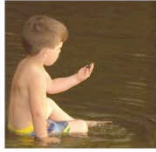


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# Enteric Viruses in Recreational Waters

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This presentation will cover the current trends and gaps in knowledge of the incidence of recreational waterborne viral diseases. The presentation also addresses the lack of an appropriate viral indicator organism and offers potential indicators which correlate with the presence of viruses.

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## Justification

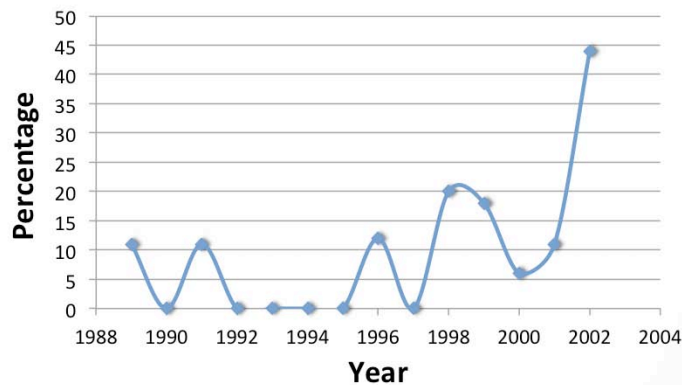
- Unknown causation of recreational water-based diseases
  - Bacterial
  - Protozoan
  - Viral
- No active surveillance system for recreational waterborne diseases
- No appropriate indicator organism for viruses

The current published literature would suggest that the major etiology of recreational water-based diseases are bacterial and protozoan based. This can be especially illustrated in publications focusing on various protozoa which are resistant to chlorination and have low infectious doses in humans (Sinclair, Jones, and Gerba, 2009). Unfortunately, it is believed that viruses are also a significant cause of recreational water-based diseases. Due to the low infectious dose, lack of culture-based detection methods, and only indirect disease surveillance systems for recreational water, there is a lack of knowledge of the actual disease incidence from viruses in recreational water.

In the United States, the surveillance of recreational waterborne outbreaks is performed by the U.S. Environmental Protection Agency (EPA), the Council of State and Territorial Epidemiologists, and the Centers for Disease Control (CDC). The state and local public health departments are responsible for investigating and reporting outbreaks to CDC, so while surveillance systems are in place, they are passive systems which rely on the local investigating agencies to recognize the occurrence of an outbreak, identify the source, and report the findings to the CDC. The challenges involved in identifying and detecting viruses result in minimal surveillance data (EPA, 2009). This lack of knowledge is compounded by the absence of an appropriate viral indicator organism in the current practices used to determine water quality and safety (Sinclair, Jones, and Gerba, 2009).

## Viral Trends

**Percentage of Virus-Based Gastroenteritis Outbreaks in Recreational Water**



In the depicted line graph, it can be seen that the percentage of virus-related recreational waterborne disease outbreaks has increased from 1989 to 2002. The graph, which was adapted from Sinclair, Jones, and Gerba (2009) as well as information from WHO (2011), indicates that the increase in viral-based outbreaks has not occurred steadily. This increasing trend may indicate a rise in viral-borne diseases, which may be the case due to increased popularity of group-based water activities including all-inclusive resorts, cruise ships, and water parks, or an increase in detection of viruses due to the CDC's inclusion of RT-PCR techniques in their surveillance laboratories. The inclusion of RT-PCR techniques began in 1993 and has led to the detection of many viruses, including Norovirus in numerous outbreaks (CDC, 2003).

## Major Viral “Offenders”

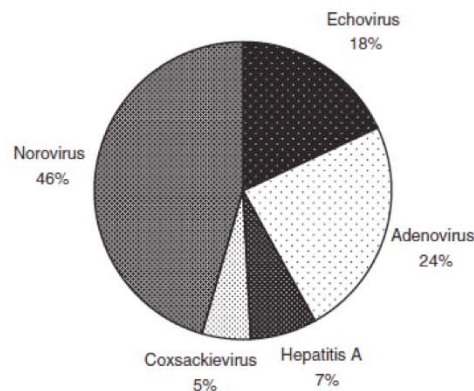


Figure 2 Outbreaks by type of recreational water,  $n = 55$ .

