swimming at both locations, because of a lower consumption rate used for wading activities (10 ml/event). Risk estimates and duration of elevated risk for wading downstream from CSO discharge point are summarized in Table 4.8 and 4.9. In general, infection risk using rotavirus model was 1-2 $\log_{10}$ higher than infection risk when echovirus 12 model was applied. At the 25-km location, mean rotavirus infection risk were 8.7 x 10^{-2} and 2.2 x 10^{-1} for low (60 viruses/ml) and high (1322 viruses/ml) CSO virus concentrations, respectively. For echovirus 12 model, mean infection risk was 8.0 x 10^{-4} for the low virus concentration and 1.54 x 10^{-2} for the high virus concentration. If echovirus 12 dose response was used a reference for risk estimates, infection risks were lower than the US EPA tolerable recreational risk level (Pi: 1.6 x 10^{-2}) at both locations (i.e. 25- and 50-km) when CSO discharge contained 60 viruses/ml, which means wading activities down stream are not affected by CSO discharge at all when the CSO virus level is 60 viruses/ml or less.

Overall, point risk estimates based on rotavirus dose response model (representing highly infectious viruses) gives more conservative results and are more protective of public health than risk estimates based on the echovirus 12 dose response model (representing moderately infectious viruses). Rotavirus model gives a longer duration of elevated risk compared to the echovirus model when the same initial virus

concentration was used. Point risk estimates based on rotavirus dose response show that, swimming and wading activities are not advisable at the beginning of the 12th h through the 34th h post CSO discharge 25 km downstream from discharge point and between the 27th h and the 55th h 50 km from discharge point; whereas point risk estimates based on echovirus 12 dose response gives a shorter duration of elevated risk, swimming and wading activities are not advisable at the beginning of the 14th h through the 28th h post CSO discharge 25 km downstream from discharge point and between the 31st h and the 48th h 50 km from discharge point (Table 4.6 and Table 4.7). In general, duration of elevated risk is dependent on initial virus concentration and the potency of the virus (dose-response model) used in the risk estimations.

Table 4.6 Single event and annual risk of rotavirus and echovirus 12 infection, morbidity, and mortality for swimming 25 km downstream of a CSO discharge point. Duration of elevated risk refers to time after virus release. Annual risks were calculated based on a swim season between May 31 and September 1, with a maximum exposure of 12 days and one swim event per day.

Virus	Low virus concentration (60 viruses/ml)				High virus concentration (1322 viruses/ml)			
	Mean Daily Risk	Peak Daily Risk	Annual risk	Duration of elevated risk (h)	Mean Daily Risk	Peak Daily Risk	Annual risk	Duration of elevated risk (h)
Rotavirus								
Risk of infection	1.43×10^{-1}	5.7×10^{-1}	8.42×10^{-1}	10-38[a]	2.79×10^{-1}	8.2×10^{-1}	9.80×10^{-1}	9.5-39[a]
Risk of morbidity	7.13×10^{-2}		5.88×10^{-1}	12.5-31.5[b]	1.40×10^{-1}		8.36×10^{-1}	12-33.5[b]
Risk of mortality	7.13×10^{-5}		8.55×10^{-4}		1.40×10^{-4}		1.67×10^{-3}	
Echovirus 12								
Risk of infection	3.90×10^{-3}	3.0×10^{-2}	4.58×10^{-2}	12.5-32[a]	5.39×10^{-2}	3.0×10^{-1}	4.86×10^{-1}	11.5-34.5[a]
Risk of morbidity	1.95×10^{-3}		2.32×10^{-2}	18-23[b]	2.70×10^{-2}		2.80×10^{-1}	14.5-27.5[b]
Risk of mortality	1.95×10^{-6}		2.34×10^{-5}		2.70×10^{-5}		3.23×10^{-4}	

[a] Duration of elevated risk based on annual infection risk of less than 1.0×10^{-4} (US EPA drinking water standard).

[b] Duration of elevated risk based on a morbidity risk of 8.0×10^{-3} (US EPA fresh recreational water standard).

Table 4.7 Single event and annual risk of rotavirus and echovirus 12 infection, morbidity, and mortality for swimming 50 km downstream of a CSO discharge point. Duration of elevated risk refers to time after virus release. Annual risks were calculated based on a swim season between May 31 and September 1, with a maximum exposure of 12 days and one swim event per day.

Virus	Low virus concentration (60 viruses/ml)				High virus concentration (1322 viruses/ml)			
	Mean Daily Risk	Peak Daily Risk	Annual risk	Duration of elevated risk (h)	Mean Daily Risk	Peak Daily Risk	Annual risk	Duration of elevated risk (h)
Rotavirus								
Risk of infection	8.61×10^{-2}	5.6×10^{-1}	6.61×10^{-1}	25-59.5[a]	1.92×10^{-1}	8.0×10^{-1}	9.23×10^{-1}	23.5-62[a]
Risk of morbidity	4.31×10^{-2}		4.10×10^{-1}	29-51.5[b]	9.61×10^{-2}		7.02×10^{-1}	27-55[b]
Risk of mortality	4.31×10^{-5}		5.16×10^{-4}		9.61×10^{-5}		1.15×10^{-3}	
Echovirus 12								
Risk of infection	2.10×10^{-3}	2.0×10^{-2}	2.49×10^{-2}	27.5-53.5[a]	3.19×10^{-2}	2.4×10^{-1}	3.22×10^{-1}	26-57[a]
Risk of morbidity	1.05×10^{-3}		1.25×10^{-2}	37.5-40.5[b]	1.60×10^{-2}		1.75×10^{-1}	31-48[b]
Risk of mortality	1.05×10^{-6}		1.26×10^{-5}		1.60×10^{-5}		1.91×10^{-4}	

[a] Duration of elevated risk based on annual infection risk of less than 1.0×10^{-4} (US EPA drinking water standard).

[b] Duration of elevated risk based on a morbidity risk of 8.0×10^{-3} (US EPA fresh recreational water standard).

Table 4.8 Single event and annual risk of rotavirus and echovirus 12 infection, morbidity, and mortality for wading 25 km downstream of a CSO discharge point. Duration of elevated risk refers to time after virus release. Annual risks were calculated based on an angling season between May and September, with a maximum exposure of 20 days and one wading event per day.

