NOROVIRUS IN RECREATIONAL WATERS IN ARIZONA

by

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Ellen L. Jones entitled Norovirus in Recreational Waters in Arizona and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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STATEMENT BY AUTHOR

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DEDICATION

This work is dedicated with much love to my parents.

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ABSTRACT

Noroviruses are the leading cause of gastroenteritis in the United States, causing an estimated 23 million illnesses, 50,000 hospitalizations and 300 deaths per year. This virus is transmitted via the fecal-oral route and infections can occur from drinking contaminated water or eating contaminated food, contact with contaminated fomites, inhalation of aerosolized vomitus, or person to person spread. Outbreaks of norovirus following exposure to contaminated recreational water have been documented, as swimmers and others engaged in water recreation often consume recreational water, either purposefully or accidentally, during their activities. In Arizona there have been several outbreaks of norovirus gastroenteritis among people using the recreational waters. In the past four years there have been four consecutive outbreaks of norovirus among rafters on the Colorado River, and in 2003 there was an outbreak of norovirus among a large group of houseboaters on Lake Powell.

Norovirus is an emerging pathogen in the state of Arizona. In an effort to better understand its epidemiology, and with the aim of providing recommendations for prevention or minimization of future outbreaks, two investigations were undertaken: an investigation of the outbreak of norovirus among houseboaters on Lake Powell, and a survey of water quality and possible sources of norovirus in the Colorado River. Additionally, historical Colorado River water quality data was reviewed and outbreak epidemiology assessed. To better understand the role of viruses in waterborne

recreational disease outbreaks, a review of the literature was conducted and an analysis of 48 recreational waterborne disease outbreaks was done.

INTRODUCTION

Problem Definition

Over 22,000 people a year raft the Colorado River in the Grand Canyon in Arizona (NPS, 2005). Over 3 million people visit Lake Powell, Arizona each year (NPS, 1997). Arizona's recreational waters are utilized by many people. In recent years, increasing numbers of norovirus outbreaks among recreational water users in Arizona have made it apparent that norovirus is an emerging pathogen in this state. Noroviruses cause an estimated 23 million gastroenteritis illnesses, 50,000 hospitalizations and 300 deaths per year (Mead, *et al.*, 1999) in the United States. Epidemiology of this emerging pathogen is poorly understood, and additional information about its role in recreational waterborne disease outbreaks is needed.

Dissertation Format

This dissertation consists of three manuscripts prepared for publication and presented as appendices. Appendix A is a review article that will also function as the literature review for this dissertation. This review is an assessment of recreational waterborne disease outbreaks caused by viruses. It will be submitted for publication to *Environmental Science and Technology*. Appendix B is an investigation of an outbreak of norovirus among houseboaters on Lake Powell, Arizona. This manuscript has been submitted to *International Journal of Environmental Health Research*. Appendix C is both a review of the history and epidemiology of norovirus outbreaks among rafters on the Colorado River, and an assessment of Colorado River water quality and potential

sources of norovirus contamination in the river. This manuscript will be submitted for publication to *Wilderness and Environmental Medicine*.

The dissertation author was responsible for all of the research presented in the manuscripts, with the following exceptions: Appendix B: sample collection was done by Marlene Gaither and Adam Kramer; Appendix C: historical water quality data presented (1995-1998) was collected by Marlene Gaither. Marlene Gaither and Dr. Kelly Bright assisted in sample collection for water quality studies of the Colorado River and nearby sites. Marlene Gaither collected stool samples from rafters and on-board toilet cans.

PRESENT STUDY

The objective of the present study was to investigate and elucidate the changing epidemiology of norovirus in Arizona recreational waters. Norovirus is an emerging pathogen in this state. The methods, results, conclusions, and recommendations of this study are presented in the manuscripts appended to this dissertation. The following is a summary of the most important findings in this document.