The pie chart shows that forty-six percent of all enteric virus-based outbreaks of known etiology were associated with noroviruses. A total of 55 outbreaks have been identified and investigated since 1989 and with noroviruses being associated with 25 of these outbreaks, this agent represents an important disease agent in recreational waters. This trend is understandable since it is estimated that noroviruses cause 23 million cases of food- and water-borne illness per year in U.S. (Podewils et al., 2007). The second most prevalent etiological agent was found to be adenovirus, which was associated with 13 outbreaks. Ten outbreaks were associated with echoviruses. Hepatitis A virus was responsible for 4 outbreaks and coxsackieviruses caused 3 of the 55 outbreaks (Sinclair, Jones, Gerba, 2009).

The represented data only includes outbreaks where the disease etiology was investigated and determined. Therefore, this data is not believed to be completely representative of all viral-borne recreational water illnesses due to the expected underreporting associated with the data.

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## Enteric Viruses

- Causative agents of respiratory, ocular, and gastroenteritis illnesses
- Primary manifestation is diarrhea
- Viruses replicate in intestinal system and shed through feces
  - Transmission from person-to-person
  - Transmission through surfaces-to-person
  - Shedding concentrations of  $10^6$  viruses/gram

Enteric viruses are associated with a wide range of diseases in humans, including respiratory, ocular, and gastrointestinal illnesses. The at-risk groups for increased incidence of enteric viral diseases include children, the elderly, and the immunocompromised (Griffin et al., 2003). The increased incidence in the young will be covered more in-depth later in the presentation.

The common disease symptom associated with enteric viruses is diarrhea, which has two major consequences. Firstly, the onset of diarrhea does not usually cause people to seek medical attention unless there is a pre-existing underlying condition. This leads to an underreporting of disease incidence and outbreaks. Secondly, the viral agents often replicate in the intestinal tract and are then “shed” from the body through feces. This shedding process can occur for weeks, further spreading the illness within households through personal and formite contact. Viral shedding through feces can reach concentrations of  $10^{10}$  viruses/gram, but on average occurs at  $10^6$  viruses/gram (Griffin et al., 2003).

## Implicated Areas of Recreational Water

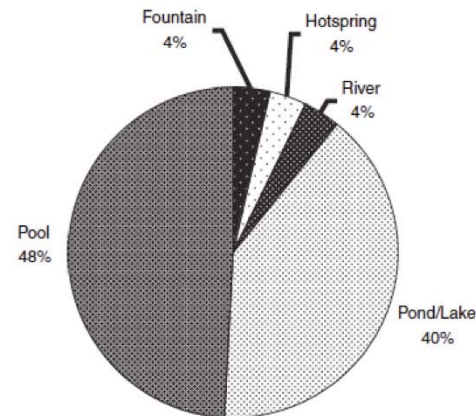


Figure 1 Outbreaks by an aetiological agent, n = 55.

Twenty-seven of the 55 investigated outbreaks occurred in swimming pools and 22 outbreaks were associated with lakes and ponds. Two outbreaks were associated with each rivers, hot springs, and recreational fountains (Sinclair, Jones, and Gerba, 2009).

It must be noted that as the case with the disease etiology data, this is not believed to be a true representation of disease incidence due to underreporting. A further compounding factor is that diseases associated with swimming pools are more likely to be reported. This is because they often represent a specific population of those who attended the specific pool, as well as defined groups such as swim teams where there is communication between the team members.

## Norovirus Outbreaks

- Estimated 23 million cases of food- and water-borne illness per year in U.S.
- No known nonhuman reservoirs of human norovirus
- Identification of norovirus primarily occurred after 1993 with the use of RT-PCR detection methods by CDC

Outbreak Location	Norovirus Cases
Swimming Pool	7
Lakes	14
Rivers	2
Recreational Fountains	1
Hot Springs	1

Norovirus is highly contagious and only spreads through food, water, formite, and person-to-person contact since there is no known reservoir of the human strain of the virus. The virus mostly commonly causes vomiting and diarrhea within 10-20 hours after exposure. This action aerosolizes the virus particles through vomit or spreads the virus through fecal shedding, causing the illness to quickly spread. The resistance of Norovirus to chlorination is debated in the literature, but the virus tends to be more associated with untreated freshwater including lakes.