	Low virus concentration (60 viruses/ml)				High virus concentration (1322 viruses/ml)			
Virus	**Mean Daily Risk**	**Peak Daily Risk**	**Annual**	**Duration of elevated risk (h)**	**Mean Daily Risk**	**Peak Daily Risk**	**Annual**	**Duration of elevated risk (h)**
Rotavirus								
Risk of infection	8.71×10^{-2}	4.1×10^{-1}	8.38×10^{-1}	11-35.5[a]	2.24×10^{-1}	7.3×10^{-1}	9.94×10^{-1}	10-38[a]
Risk of morbidity	4.36×10^{-2}		5.90×10^{-1}	14-29[b]	1.12×10^{-1}		9.07×10^{-1}	12.5-31.5[b]
Risk of mortality	4.36×10^{-5}		8.71×10^{-4}		1.12×10^{-4}		9.07×10^{-4}	
Echovirus 12								
Risk of infection	7.96×10^{-4}	5.0×10^{-3}	1.58×10^{-2}	12.5-30.5[a]	1.54×10^{-2}	9.0×10^{-2}	2.67×10^{-1}	11.5-33.5[a]
Risk of morbidity	3.98×10^{-4}		7.93×10^{-3}	NA[b]	7.70×10^{-3}		1.43×10^{-1}	15.5-25.5[b]
Risk of mortality	3.98×10^{-7}		7.96×10^{-6}		7.70×10^{-6}		1.54×10^{-4}	

[a] Duration of elevated risk based on annual infection risk of less than 1.0×10^{-4} (US EPA drinking water standard).

[b] Duration of elevated risk based on a morbidity risk of 8.0×10^{-3} (US EPA fresh recreational water standard).

Table 4.9 Single event and annual risk of rotavirus and echovirus 12 infection, morbidity, and mortality for wading 50 km downstream of a CSO discharge point. Duration of elevated risk refers to time after virus release. Annual risks were calculated based on an angling season between May and September, with a maximum exposure of 20 days and one wading event per day.

Virus	Low virus concentration (60 viruses/ml)				High virus concentration (1322 viruses/ml)			
	Mean Daily Risk	Peak Daily Risk	Annual risk	Duration of elevated risk (h)	Mean Daily Risk	Peak Daily Risk	Annual risk	Duration of elevated risk (h)
Rotavirus								
Risk of infection	5.56×10^{-2}	3.6×10^{-1}	6.81×10^{-1}	25.5-58.5[a]	1.59×10^{-1}	7.0×10^{-1}	9.69×10^{-1}	24-62.5[a]
Risk of morbidity	2.78×10^{-2}		4.31×10^{-1}	30.5-49.5[b]	7.96×10^{-2}		8.09×10^{-1}	28-53[b]
Risk of mortality	2.78×10^{-5}		5.56×10^{-4}		7.96×10^{-5}		1.59×10^{-3}	
Echovirus 12								
Risk of infection	4.20×10^{-4}	4.0×10^{-3}	8.37×10^{-3}	28.5-52.5[a]	8.40×10^{-3}	7.0×10^{-2}	1.55×10^{-1}	26.5-55.5[a]
Risk of morbidity	2.10×10^{-4}		4.20×10^{-3}	NA[b]	4.20×10^{-3}		8.07×10^{-2}	33.5-45[b]
Risk of mortality	2.10×10^{-7}		4.20×10^{-6}		4.20×10^{-6}		8.40×10^{-5}	

[a] Duration of elevated risk based on annual infection risk of less than 1.0×10^{-4} (US EPA drinking water standard).

[b] Duration of elevated risk based on a morbidity risk of 8.0×10^{-3} (US EPA fresh recreational water standard).

Interval risk estimates

Interval risk estimates associated with swimming and wadding in virus-polluted water were computed using rotavirus (represented highly infectious viruses) and echovirus 12 (represented moderately infectious viruses) dose response models. For volume consumed during swimming activity, a lognormal distribution with a mean of 38 ml and 19 ml were used for non-adult and adult swimmers, respectively (Dufour et al. 2006). Volume consumed during wading activity was set at 10 ml per event. River discharge, CSO discharge volume and CSO discharge duration values in summer along with their means, distributions and standard deviations were used in all simulations unless specified otherwise (refer to Table 4.4). Other parameters and their values used are listed in Table 4.4. A Monte Carlo analysis consisting of 10,000 trials was performed for each simulation.

Rotavirus infection risk distributions obtained from the Monte Carlo analysis did not follow a normal distribution and are highly right-skewed (Fig. 4.2), resulting in mean infection rates that are much higher than the median and 75th percentile risk values in some cases. However, in order to maintain uniformity and not to underestimate health risk, the mean rotavirus infection probability for each exposure group was used for reporting and comparisons. Mean rotavirus infection risks correlate positively to water ingestion volume and negatively with distance from discharge point. Mean rotavirus infection risks were higher than the US EPA tolerable risk level for freshwater 25 km downstream from discharge point for at least 100 h after virus release with infection risk peaked between the 18th and the 30th h after virus release (Fig. 4.3). At this location, the

peak rotavirus infection risks were the highest for non-adult swimmers (1.73×10^{-1}), followed by adult swimmers (1.41×10^{-1}) and waders (1.27×10^{-1}).

For the 50-km location, mean rotavirus infection risks were below the US EPA tolerable risk level for recreational freshwater for the first 10 h after virus release. At this location, mean rotavirus infection risk peaked between the 30^{th} and the 48^{th} h after virus release (Fig. 4.4). Peak rotavirus infection risks for the 50-km location follow a similar trend as for the 25-km location though mean peak infection levels were lower than those observed at the 25-km location. The mean probability of rotavirus infection was the highest for non-adult swimmers (1.06×10^{-1}), followed by adult swimmers (8.39×10^{-2}) and waders (7.47×10^{-2}). This trend can be explained by the consumption rate for each population group during recreational activities, non-adult swimmers tend to consume more water than adult swimmers and waders.

The length of duration of elevated risk was positively associated with distance from discharge point. For the 25 km location, the probability of rotavirus infection remains elevated (i.e. above US EPA tolerable risk level of 8 illnesses/1000 events) for at least 96 h after virus discharge (Fig 4.3). Duration of elevated risk 50 km downstream of the discharge point has a longer tail (slower decline) than duration of elevated risk curve at the 25-km location, which is in coherent with the virus transport pattern observed in bacteriophage P22 tracer study (Chapter 3). At this location, rotavirus illness risk level remains elevated for at least 140 h (Fig 4.4).