The manuscript, "Viruses in Recreational Waterborne Disease Outbreaks: A Review" in appendix A is an examination of 48 outbreaks of recreational waterborne viral disease. This review supports the observations and conclusions drawn from epidemiological studies of recreational waterborne disease outbreaks. In the outbreaks reviewed, children were the primarily affected population in over half (54%). Also, inadequate disinfection was an important factor in outbreak causation; 68% of outbreak reports reviewed included mention of inadequate chlorination or other disinfection. Disinfection failure due to equipment malfunction was found to have caused some outbreaks (Caldwell *et al.*, 1974; Martone *et al.*, 1980; Turner *et al.*, 1987; Papapetropoulou *et al.* 1996; Harley *et al.*, 2001; CDC, 2004). Additionally, several outbreak investigations showed that head immersion is an important factor in increased risk of illness, indicating that there is a behavioral component to disease transmission among swimmers (Seyfried *et al.*, 1985; Parkin *et al.*, 2002). A marked increase in the number of echovirus outbreaks reported was noted to have occurred over approximately the last ten years. Whether this is the result of increased detection and reporting or an

actual increase in incidence of echovirus is unclear and warrants further investigation. Sixty-seven percent of echovirus outbreaks occurred in swimming pools, suggesting that assessment of current disinfection guidelines and their efficacy for echovirus may be needed.

The manuscript in Appendix B, "Role of Fomite Contamination During an Outbreak of Norovirus on Houseboats" is a report of an outbreak investigation conducted after an outbreak of norovirus among houseboaters on Lake Powell, Arizona. Twenty people became ill during this outbreak. This investigation demonstrated widespread fomite contamination in the houseboats, while samples of drinking water were negative for the virus. It was also learned that one of the participants on the first boating trip arrived displaying symptoms of gastrointestinal illness prior to boarding the boat, and this participant likely spread the virus to subsequent groups who used the same houseboat via fomite contamination and other modes of transmission. Due to the close quarters, shared bathrooms, and shared meals, it is presumable that person-to-person and foodborne transmission and environmental contamination all played a role in disease transmission. Negative results from the testing of the drinking water supply and the identification of an index case support the idea that this outbreak was not waterborne. Education of the public regarding norovirus transmission in confined spaces may aid people in making important decisions about excluding themselves from activities and trips when they are ill. This outbreak may have been minimized or prevented if the index case had been excluded from the trip based on evidence of symptoms of norovirus gastroenteritis, or if the index case had excluded himself on the basis of those symptoms.

The manuscript, "Recurring Outbreaks of Norovirus Among Rafters on the Colorado River" is presented in Appendix C. This manuscript is an analysis of the water quality along the Colorado River between Glen Canyon Dam and the convergence of the Colorado River with Diamond Creek, near Peach Springs, Arizona, including specifically testing for the presence of human norovirus by reverse transcriptase polymerase chain reaction (RT-PCR). Potential sources of norovirus contamination in the Colorado River were also assessed for norovirus content, including Glen Canyon Dam Wastewater Treatment Plant, Page, Arizona Wastewater Treatment Plant, and Wahweap Wastewater Treatment Plant. Four field studies were conducted for sample collection: July, 2003; August, 2003; May, 2004; July, 2004. Stool samples from ill patients and composite stool samples from on-board toilet-cans were analyzed for the presence of human norovirus during the 2003 and 2004 outbreaks. Of the six norovirus outbreaks reported, four have occurred in the consecutive previous four years.

Historical water quality data from points along the Colorado River revealed that overall water quality in the river has been high, with only 6/64 test samples (9.4%) above compliance with guidelines for recreational waters (<200 fecal coliform/100ml). Upon examination of the six occasions when the limits were exceeded, the contamination appears sporadic, and does not indicate specific ongoing sources of contamination in one or several areas, rather it points to localized random events.

Potential sources of norovirus outbreaks among rafters include drinking contaminated river water, consuming contaminated food stuffs, rafter importation of the virus and subsequent person to person spread, and contaminated campsites or equipment.