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## Norovirus Case Study

- On 3 February 2004, the Vermont Department of Health received reports of acute gastroenteritis
- Interviews showed a possible connection between the ill and a local swimming facility
- 53 of 189 people interviewed showed symptoms of vomiting and/or diarrhea
- 5 specimens positive for Norovirus
  - 3 had identical sequences

A retrospective cohort study was performed by the Vermont Department of Health to determine the causative agent of reported cases of acute gastroenteritis. Reports of the gastroenteritis were received by the Department of Health and from the initial questioning performed on February 3<sup>rd</sup>, 2004, it was found that a common swimming facility was visited by many of the ill. The investigating team questioned 189 people who had visited the swimming pool between January 30 and February 2. The team found that 53 (28%) of the pool's visitors had vomiting or diarrheal symptoms within 72 hours of being at the pool. There were 5 positive specimens for norovirus, three of which were identical sequences. The cause of the outbreak was determined to be insufficient chlorination and inadequate maintenance of the facility (Podewils et al., 2007).



## Adenovirus Outbreaks

- First described in 1953
- DNA genetic code
- Significant cause of acute upper respiratory tract infections
  - Conjunctivitis and pharyngoconjunctival fever
- 48 serotypes
  - 40 and 41 linked to gastroenteritis symptoms

Outbreak Location	Norovirus	Adenovirus
Swimming Pool	7	11
Lakes	14	2
Rivers	2	0
Recreational Fountains	1	0
Hot Springs	1	0

Adenoviruses differ from the other viral agents presented here in that the genetic code is DNA-based rather than RNA-based. This allows the identification of the virus through standard PCR, rather than RT-PCR which is more costly. The first outbreak appeared in 1953, where viral pharyngoconjunctival fever was reported to have spread from a common swimming area. Since this time, 13 other outbreaks have been identified. The adenovirus can be associated with gastroenteritis symptoms, but only 2 of the 48 serotypes cause this disease manifestation. Therefore, it is far more common to see recreational water-based adenovirus outbreaks result in pharyngoconjunctival fever symptoms (WHO, 2011).

## Adenovirus Case Studies

- Resistant to chlorination
- Possible decrease in incidence
  - 9 outbreaks prior to 1980, only 4 since
- Majority of cases of serovars 40 and 41 affect children
- Seasonal occurrence
  - Most prevalent in late autumn (WHO, 2011)
- Present in indoor and outdoor pools
  - 4 of 26 and 3 of 28
  - 3 of 28

Adenovirus is often linked to inadequately chlorinated swimming pools. This is due to the virus' resistance to chlorination. The virus is able to survive in properly chlorinated pools, but is able to thrive in less maintained pool facilities causing outbreaks (Sinclair, Jones, and Gerba, 2009). There may be a possible decrease in the incidence of adenovirus, with only 4 outbreaks occurring since 1980. Despite lower incidence of the virus, the majority of cases from the gastroenteritis serotypes affect children more than any other group, with many outbreaks associated with swim teams.

Adenoviruses also differ from the other viral agents being covered in that they have a higher prevalence in the autumn season as opposed to just the summer months (WHO, 2011). In the autumn months, three swimming pools were tested for adenovirus, of the two indoor pools as well as the outdoor pool each had positives of adenovirus (van Heerden, Ehlers and Grabow, 2005)

## Echovirus Outbreaks

- Estimated 30 million cases of illness in U.S. per year (WHO, 2004)
- Highly associated with healthy children
  - 26 cases of encephalitis
  - Outbreak in special care nurseries
- Many cases of asymptomatic

Outbreak Location	Norovirus	Adenovirus	Echovirus
Swimming Pool	7	11	6
Lakes	14	2	4
Rivers	2	0	0
Recreational Fountains	1	0	0
Hot Springs	1	0	0