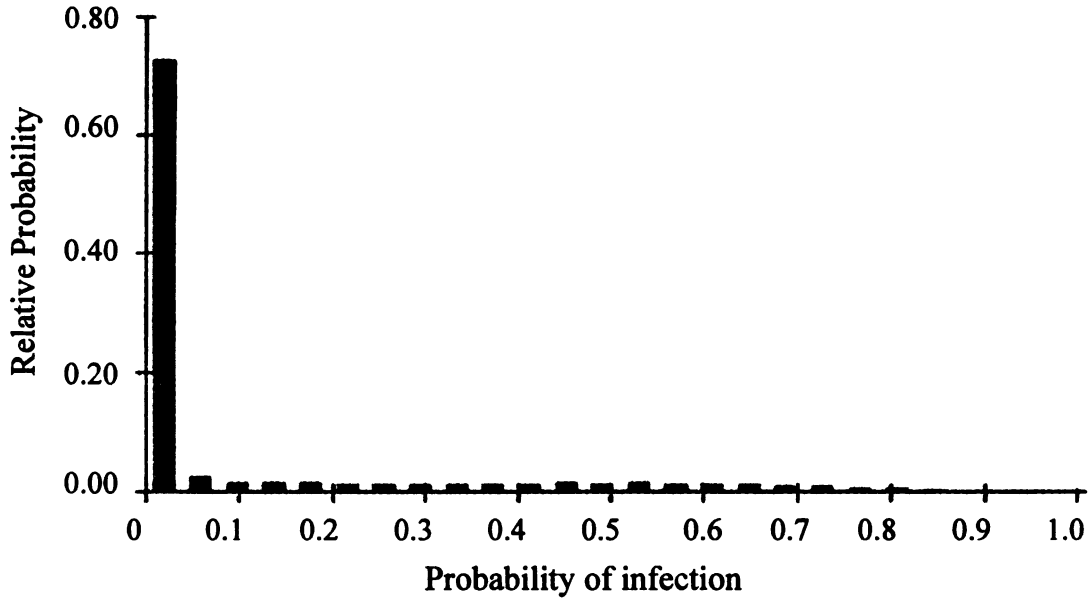

Figure 4.2 Distribution of rotavirus probability of infection by its relative probability (occurrence), 24-48 h after release, 50 km downstream of discharge point. Median probability of infection is lower than mean probability of infection.

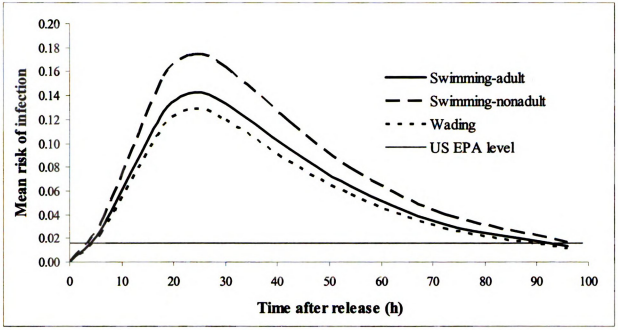

Figure 4.3 Mean risk of rotavirus infection for recreational activities within hours after CSO discharge, 25 km downstream from discharge point. Mean risk of infection peaked between 18th and 30th h after release. Mean infection risk was higher than 1.6 x 10^{-2} (calculated from US EPA tolerable recreational illness risk level of 8 x 10^{-3}) for at least 96 h after virus release.

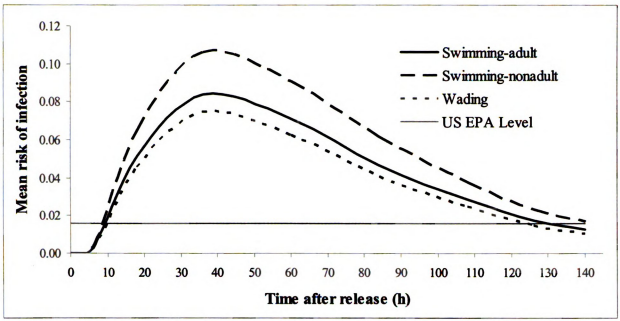

Figure 4.4 Mean risk of rotavirus infection for recreational activities within hours after CSO discharge, 50 km downstream from discharge point. Mean infection probability peaked between 30^{th} and 48^{th} h after release. Mean infection risk was higher than 1.6×10^{-2} (calculated from US EPA tolerable recreational illness risk level of 8×10^{-3}) for at least 140 h after virus release.

Compared to point estimate, interval estimate has an added advantage as one can include uncertainties associated with each parameter in a single simulation and an overall picture of risk (i.e. distribution, and range) can be obtained. A Monte Carlo simulation takes into account uncertainties and variability associated with each input parameter based on their distribution types. When compared to results from point estimate, it was observed that interval risk estimate based on mean rotavirus infection risk gave an extended duration of elevated risk. For the 25-km location, duration of elevated risk for rotavirus infection by the point estimate (based on an initial CSO virus concentration of 1322 viruses/ml) began at 27 h after virus release and lasted to 55 h after virus release, whereas for the interval estimate, the duration of elevated risk began approximately four hours after virus release and infection risk was elevated till at least 96 h after virus release (Table 4.6 & Fig 4.3). In other words, interval risk estimate gave a more conservative estimation; rotavirus infection risk level was elevated beyond the US EPA tolerable recreational freshwater level for 28 h and 92 h, according to the point estimate and the interval estimate, respectively. For the 50-km location, risk level for rotavirus infection was elevated between the 27^{th} and the 55^{th} h after virus release by point estimate, while it was elevated beginning at the 10^{th} h and declined below the US EPA risk level at approximately 140 h based on the interval estimate (Table 4.8 & Fig 4.4).

By defining a large range for the "time after release" parameter, sporadic "elevated risk event" at time ranges beyond the duration of elevated risk calculated based on the mean infection risk can be observed and may be included in risk management (Fig 4.5 and Fig 4.6) . For the 25-km location, it was shown that a random swimmer may have a 30 or 40% chance of getting a rotavirus infection from swimming in the contaminated

water approximately 140 hours after the discharge event (Fig 4.5). For the 50-km location, a sporadic elevated risk event may happen for more than 300 h after the discharge event (Fig 4.6).