To reduce the probability of infection among rafters, additional testing of point-of-use water filters that are effective at virus removal should be conducted. These devices would lessen or eliminate the need for chemical treatment of the river water, and could reduce incidence of infection. Continued examination of the epidemiology of norovirus outbreaks among rafters on the Colorado River is needed, because the source of most outbreaks is unknown. Ethanol solutions of 70% and 90% have been proven effective at killing 99% of feline calicivirus (a norovirus surrogate) within a contact time of just one minute (Malik *et al.*, 2006). The use of 70% ethanol based hand-sanitizing gels is advised on rafting trips for use when clean water for hand washing is not available to minimize spread of the virus.

Testing the river for noroviruses is problematic because the total volume of the river is so high ($1.7x10^{11}$ liters between Glen Canyon Dam and the confluence with Diamond Creek) that the virus becomes very dilute and difficult to detect. Rafter exposure to this water is high, up to 4 liters of river water per day, and there may be 200 to 500 rafters on the river on any given day (NPS, 2005). There are between 10^6 and 10^{11} noroviruses in just one gram of fecal matter (Lund and Lindqvist, 2004; Pang *et al.*, 2004). At an average of 10^9 viruses per gram, an inoculum of 500g of norovirus containing stool into $1.7x10^{11}$ liters of water would result in a level of contamination of 3 viruses per liter, assuming even dispersion. Because of the volume of water consumed and the infectiousness of the virus, the level of contamination required to make one person ill on the Colorado River is small. At this time, no human dose-response data or human dose-response model fitting has been published for norovirus. Consequently,

extrapolations and microbial risk assessments are difficult or impossible to perform for this virus.

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APPENDIX A

LITERATURE REVIEW

VIRUSES IN RECREATIONAL WATERBORNE DISEASE OUTBREAKS: A REVIEW

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Abstract

This review is an examination of forty-eight outbreaks of viral disease originating from exposure to recreational waters. It includes outbreaks reported in peer-reviewed journals, reliable accounts of outbreaks reported by the popular media, and outbreaks reported to the Centers for Disease Control (CDC) and published in Morbidity and Mortality Weekly (MMWR) or MMWR-Surveillance Summaries. It includes outbreaks from around the world that have been reported since 1951. Use of recreational waters for swimming and water activities has long been observed to be related to increased risk of morbidity, including gastrointestinal, respiratory, and ocular illnesses. Numerous epidemiological studies over the past several decades conducted at bathing beaches and swimming pools around the world have confirmed that a significant increased risk of illness exists among swimmers, especially children and those who submerge their heads below water. While bacteria and protozoa do cause recreational water associated illnesses, viruses are also a significant cause. In this review, noroviruses caused the largest proportion of outbreaks by etiology, 42% (n = 20), while the second most prevalent type of infection was adenovirus, which caused 27% of outbreaks (n = 13). Nine outbreaks, or 19%, were caused by echoviruses. Hepatitis A virus was responsible for four outbreaks, or 8% of the total, and coxsackieviruses caused just two outbreaks or 4% of the total. Half of the outbreaks occurred in swimming pools (n = 24). While the second largest percentage, 40%, occurred in lakes or ponds (n = 19). The percent of viral vs. bacterial/parasitic recreational waterborne gastrointestinal disease outbreaks reported to Centers for Disease Control for the years 1989 to 2002 was examined and found to

vary from 0 to 44%, with an average of 6% for the decade of the 1990s. Fifty-four percent of outbreaks were reported to primarily affect children (n = 26) vs. 19% of outbreaks that affected all ages (n = 9). In 15 outbreaks (31%), bacterial indicators of fecal contamination could be detected in the source water. However, in three outbreaks, disease occurred even in the absence of bacterial indicators. Inadequate disinfection was found in 68% (n = 17) of outbreak investigations, indicating that inadequate disinfection is still a major factor in swimming pool outbreaks. A lack of required reporting and non-uniform water quality and chlorination/disinfection standards continues to contribute to waterborne recreational disease outbreaks.