Several studies have shown that echovirus may cause sporadic cases or small outbreaks of severe or fatal encephalitis in otherwise healthy children. Echovirus 7 was reported as the predominant virus isolated from 26 clinically diagnosed cases of encephalitis in Pondicherry, India. Several of these cases died within a few hours of admission but no further clinical details were available. Fatal echovirus 7 infection has been reported in infants during outbreaks in special care nurseries (WHO, 2011). Echovirus and other enteroviruses account for 30 million infections in the United States each year (WHO, 2004). It has been suggested that the high prevalence of echovirus 13, which manifests as meningitis, may indicate this virus has the potential to act as an emerging type. Furthermore, many echovirus infections are asymptomatic (approximately 43%) therefore it is difficult to determine the true incidence of infection (WHO, 2011)

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## Coxsackievirus Outbreaks

- Estimated 30 million cases of illness in U.S. per year (WHO, 2004)
- 3 Outbreaks
  - 1970's
  - 2004
- Associated with lake water
  - Fresh and marine water survival

Three outbreaks of recreational water-borne coxsackievirus were identified. Two of the outbreaks took place over 30 years ago and one in 2004. The first occurred in 1972 in Vermont at a boys' summer camp and the second occurred in France in 1974. Both outbreaks were caused by polluted freshwater lakes where children swam. A recent outbreak was reported from 21 persons returning from a school-organized trip to Mexico. The onset suggested a point source exposure likely from swimming in sewage-contaminated water (Sinclair, Jones, Gerba, 2009).

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## Hepatitis Outbreaks

- Hepatitis A
  - 4 outbreaks
  - Associated with surface waters
- Hepatitis E
  - Contributes to viral load in sewage
  - No reported outbreaks



Four outbreaks of recreational water based hepatitis A have occurred at a rate of one per decade since the 1960's. The earliest report was from an outbreak in 1969 in South Carolina affecting 14 people, and the most recent outbreak took place in Australia in 1997 and affected six people. The relative morbidity contributed by these four outbreaks is low (96 cases) (Sinclair, Jones, and Gerba, 2009). Hepatitis E is excreted through feces as well as urine and contributes a majority of viral proportions found in sewage. Despite this, no reports of recreational water based illness are reported (WHO, 2011).

## Enteric Virus Detection

- Hawaii
  - 8% beaches positive for enteroviruses
  - Wastewater
- Florida
  - Charlotte Harbor
    - 75% positive
  - Sarasota
    - 81% positive
  - Septic tank system leakage

A Hawaiian water quality study found that 8% of beach samples were positive for enteroviruses with concentrations ranging from 3.7-10 viruses/100 L. Concentrations were on average 3.7 PFU/100 liters, with the highest level detected at 10 PFU/100 liters. This study suggested the viral pollution was originating from a wastewater outfall that received only primary treatment. This work led to recommendations that better sewage treatment be implemented.

Extensive studies in Florida have shown that enteroviruses and Hepatitis A can be routinely detected in near shore marine environments. In Charlotte Harbor on the Gulf Coast of Florida, enteroviruses were detected in 75% of the sites screened. In Sarasota, Fla., just north of Charlotte Harbor, enteroviruses were detected in 81% of the samples. Their presence was attributed to the high density of septic tanks in this coastal community (Griffin et al., 2003).

## Enteric Virus Detection

- Florida Keys
  - 79% sites positive for enteroviruses
  - 63% positive for Hepatitis A
  - 11% positive for Norwalk
- Swimming pools and wading pools of Houston, TX
  - 10 of 14 positive for viruses
  - 3 of 10 adequately chlorinated
  - 6 of 10 negative for coliforms
  - All 7 wading pools positive

Extensive residential development in the Florida Keys, without the development of municipal wastewater treatment and disposal systems, has led to the majority of canal front homes using septic tanks for waste disposal. In these canals and in near shore water sites of the Florida Keys, 79% of sample sites were positive for enteroviruses, 63% were positive for Hepatitis A, and 11% were positive for Norwalk when screened for RT-PCR (Griffin et al., 2003).