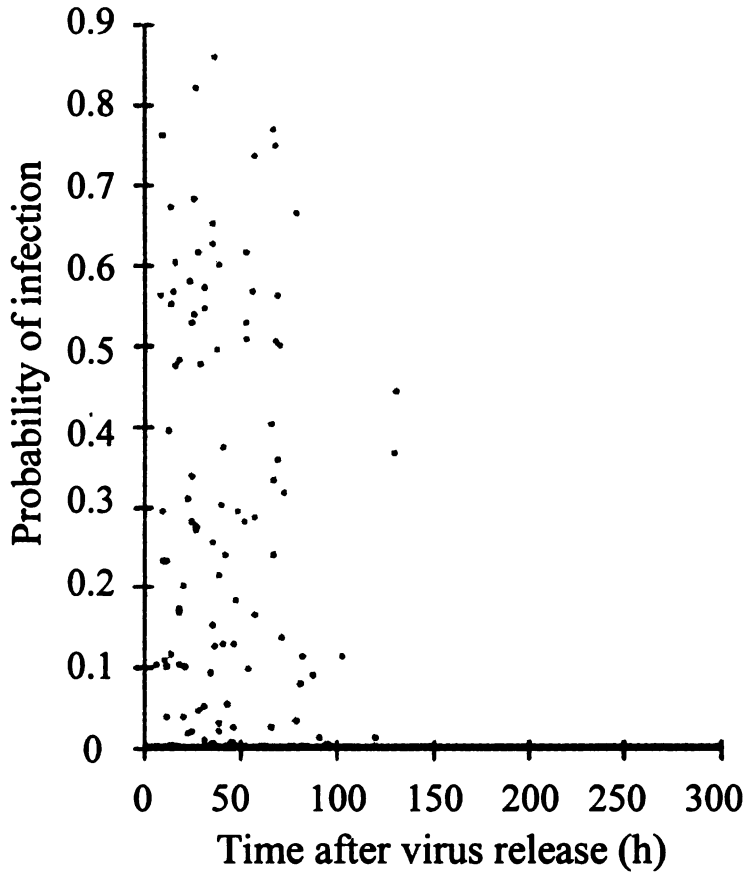

Figure 4.5 Probability of rotavirus infection versus time after release (h) 25 km downstream from discharge point. Elevated risk event could happen after more than 100 h after virus release. River discharge and CSO variables in summer were used for this simulation.

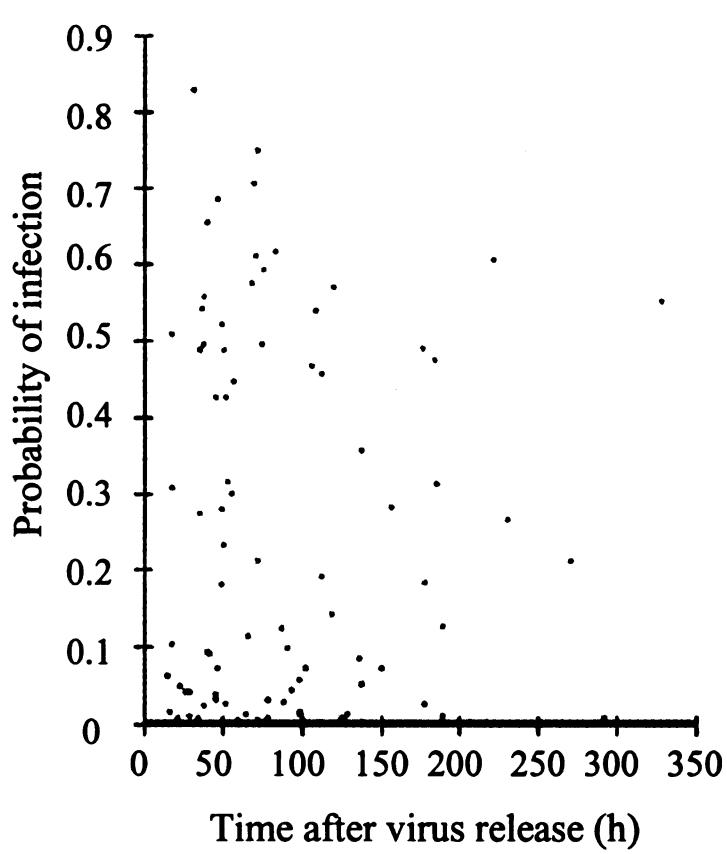

Figure 4.6 Probability of rotavirus infection verses time after release (h) 50 km downstream from discharge point. Elevated risk event could happen after approximately 300 h after virus release. River discharge and CSO variables in summer were used for this simulation.

Sensitivity analysis showed that river discharge is the primary contributor to uncertainty in probability of virus infection for both swimming and wading activities. A trend between river discharge and probability of rotavirus infection 25 km downstream from discharge point was found (Fig 4.7). In Fig 4.7, probability of rotavirus infection 25 km downstream of virus discharge point (time after virus release was set between 30-40 hours after virus release) peaks when river discharge is approximately 35 m^3/ s. The relationship between river discharge and probability of virus infection is dependent upon

distance from discharge point and time after contaminant release. For example, summer river discharge contributed to approximately 33 % of the single event rotavirus infection risk and 40 % of annual rotavirus infection risk in adult swimmers 25 km downstream of virus discharge point between 30-40 h after virus release. CSO duration contributed to 15% of single event infection risk and 18% of annual infection risk in adult swimmers under the same distance and time conditions.

In addition, the river discharge also highly influenced the duration of the elevated risk at each location. As river discharge increases, the duration of elevated risk decreases. In this case, when river discharge values for the summer (mean: 66.87 m^3/s, std dev: 50.31) was substituted with the overall mean river discharge values (mean: 106.64 m^3/s, std dev: 33.50), which has a higher mean and a lower variance compared to river discharges in the summer in risk simulation, duration of elevated risk at both locations (i.e. 25 and 50-km downstream) decreased. Mean infection probability peaked between the 14^{th} and 16^{th} h after release. For the 25-km location, mean rotavirus infection risk was higher than 1.6×10^{-2} (calculated from US EPA tolerable recreational illness risk level of 8×10^{-3}) for less than 40 h after virus release (Fig 4.8), compared to approximately 96 h after virus release when river discharge for summer was used (Fig 4.3). Peak mean rotavirus infection risks were also higher in this simulation. They were 2.71×10^{-1}, 3.28×10^{-1} and 2.47×10^{-1} for adult swimmers, non-adult swimmers and waders, respectively, compared to 8.39×10^{-2} (adult swimmers), 1.06×10^{-1} (non-adult swimmers), and 7.47×10^{-2} (waders) when river discharge values for summer were used. Other parameters such as seasonal virus concentration in CSO discharge and CSO discharge

duration, do not show significant influence on duration of elevated risk and risk distribution.

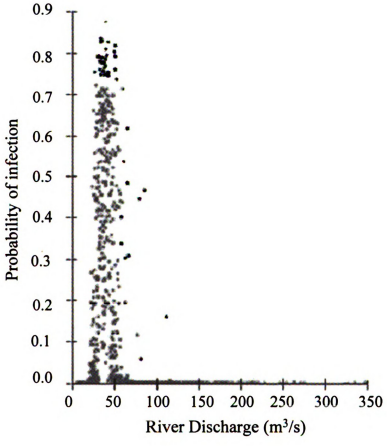

Figure 4.7 Scatter plot of probability of rotavirus infection versus river discharge (m^3/s) 25 km downstream of virus discharge point between 30 and 40 hr after virus release. The probability of infection peaks when river discharge is approximately $35 m^3/s$.