Key Words

waterborne, outbreak, viral, recreation, swimming pool, lake, pond, river, norovirus, adenovirus, hepatitis A, echovirus, coxsackievirus, enteroviruses

Introduction

Waterborne disease can be acquired during water-related recreational activities such as swimming, boating, or other water sports. Many epidemiological studies conducted at both marine and freshwater bathing beaches have shown that there is a significant increase in incidence of illness, including gastrointestinal, respiratory, ear and ocular, and skin or wound infection among those who engage in water based recreational activities (Cabelli *et al.*, 1979; Cabelli *et al.*, 1982; D'Alessio *et al.*, 1981; Seyfried *et al.*, 1985). Several viruses including coxsackieviruses, adenoviruses, echoviruses, hepatitis A virus, astroviruses, and noroviruses have all been shown to cause recreational waterborne disease outbreaks (Table 1). Some studies have found an association between certain bacterial water quality indicators and rates of illness among bathers (Cabelli *et al.*, 1982; Corbett *et al.*, 1993). Other studies have found that even water that is only marginally polluted or meets state or local water quality requirements can be the source of outbreaks of disease or can contain enteric viruses (Cabelli *et al.*, 1979; Cabelli *et al.*, 1982; Rose *et al.*, 1987). There is inconsistency among the numerous epidemiological studies as to which indicator organisms best correlate with incidence of illness (Corbett *et al.*, 1993), and some studies have found illness in the absence of indicator organisms (Foy *et al.*, 1968; Hauri *et al.*, 2005; Papapetropoulou *et al.*, 1998). The lack of a consistent correlation between indicator organisms and disease may be particularly troubling with respect to viral pathogens since bacterial indicators have been found to be unreliable indicators of the presence of virus. Studies showing the presence of human enteric viruses in recreational waters and/or a positive correlation between swimming in

recreational waters and increased risk of disease have been conducted at bathing venues around the world including: Sydney, Australia (Corbett *et al.*, 1993), Blackpool Beach, United Kingdom (Alexander *et al.*, 1992), Northern Ireland (16 sites) (Hughes *et al.*, 1992), Ontario, Canada (Seyfried *et al.*, 1985), Israeli coastal beaches (Fattal *et al.*, 1991), Lake Pontchartrain, New Orleans (EPA, 1981), and Hong Kong coastal beaches (9 sites) (Cheung *et al.*, 1990).

The Centers for Disease Control (CDC), the U.S. Environmental Protection Agency (EPA), and the Council of State and Territorial Epidemiologists have collaborated to maintain a surveillance system for recreational waterborne disease outbreaks since 1978. The CDC periodically reports the data from this surveillance system in the Morbidity and Mortality Weekly Report Surveillance Summaries (MMWR-SS). Reports of waterborne disease outbreaks to the CDC are voluntary on the part of the states. For this reason, many outbreaks go unreported and are not accounted for by the surveillance system. In an effort to obtain the most information possible about recreational waterborne disease outbreaks, a literature review was conducted to include information about outbreak investigations not reported to the CDC. Also, because many outbreaks are not published, the popular media was searched for reliable accounts of waterborne recreational disease outbreaks.

Inclusion Criteria for Data Set Creation

Construction of the set of outbreaks was accomplished by searching Medline, ScienceDirect, and LexisNexis databases for published journal articles and reports in the popular press, and using the Google search engine to search websites on the World Wide Web. The following virus names: norovirus, norwalk virus, adenovirus, echovirus, hepatitis A, hepatitis E, coxsackievirus, and enterovirus were used individually in combination with the following terms: water, recreation, outbreak, swimming, pool, lake, and river. These terms were connected where possible with the Boolean operator "or", and combined with virus names one at a time where Boolean operators were not useable. Additionally, the reference sections of all articles were searched for references to additional outbreaks that met inclusion criteria. Inclusion criteria for outbreaks were as follows: viral disease outbreaks originating from water recreation and not linked to transmission via drinking water or contaminated food. A total of 48 outbreaks were identified for inclusion, occurring between 1951 and 2005, using these criteria. This collection of outbreaks clearly does not represent a random sample of outbreaks. Only a small fraction of outbreaks are published in journals and in the popular press or reported to the CDC. Published outbreaks represent those outbreaks that were detectable and those outbreaks where both sufficient expertise and sufficient funds to investigate were available. Consequently, outbreaks in United States counties or countries with fewer resources will be underrepresented.