Municipal swimming pools and wading pools in Houston, Texas were examined for the presence of human enteric viruses using a portable virus concentrator at the site to concentrate viruses from 100-gallon to 500-gallon samples. Ten of 14 samples contained viruses; three of these were positive for virus in the presence of residual free chlorine. Enteroviruses were isolated from two pools which exceeded the 0.4 ppm free residual chlorine standard. This study appears to be supportive of recent evidence that indicates a higher incidence of enterovirus infection among bathers. All seven wading pool samples contained virus. Total coliform bacteria were not adequate indicators of the presence of virus, as six of the samples were positive for virus but negative for coliforms (Keswick, Gerba, and Goyal, 1981).

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## Indicator Organisms

- *Escherichia coli*
  - **Advantages**
    - Typically non-pathogenic
    - Easy to identify
    - Present in higher concentrations than pathogens
  - **Disadvantages**
    - Not unique to humans
    - Multiplies in contaminated waters
    - Isolated from pristine waters



*E. coli* is the most common and typical indicator organism used for detection of fecal contamination in water. The bacteria is easy to identify through cultural methods, which are fast and cheap and many strains are non-pathogenic. The pathogen also grows quickly, so will be seen at higher concentrations than most other fecal pathogens. Unfortunately, there is not a direct correlation between the presence of *E. coli* and human waste contamination. Furthermore, the presence of *E. coli* does not always correlate with the occurrence of fecal contamination (Maier, Pepper, and Gerba, 2009).



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## Indicator Organisms

- *Enterococcus faecalis* and *Enterococcus faecium*
  - **Advantages**
    - Highly associated with humans
    - Tolerant to high salinity (6.5% NaCl)
  - **Disadvantages**
    - Does not persist in soil or water environment as long as *E. coli*

*Enterococcus* spp. are also included as fecal contamination indicator organisms for their ability to survive in marine environments. This indicator does not need to be tested for in freshwater conditions. Unfortunately, the bacterium does not persist in the environment for very long, so a false negative test may result if the sampling is done long after the contamination event has occurred in the water system (Maier, Pepper, Gerba, 2009).

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## Appropriate Viral Indicators

- *Bacteroides fragilis*
  - Produces bacteriophage HSP 40 strain
  - Only associated with human fecal matter
  - Better survival in sediment and water than enteroviruses
- Not detectable at low levels

*B. fragilis* produces a bacteriophage which can be used as an indicator for virus presence in recreational waters. The bacteriophage is more concentrated in presence of sewage than enterovirus with 72% positive samples for the bacteriophage and 56% positive enterovirus samples from water and sediment. Furthermore, the bacterium is only associated with the intestinal tract of humans, which is important in the case of virus detection since viruses are very host specific, there is no reason to cause alarm over a non-human contamination event with respect to most viruses. The major concern with this bacteriophage is that it is not detectable at low levels in the marine environment (Maier, Pepper, Gerba, 2009).

## Conclusions

- Illnesses more prevalent in children
- Pool associated illness likely over-represented
- There is no perfect indicator organism
- Need more accurate reporting



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Children are more susceptible to many of the enteric viruses, particularly adenovirus, which young children are very sensitive to. Furthermore, children tend to have a longer and more common exposure to recreational waters than most adults (WHO, 2011). The outbreak data and reports are also incomplete. Only information on investigated outbreaks where the disease etiology was determined were included, so this is highly underreported data. There is also a high incidence of pool-related outbreaks, this again may be because pools have a defined population using them and there are numerous agents which are chlorine resistant, affecting those in the pool environment. Pools are also enclosed bodies of water which are apt to have human-based contamination events. Once these events occur, especially from children, the pathogenic agents are not diluted as they are in open water systems. Furthermore, the use of an additional indicator organism to represent viral loads in open water systems would be helpful since current indicators have no correlation with viral agents. Unfortunately, there is no perfect indicator organism for all contaminants since bacteriophage cannot be detected at low levels, but it is better than the current methods.

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