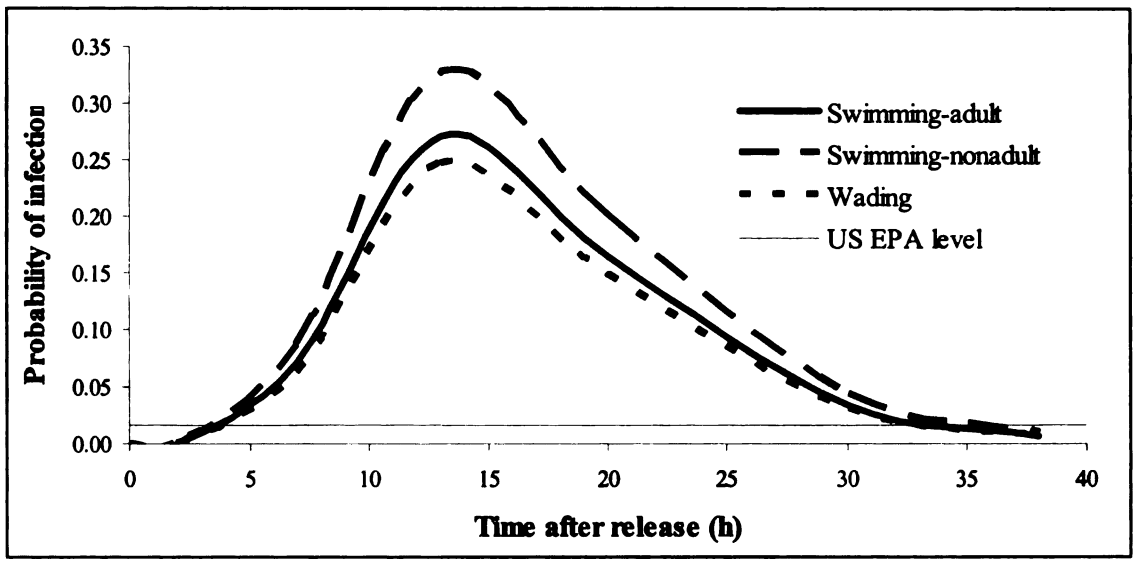

Figure 4.8 Mean risk of rotavirus infection for recreational activities within hours after CSO discharge, 50 km downstream from discharge point, simulated with the overall river discharge values (including all seasons, mean: 106.64 m^3/s, std dev: 33.50). Mean infection probability peaked between the 14^{th} and 16^{th} h after release. Peak mean rotavirus infection risks were $2.71x10^{-1}$, $3.28x10^{-1}$ and $2.47x10^{-1}$ for adult swimmers, non-adult swimmers and waders, respectively. Mean infection risk was higher than 1.6 x 10^{-2} (calculated from US EPA tolerable recreational illness risk level of 8 x 10^{-3}) for less than 40 h after virus release.

The last part of this risk analysis evaluated risk for swimming in the lower Grand River on days without a CSO event. For this scenario, outcomes from point estimate were compared to those of interval risk estimates. Only two parameters: ingestion rate during swimming and virus concentration were used in the analysis. In point estimate, ingestion rates of 50 ml and 10 ml were used for swimming and wading, respectively. Dose

response models for rotavirus and echovirus 12 were used for risk comparisons between highly infectious viruses and moderately infectious viruses. An average virus concentration of 7.76 viruses /ml was used. The annual risk for swimming was estimated based on a maximum of 94 days and the annual risk for wading was based on an annual exposure of 140 days. For the partial Monte Carlo analysis, distributions for virus concentration in surface water (lognormal; mean: 7.76 viruses/ml, standard deviation: 19.8) and maximum exposure days for both swimming (min:1, mode:12, max:94) and wading activities (triangular distribution: min:1, mode:27, max:140) were added. In the full Monte Carlo analysis, distributions of water ingestion rate during swimming for adults (mean: 19 ml, standard deviation: 19 ml) and non-adults (mean: 38 ml; standard deviation: 31 ml) were included in the simulation. All parameters used are listed in Table 4.5.

In point estimate, a single swimming event risk of $8.4x10^{-1}$ and $3.4x10^{-1}$ was computed for rotavirus and echovirus 12, respectively (Table 4.10). For a single wading event, infection risk were slightly lower, $7.5\ x10^{-1}$ for rotavirus and $1.2x10^{-1}$ for echovirus 12. These risk values shows that even during normal days, risk of virus infection is elevated in the lower Grand River. However, it must be noted that the use of the average virus concentration in surface water for this risk calculation most likely caused overestimations because only 6 out of 20 surface water samples analyzed were positive for adenovirus DNA (non-detects were substituted with detection limit*0.50). In addition, the assumption that one virus DNA copy represents one viable virus may contribute to risk overestimation as well. Moreover, other factors such as virus attenuation in the water were not included in the model.

Results from partial Monte Carlo and full Monte Carlo analyses were less conservative (have lower mean risk values) than results from point estimate, demonstrating the influence of uncertainties associated with virus concentrations in surface water and ingestion volume. However, with partial and full Monte Carlo analyses, distribution and range of infections could be obtained. Partial Monte Carlo analysis gives a mean single event risk of infection from swimming of 7.7×10^{-1} (min: 3.7×10^{-1}; max: 9.3×10^{-1}) and 2.3×10^{-1} (min: $<1.0 \times 10^{-3}$; max: 7.9×10^{-1}) for rotavirus and echovirus 12, respectively (Table 4.10). In partial Monte Carlo estimation, single event risks for wading are lower than point estimates for both viruses as well, 6.5×10^{-1} (min: 5.0×10^{-2}; max: 9.2×10^{-1}) for rotavirus and 9.0×10^{-2} (min: $<1.0 \times 10^{-3}$; max: 7.2×10^{-1}) for echovirus 12. Annual infection risks for rotavirus were higher than 9.9×10^{-1} for all scenarios (both swimming and wading activities) while annual infection risks for echovirus 12 was 9.1×10^{-1} for swimming and 7.8×10^{-1} for wading.

Full Monte Carlo analysis was conducted to evaluate the influence of uncertainty in ingestion volume, in addition to uncertainty associated with virus concentrations in surface water. Ingestion volume data for adult and non-adult swimmers were extracted from Dufour et al. 2006. Full Monte Carlo analysis gave a slightly lower risk estimates than those from partial Monte Carlo, showing minimal influence of ingestion volume on virus infection risk. The overall rotavirus and echovirus 12 infection risk from swimming are 7.2×10^{-1} (adult: 6.7×10^{-1}; non-adult: 7.3×10^{-1}) and 1.7×10^{-1}(adult: 1.2×10^{-1}; non-adult: 1.8×10^{-1}), respectively (Table 4.10). Full Monte Carlo analysis was not performed for wading activity because a distribution for ingestion rate was not available. Annual infection risks for rotavirus were higher than 9.9×10^{-1} for all scenarios (both

swimming and wading activities) while annual infection risks for echovirus 12 ranged between 7.2×10^{-1} and 8.3×10^{-1}. In all analyses, single event infection risks estimated using both rotavirus and echovirus 12 dose response models were higher than US EPA tolerable risk level for recreational freshwater. This suggested that the lower Grand River is contaminated by enteric viruses at levels where by the risk of contracting viral-induced acute gastrointestinal illness from recreating in the river without a CSO event is higher than 8×10^{-3}.