Adenovirus Outbreaks

Adenoviruses are members of the family *Adenoviridae* and the genus *Mastadenovirus*, which contains all human serotypes. They are icosahedral in shape, 80-90 nm in size, and contain double stranded DNA. Adenoviruses can cause a myriad of clinical illnesses including respiratory infections, ocular infections, enteric infections (gastroenteritis), encephalitis, pneumonia, and even genitourinary infections. Adenoviruses 40 and 41 have been shown to cause gastroenteritis specifically and are spread through the fecal oral route. Respiratory spread via droplets or contact occurs in the case of types 3, 4, and 7, and these are the serotypes responsible for swimming pool outbreaks of conjunctivitis or pharyngoconjunctival fever (White and Fenner Ed., 1994).

The present review identified 13 published adenovirus outbreaks spanning six decades (Table 2). The first outbreak report appeared in 1953 (Cockburn, 1953), and represented the first time spread of viral pharyngoconjunctival fever was reported to have occurred from swimming. The most recent outbreak occurred in Australia in 2000 and was a primary school outbreak of pharyngoconjunctival fever caused by adenovirus type 3 (Harley *et al.*, 2001) in which transmission occurred via an inadequately chlorinated swimming pool at a school camp. There have been 11 other outbreaks reported in the years between the first report and the most recent. They have occurred in 5 different countries, and 5 different states in the United States. Most of these outbreaks occurred in swimming pools (n = 11), while two occurred in a lake or pond (Kjellén *et al.*, 1955; Moore *et al.*, 1991). Just over half (54%) of the outbreaks were caused by adenovirus type 3 (n = 7). The incidence of adenovirus outbreak reports has declined over the

decades examined in this review. Nine of the outbreaks (69%) occurred prior to 1980, and there have only been four reported outbreaks in the decades since 1980. The largest outbreak, however, did take place in 1991 in North Carolina (Moore *et al.*, 1991). This outbreak caused a reported 595 cases of adenovirus type 3 infections. The overall reported morbidity caused by all adenovirus outbreaks is 1,648 cases.

Coxsackievirus and Echovirus Outbreaks

Coxsackieviruses and echoviruses belong to the genus *Enterovirus* which is in the family *Picornaviridae*. These viruses are small in size (30 nm diameter) and have a nonenveloped capsid that is icosahedral in shape. They are comprised of a linear, plus sense single stranded RNA genome. Together with polioviruses and enteroviruses, coxsackieviruses and echoviruses are commonly referred to collectively as "enteroviruses". Enteroviruses enter the body via ingestion, and grow both in the throat and intestinal tract and are subsequently shed in the feces. They can cause conjunctival, respiratory, or gastrointestinal illness, but can also cause more serious diseases such as meningitis, paralysis, myocarditis, or hand-foot-and-mouth disease (White and Fenner Ed., 1994).

Two outbreaks of recreational waterborne coxsackievirus were identified (Table 3). Both outbreaks took place over 30 years ago. One occurred in 1972 in Vermont at a boy's summer camp (Hawley *et al.*, 1973), and the other occurred in France in 1974 (Denis *et al.*, 1974). Both outbreaks were caused by polluted freshwater lakes where children swam. No similar coxsackievirus outbreak has been reported in the literature

since. However, nine echovirus outbreaks were identified (Table 4). The first reported outbreak occurred in 1992 in Ireland (Kee *et al.*, 1994), and the most recent outbreak was a large community wide outbreak in Siberia in 2004 (Manenkov, 2004). Six of the nine outbreaks (67%) were the result of contaminated swimming pools. The other three outbreaks were the result of contact with other bodies of water, such as a lake, a pond, and a reservoir (CDC, 2000; Hauri *et al*., 2005; Manenkov, 2004). All nine outbreaks have occurred in the last decade and the first half of the present decade. Whether this represents more diligent reporting or improved surveillance and detection, or whether it represents an actual increase in incidence is unclear, but merits investigation. The total number of reported cases from all coxsackievirus outbreaks is just 26; the total number of cases from all echovirus outbreaks is unknown, but is much greater than 5,000.

Hepatitis A Virus Outbreaks

Hepatitis A belongs to the family *Picornaviridae* and has its own genus, *Hepatovirus*. Hepatitis A virus is a 27 nm icosahedral virus that is a single stranded RNA virus. It enters the body via ingestion, multiplies in the intestine, and then spreads to the liver through the blood stream. The incubation period for hepatitis A virus is long, about four weeks with a range from two to six weeks. This makes it more difficult to isolate virus from patients when they present for treatment (White and Fenner Ed., 1994).

Four outbreaks of recreational waterborne hepatitis A were identified (Table 5). These outbreak reports have occurred at a rate of one per decade since the 1960s. The earliest report was from an outbreak in 1969 in South Carolina affecting 14 people

(Bryan *et al.*, 1974), and the most recent outbreak took place in Australia in 1997 and affected 6 people (Tallis *et al.*, 1997). The relative morbidity contributed by these four outbreaks is low when compared to outbreaks of other etiologies. The total number of reported cases from all hepatitis outbreaks is 96.

Norovirus Outbreaks

Noroviruses are the most common etiological agent of gastroenteritis in the United States, causing an estimated 23 million cases per year (Mead *et al*., 1999). Noroviruses are members of the family *Caliciviridae* and the genus *Norovirus*. They are 27-32 nm single-stranded nonenveloped RNA viruses (Heymann, 2004). Noroviruses cause acute onset of projectile vomiting and diarrhea, sometimes with low grade fever, headache, and malaise (Dolin *et al*., 1972). Symptoms are usually self-limited, lasting 24 to 72 hours. The incubation period is usually 24 to 48 hours, but onset of symptoms as soon as 10 hours after exposure has been reported (Heymann, 2004). Noroviruses are extremely contagious. Norovirus gastroenteritis causes rapid dehydration, which is of particular concern among the elderly and very young, as well as those who are engaged in physically demanding activities which hasten dehydration, such as during outbreaks among soldiers during deployment (Matson, 2005). There are no known non-human reservoirs for human norovirus, and no long-term immunity is gained from infection (Parrino *et al*., 1977). It is not yet possible to grow noroviruses in cell culture. Reverse transcriptase polymerase chain reaction (RT-PCR) identification of norovirus has only

been possible since the early 1990's, and the test has only become widely available in the last decade.

Twenty recreational waterborne norovirus outbreaks were identified, occurring between 1977 and 2004 (Table 6). Outbreaks occurred in a wide variety of types of recreational water. Six outbreaks (30%) resulted from swimming pools, ten outbreaks (50%) resulted from lakes, two from rivers (10%), and one each from a recreational fountain and a hot spring. The Centers for Disease Control adopted reverse transcriptase polymerase chain reaction (RT-PCR) for routine testing in 1993, and after that public health laboratories around the country adopted this testing method as well. Consequently, norovirus outbreaks were not frequently reported until the late 1990's, though many outbreaks may have occurred and gone undetected prior to the availability of a rapid, easy, and specific test. At the same time there has been a marked increase in incidence of norovirus outbreaks in recent years (CDC, 2002), and this may be an actual increase not entirely explained by increased testing.