With the use of Monte Carlo analysis, difference in risk distribution for both viruses can be observed. In interval risk estimation, distribution of risk is important in determining a reference risk value to be utilized. Distributions of both rotavirus and echovirus 12 infection risk were fitted using Risk Solver®. In the full Monte Carlo analysis, probability of rotavirus infection is heavily left-skewed (i.e. has relatively few low values) and fits into a MinExtreme distribution. For example, the probability of rotavirus infection for adult swimmers has a mean (6.7×10^{-1}) that is lower than its median (7.0×10^{-1}) and mode (7.5×10^{-1}) (Fig 4.9). Probability of echovirus 12 infection, on the other hand, is heavily right-skewed and fits an exponential distribution. The probability of echovirus 12 infection for adult swimmers has a mean (1.2×10^{-1}) that is higher than its median (6.8×10^{-2}) and mode (6.0×10^{-3}) (Fig. 4.10). So, depending on the desired level of protection, mean, median, 95th percentile or other risk percentile from a risk distribution may be used in risk reporting and evaluation.

Table 4.10 Comparison of mean risk of infection for recreational activities using point estimate, partial Monte Carlo and full Monte Carlo for rotavirus and echovirus 12 in contaminated surface water in the lower Grand River.

Rotavirus	Mean risk	Min	Max	Echovirus 12	Mean risk	Min	Max	Annual risk***
Point estimate				**Point estimate**				
Swimming	$8.4x10^{-1}$			Swimming	$3.4x10^{-1}$			$9.9x10^{-1}$
Wading	$7.5x10^{-1}$			Wading	$1.2x10^{-1}$			
Partial Monte Carlo*				**Partial Monte Carlo**				
Swimming	$7.7x10^{-1}$	$3.7x10^{-1}$	$9.3x10^{-1}$	Swimming	$2.3x10^{-1}$	$<1.0x10^{-3}$	$7.9x10^{-1}$	$9.1x10^{-1}$
Wading	$6.5x10^{-1}$	$5.0x10^{-2}$	$9.2x10^{-1}$	Wading	$9.0x10^{-2}$	$<1.0x10^{-3}$	$7.2x10^{-1}$	$7.8x10^{-1}$
Full Monte Carlo**				**Full Monte Carlo**				
Swimming-overall	$7.2x10^{-1}$	$8.0x10^{-2}$	$9.5x10^{-1}$	Swimming-overall	$1.7x10^{-1}$	$<1.0x10^{-3}$	$8.5x10^{-1}$	$8.1x10^{-1}$
Adult	$6.7x10^{-1}$	$1.2x10^{-1}$	$9.2x10^{-1}$	Adult	$1.2x10^{-1}$	$<1.0x10^{-3}$	$7.4x10^{-1}$	$7.2x10^{-1}$
Nonadult	$7.3x10^{-1}$	$2.4x10^{-1}$	$9.4x10^{-1}$	Nonadult	$1.8x10^{-1}$	$<1.0x10^{-3}$	$8.0x10^{-1}$	$8.3x10^{-1}$

* partial Monte Carlo was conducted using an average ingestion volume of 10 ml/wade, 50 ml/swim and lognormal distribution of virus concentration in surface water (mean: 7.76 virus/ml, standard deviation: 19.8). Triangular distributions for days of exposure for wading (min=1, mode=27, max=140) and swimming (min=1, mode=12, max=94) were used for estimating annual risks.

** full Monte Carlo was conducted using lognormal distribution for both virus concentration and ingestion volume per swim (data from Dufour et al. 2006).

*** annual risk for rotavirus infection > 9.9×10^{-1} for all scenarios.

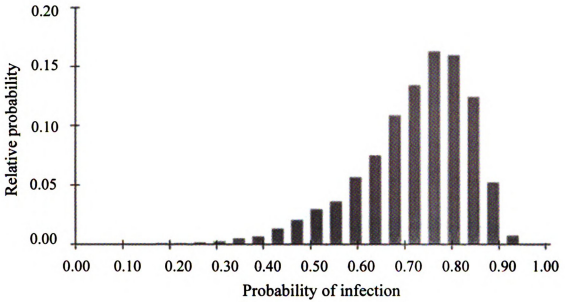

Figure 4.9 Relative probability of risk level versus probability of rotavirus infection for adult swimmers in the full Monte Carlo analysis shows distribution of rotavirus infections in surface water without a CSO event. Mean: 6.7×10^{-1}, median: 7.0×10^{-1}, mode: 7.5×10^{-1}.

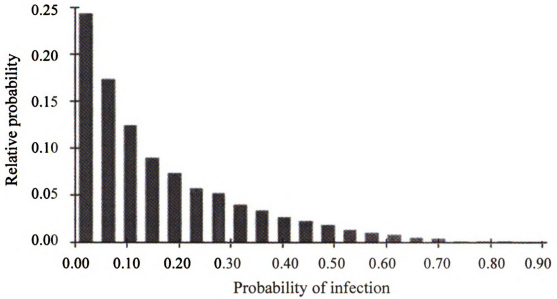

Figure 4.10 Relative probability of risk level versus probability of echovirus 12 infection for adult swimmers in the full Monte Carlo analysis shows distribution of echovirus 12 infections in surface water without a CSO event. Mean: $1.2x10^{-1}$, median: $6.8x10^{-2}$, mode: $6.0x10^{-3}$.

Discussion

Recently, quantitative microbial risk assessment models have been used to evaluate and describe risk associated with exposure to pathogens in different environmental matrices (Donovan et al. 2008; Eisenberg et al. 2008; Rose et al. 1991; Ryu and Abbaszadegan 2008; Soller et al. 2003; van Heerden et al. 2005). This risk analysis sought to fill gaps in previous risk analyses associated with virus contaminated water. This is the first risk analysis that incorporated a virus transport model to estimate virus attenuation downstream from discharge point. In addition, while risk analysis have been performed to estimate health risk of CSO discharge, this is the first study that

measured the concentration of a human virus pathogen in CSO discharges and applied the concentration in a risk assessment.

As with any microbial risk assessment model, this risk assessment comes with several limitations and contains assumptions on pathogen, host behavior and efficiency of pathogen detection assays. The most important limitation of this risk assessment is limited field data. In this assessment, average virus concentrations in CSO events were computed from only six samples collected in winter (one event) and spring (five events) 2008. As reported by other virus monitoring studies, virus concentrations may vary from season to season, affected by differences in temperatures, host discharge rates, and microbial composition of water (Krikelis et al. 1985; Skraber et al. 2004; Tani et al. 1995). In order to better characterize seasonal trend and distribution of virus concentrations for more accurate estimation of health risk, long-term monitoring of virus concentration in CSOs is warranted.