Viral Outbreaks vs. Bacterial/Protozoan Outbreaks

It is clear that the majority of disease burden from recreational waterborne disease results from infections that are bacterial or protozoan. Several of the protozoan pathogens responsible for the highest disease burden are quite resistant to disinfection by chlorination. The relative numbers of viral vs. bacterial/parasitic recreational waterborne gastrointestinal disease outbreaks reported to Centers for Disease Control and the percent of these outbreaks that are of viral etiology for the years 1989 to 2002 are shown in Table

7. The percent of reported outbreaks that have a viral etiology has been zero for 6 of the 14 years examined. However, from 1998 to 2002 (the most recent year for which data is available) viral outbreaks have been reported every year. Also, the highest contribution (44%) of viral outbreaks occurred in 2002. It seems that viral outbreaks are beginning to comprise more of the total recreational waterborne outbreaks reported to the CDC which is most likely the result of simplification of testing methods, such as the advent of the use of PCR for detection of environmental pathogens, but may also represent, at least partially, a true increase in incidence due to changes in viral epidemiology or changing host behavioral patterns, such as increased participation in high-density recreation like travel on cruise ships, participation in "package vacations" at all-inclusive resorts, and the increasing popularity of water parks.

Outbreaks by Etiologic Agent

Forty-two percent (n = 20) of all outbreaks in this review were caused by noroviruses (Figure 1). This represents the largest proportion of outbreaks by etiology. The second most prevalent type of infection was adenovirus, which caused 27%, or 13 outbreaks. Nine outbreaks, or 19%, were caused by echoviruses. Hepatitis A virus was responsible for four outbreaks or 8%, and coxsackieviruses caused two outbreaks or 4%.

Outbreaks by Type of Recreational Water

Half of the outbreaks occurred in swimming pools (n = 24). While the second largest percentage, 40%, occurred in lakes or ponds (n = 19). Two outbreaks were

reported following exposure to river water and two occurred after exposure to hot springs. One outbreak was the result of a contaminated recreational fountain, designed for children to play in (Figure 2). Outbreaks in swimming pools are more likely to be reported because they represent a limited defined population; these outbreaks may also be more likely recognized among groups such as swim teams where members involved know each other. Ponds and lakes may be larger in volume and are not disinfected.

Children and Recreational Waterborne Disease

Higher rates of illness among children swimmers than adult swimmers have been shown repeatedly (Seyfried *et al.* 1985, Fattal *et al.* 1991). In this review, 54% of outbreaks were reported to primarily affect children (n = 26) vs. 19% of outbreaks that affected all ages (n = 9) and 27% where the age distribution was unknown or not reported (n = 13) (Figure 3). Children are disproportionately affected by waterborne recreational outbreaks. This is likely due to immunological, physiological, and behavioral factors. Children are more susceptible to infection due to their naïve immune systems. For the same reason they can also experience more severe symptoms than adults. Physiological differences between children and adults also leave children more vulnerable to some forms of infection. Also due to their small size, children are more vulnerable to rapid dehydration from vomiting and diarrhea, making them more likely to have poor-prognosis gastroenteritis infections. Behavioral aspects also put children at increased risk of infection. Children may also be less likely than adults to bathe after swimming or

even to wash their hands between swimming and eating. Children are also more likely to put their heads underwater or ingest recreational water (Parkin *et al.* 2002).