The high background risk estimated by this study ($>6.7x10^{-1}$ for rotavirus and $>1.2x10^{-1}$ for echovirus 12) based on average virus concentration in surface water is probably due to the high detection limit and insufficient field data (n = 20). The virus detection method used in this study has a quantification limit of 10 viruses/L, an improved virus detection method with a lower detection limit is essential for getting a more accurate picture of virus concentrations in impacted and contaminated surface water. In addition, because of the limited number of samples collected, a good distribution of virus concentration cannot be determined. A more frequent and rigorous monitoring of enteric virus concentrations in the lower Grand River is necessary for a more precise estimation as well as identification of pathogen hot spots (e.g. illicit

contaminant discharge point or transient storage area) along the river. Furthermore, because of a lack of knowledge on enteric virus-associated illness prevalence in the population, the attributable risk by water-related recreational activities in the area cannot be determined and compared with results from this assessment.

This risk assessment was built on several assumptions that may cause underestimations or overestimations of risks. Assumptions regarding attenuation of viral pathogen in surface water, viability of recovered viruses, infectivity of pathogens, and ingestion volume were used. Because it is impossible to inoculate river water with pathogenic microorganisms, the transport behavior and attenuation of a surrogate virus, bacteriophage P22, in surface water was modeled and used in this risk assessment. In this study, it was assumed that human viral pathogens (in our case, adenoviruses) are as persistent as bacteriophage P22 while the actual survival of these viruses in river water has not been compared.

Secondly, in order to generate a worst case scenario, it was assumed that all viruses isolated from environmental samples were infectious and that one DNA copy in PCR represents one virus. In reality, this may not be true. There is a possibility of quantification of partially degraded DNA from environmental samples as DNAs are known to outlast virus viability in the environment (Masago et al. 2008; Wetz et al. 2004).

In addition, assumptions regarding immunity or sensitivity of hosts that may cause underestimations of risk in certain population groups were used. For example, because of a general lack of dose response data, a single dose response model was used for both adult and children though children may be more vulnerable to these pathogens.

The recreational risk of other sensitive populations, such as pregnant women, elderly and immuno-compromised patients are not specified in this assessment.

This assessment is based on monitoring data for adenoviruses, although they are thought to be more prevalence and persistence than other waterborne enteric viruses, the cumulative risk from more than 100 types of enteric viruses, parasites and bacteria pathogens, which are regularly present in waste water is hard to determine. Nevertheless, this risk assessment provides a feasible framework to estimate risk from contacting with an important group of pathogens that were released into the river during a sewage spill or CSO event.

Overall, this risk assessment could be improved by a detailed study of major uncertain parameters in the model: 1) a long-term and more rigorous assessment of enteric virus concentration in the river during dry season and in CSO discharge; 2) comparison of viable virus counts to qPCR virus concentration; 3) survival of enteric virus versus bacteriophage P22; 4) survey of virus transport and dispersion at Lake Michigan beaches (plume studies).

Compared to other risk analysis on recreational water, this risk assessment estimated higher background virus infection rates, possibly due to the high background virus concentrations detected in the lower Grand River and some of the assumptions mentioned previously. In a risk assessment of adenoviruses in recreational water in South Africa, daily adenovirus infection risks of 1.71×10^{-4} and 3.12×10^{-5} were calculated for river water and dam water, respectively (van Heerden et al. 2005). The low infection risks were linked to the low adenovirus concentrations in the studied river water and dam water, virus levels were between 10^3 and 10^4 times lower than those found in this study.

Risk analyses related to recreational water have also been performed applying different methodologies. In an epidemiological study in which beachgoers were interviewed for health symptoms after recreating at four Great Lakes beaches polluted by sewage, Wade et al. 2008 found that gastrointestinal illness rate is directly correlated to daily average Enterococcus exposure. They observed a 3.4 % increase in gastrointestinal illness incidence in swimmers who were exposed to 100 *Enterococcus* compared to non-swimmers. During the 10 to 12 days follow-up period in the study, the incidence of new GI illness among swimmer and non-swimmers were 8.3% and 6.0%, respectively (Wade et al. 2008). Soller et al. (2006) took a different approach in their risk analysis, they built a water quality model based on fecal indicator data in the Newport Bay area for estimating site-specific concentrations of male specific coliphage (used as a surrogate for human enteric viruses). A much lower gastrointestinal illness rate (0.09 %) from recreating in marine beaches in South California was simulated based on a disease transmission model. This phenomenon is possibly because exceedances of the water quality standard based on bacteria indicators levels most commonly occur during the time of the year and in areas where recreational usage is low.

Until recently, few microbial risk assessments has been performed on health effects of recreation in contaminated freshwater, especially in the aftermath of a contamination event, such as a CSO event. Donovan et al. (2008) found high bacteria indicators and *Giardia* concentrations in two water samples collected within the vicinity of a CSO outlet in the Lower Passaic River but detailed analysis on other pathogens was not available. Based on their CSO data, Donovan et al. (2008) estimated annualized

infection risks of 0.88 for both *Enterococcus* and fecal *Streptococcus*, and >0.99 for *Giardia*.

Conversely, this risk analysis provides a comprehensive view of the health risk associated with recreation in surface water affected by CSO events; with sufficient monitoring and transport data, health effects associated with other pathogens can be evaluated. Different scenarios can be developed to demonstrate benefits of management choices, such as improved wastewater treatment, diversion of contaminated flow, public education or beach advisory/closure. With this model, it was suggested that duration of elevated risk and risk levels at a site are influenced by river discharge, CSO volume and CSO duration. Thus, instead of repeated measurement of microbial indicator levels, which is laborious and time consuming, a predictive system based on CSO discharge information and river discharge can be used by environmental managers for informed regulatory decision making such as duration of beach advisory or beach closure. In addition, this risk assessment framework may be used to evaluate CSO effects at other sites, provided that sufficient site-specific data (i.e. background bacteria indicators or virus concentrations, hydrogeology data, CSO discharge data) are available.

In short, this risk analysis provides valuable insights that can be applied in both regulatory and operational contexts. Environmental managers can refer to this analysis for regulatory decision-making, such as the posting of beach advisory or deciding duration of a beach closure. Wastewater operators may use this framework to examine the benefits of making operational changes or adding additional waste water treatments.

Conclusion

This risk assessment combined an analytical hydrological model with environmental data in a QMRA framework using probability of infection models. The result of this risk analysis suggests that contact with water in the lower Grand River after a CSO event poses significant health risk. However, more monitoring data are needed for evaluating seasonal risk from CSO discharges. Interval risk estimation gives a longer duration of elevated risk than point risk estimate. However, careful examination of interval risk estimation data, especially risk distribution, is important when interpreting risk. In this study, stream flow was a good predictor for duration of elevated risk. Overall, this analysis suggests that surface water quality in Grand Rapids can be highly impacted by CSO discharges and the community attention on preventing and controlling CSOs is warranted.

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CHAPTER 5.

SUMMARY AND CONCLUSIONS

Enteric viruses are important pathogens in surface water and ground water of the Great Lakes region. Enteric viruses, such as adenoviruses and enteroviruses, are capable of causing diseases affecting multiple organs in infected individuals and are major causes of water-transmitted gastrointestinal disease in swimmers (Bosch 1998). In the current study, it was hypothesized that human enteric viruses are present in high concentrations in sewage and rivers receiving sewage effluents in Michigan and waste water treatment is not efficient in removing these viruses. Real time PCR was used for adenoviruses detection and quantification in raw sewage, treated waste water and river water. Adenovirus was chosen as an index virus because of its high prevalence and persistence in aquatic environments.

Monitoring results showed that adenovirus consistently present in waters in Michigan and waste water treatment in East Lansing only physically removed less than two $\log_{10}$ of adenoviruses. The actual inactivation of adenoviruses could not be assessed because of limitation of detection method used. Average adenovirus concentrations were 1.15×10^6 viruses /L in raw sewage, 1.12×10^6 viruses/L in primary treated effluent, 1.05×10^3 and 2.0×10^4 viruses/L in secondary treated effluent and 8.3×10^4 viruses/ L in tertiary treated effluent. Enteric adenovirus type 41 (60%) was predominant in waste water tested. Other adenovirus types detected were Adenovirus type 12 (29%), type 40 (3%), type 2 (3%) and type 3 (3%). Human adenovirus concentrations were above real time PCR detection limit in 6/20 river water samples, with concentrations ranging between 8×10^1 and 6.6×10^4 viruses/L (mean: 7.76×10^3 viruses/L). Six combined sewer

overflow (CSO) samples were collected from the Market Street Retention Basin in Grand Rapids in 2008, adenovirus concentration in these samples ranged between 6 x 10^4 and 1.3 x 10^6 viruses/L (mean: 5.4 x 10^5 viruses/L). Analysis of variance (ANOVA) shows that virus concentration in CSO samples (average: 5.35 x10^5 viruses /L) were not significantly different from virus concentration in raw sewage and primary-treated effluent (p-value: 0.39). The high adenovirus concentration in CSO samples suggested that the discharge of CSO into the Grand River after heavy precipitation events could lead to adverse public health risk.

The main objective of this research was to quantify human health risk associated with enteric viruses from recreating in sewage contaminated surface water after a CSO event. In the current study, it was hypothesized that virus transport and attenuation in a river can be assessed by running a tracer study using a bacteriophage (i.e. bacteriophage P22) as a surrogate for waterborne viruses. Bacteriophage P22 morphologically resembles adenoviruses and unlike most soluble chemical tracer, it presents as suspended colloids and more adequately describes human virus behavior in water. As a part of the exposure assessment and to model virus transport and inactivation after CSO events, a tracer study using bacteriophage P22 was conducted on a 40-km reach of the lower Grand River in Grand Rapids. The transport and attenuation of bacteriophage P22 in the tracer study could be explained by sunlight inactivation, adsorption to suspended particles, transient storage and land use. It was thus determined to be a suitable tracer for this complex surface water system. From the tracer study, it was estimated that bacteriophage P22 has an inactivation range between 0.27 and 0.57 per day. An analytical virus transport model for estimating virus arrival times and concentrations after release was

then developed based on site specific information (i.e. river discharge, CSO discharge volume, and CSO discharge duration) and virus attenuation rate from the tracer study.

Using adenovirus as an index virus to represent the likelihood of virus survival through waste water treatments and virus concentration in CSO and surface water, it was hypothesized that the risks for enteric virus exposure at Great Lakes beaches are elevated to above US EPA acceptable risk level for recreational freshwater (8 illnesses per 1000 swimmers) during the swimming season, especially after a CSO event. With the field survey data and virus transport model, a quantitative microbial risk assessment was conducted to evaluate the probability of virus infection or gastrointestinal disease resulting from incidental ingestion of contaminated recreational water at Lake Michigan beaches that receive input from the lower Grand River.

The point risk assessment modeled with the lowest CSO virus concentration and CSO discharge volume estimated that the probability of rotavirus infection and gastrointestinal illness were $4.8x10^{-1}$ and $2.4x10^{-1}$, respectively. The risk assessment found that with the levels of virus discharged, contact with the water after a CSO event poses significant human health risk. The high risk associated with recreating in water receiving CSO discharge determined from this study emphasizes the importance of proper wastewater treatment and retention. For all scenarios, estimated risks were higher than the acceptable level set by U.S. EPA for recreational freshwater.

Infection risks related to swimming in beaches of the lower Grand River without a CSO event were assessed using rotavirus (representing highly infectious viruses) and echovirus 12 (representing moderately infectious viruses) dose response models. Risk of rotavirus infection related to swimming in beaches in the lower Grand River without any

CSO event ranged between $8.0x10^{-2}$ and $9.5x10^{-1}$ (mean: $7.2x10^{-1}$). The echovirus 12 dose response, on the other hand, gave infection risks range between 0.00 and $8.5x10^{-1}$ (mean: $1.7x10^{-1}$). Regardless of dose response model use, estimated risks were again higher than US EPA's acceptable risk level for recreational freshwater.

Monte Carlo simulations were used to characterize uncertainties associated with different discharge scenarios (i.e. seasonal stream flow, CSO discharge volume, virus concentration, distance from discharge point etc). River discharge and CSO duration were identified as two main factors influencing risk levels and duration of elevated risk. Summer river discharge contributed to approximately 31% of single event infection risk and 38% of annual risk of infection in adult swimmers. CSO duration contribute to 15% of single event infection risk and 18% of annual infection risk in adult swimmers. Effect of a CSO discharge may last for several days depending on river discharge and distance from CSO discharge point, i.e. up to 140 hours and 300 hours at 25 and 50 km from CSO discharge point, respectively.

In conclusion, concentrations of enteric viruses in the lower Grand River make it unfit for bodily contact recreational activities, especially after a CSO event. Analytical virus transport model used in the current study served as a good starting point for estimating virus survival, transport and associated health risk at a specific location as a result of a CSO discharge or sewage spill. Availability of CSO discharge information and site specific data (i.e. river discharge, area of channel, and slope of river) make it possible for a more accurate estimation of duration of elevated risk at a recreational site and inform improved management options.