The Role of Bacterial Indicators of Water Quality

Of the 48 outbreak reports included in this review, 61% (n = 29) did not contain information about bacterial indicators of fecal contamination in the source water (Figure 4). These outbreaks without indicator organism data represent in part outbreaks that took place prior to 1980 (n = 7) and outbreaks that were reported only briefly in Morbidity and Mortality Weekly Report (n = 7). Of the remaining outbreaks, 31% (n = 15) had bacterial indicators of fecal contamination in the source water. However, in three outbreaks, disease occurred even in the absence of bacterial indicators of fecal contamination. Foy *et al.* (1968) investigated an outbreak of adenovirus type 3 associated with intermittent chlorination of a swimming pool in 1968. No coliform bacteria could be detected in the pool water in tests performed on three consecutive days, despite the absence of detectable chlorine residual. During the investigation of an adenovirus outbreak at a municipal swimming pool in Greece in 1995, Papapetropoulou *et al.* (1998) found that they could detect adenovirus in the pool water using PCR with primers specific for the detection of adenoviruses, but all bacteriological indicators tested, total coliforms, fecal coliforms, and fecal streptococci, were negative. During a community wide outbreak of echovirus in the city of Kassel, Germany in 2001, investigators found that bathing in a particular pond, "pond A" was associated with illness. However, weekly testing of pond water for total coliforms, fecal coliforms,

enterococci, and *Staphylococcus aureus* never indicated levels above the European Union (EU) bathing water guideline limits (Hauri *et al.* 2004). Rose *et al.* (1987) reported that enterovirus and rotavirus could be readily isolated from the recreational waters of Oak Creek, Arizona despite fecal indicators within range of regulatory limits (200 CFU fecal coliform/100ml). The authors demonstrated 18 of 41 recreational water samples were positive for either enterovirus or rotavirus. This study illustrates that fecal coliforms do not always adequately reflect viral pollution of recreational waters.

One outbreak of echovirus infection associated with an outdoor swimming pool in Ireland (Kee *et al.*, 1992) was excluded from this analysis because the source of the outbreak was known to be non-fecal as two children were observed vomiting in the pool at the advent of the outbreak. Out of the 18 outbreaks where tests were conducted for fecal indicators of contamination, 15 (83%) were positive. Though bacteriological indicators of fecal contamination do not always correlate with viral contamination, they seem to be an important indicator of water quality in bathing venues and were only absent in the presence of viral outbreaks in 3 out of 18 (16.7%) outbreaks where testing was conducted. Over half of outbreak reports did not include information about bacterial indicators. Whether this is because bacterial indicators were not tested for, or because the information was not included in the report is unknown.

The Role of Inadequate Disinfection

For the purposes of this review, inadequate disinfection is defined as absent or insufficient free chlorine (≤1ppm), chlorination or disinfection equipment failure, or

inadequate method of disinfection such as improper use of hydrogen peroxide and/or UV. Only outbreaks taking place in swimming pools or other typically disinfected venues were included in this portion of the analysis; lakes, rivers and ponds were excluded. Inadequate disinfection was found in 68% (n = 17) of outbreak investigations (Figure 5), indicating that inadequate disinfection is still a major factor in swimming pool outbreaks. Seven outbreak reports (28%) either did not include information about the level of disinfection of the water or this information was unknown to the investigators. Only one report could be found in which an outbreak occurred despite adequate disinfection. This was a case of an overwhelming volume of inoculum entering a swimming pool (Kee *et al.* 1992). An outbreak of echovirus type 30 began when two children vomited into a crowded swimming pool. Despite hourly chlorine level readings all within the recommended limits, an outbreak ensued after the vomiting incidents. These findings reiterate that proper disinfection is important in controlling viral outbreaks in swimming pools, though many swimming pool outbreaks are caused by other organisms that may be more disinfection resistant, such as *Cryptosporidium parvum*. There is evidence that norovirus may be more resistant to chlorine disinfection than other enteric viruses (Barker *et al.* 2004). Of the 6 outbreaks of norovirus resulting from swimming pool exposure, four reports (67%) indicated inadequate chlorination (Kappus *et al.* 1982; Holmes *et al.* 1987; Maunula *et al.*, 2001; Blevins *et al.* 2004) and two reports did not contain information regarding disinfection. Thus, adequate chlorination can be inferred to be an important component in the prevention of norovirus outbreaks originating from swimming pools.

In 2003, the CDC reported surveillance data from swimming pool inspections in five different states, Pennsylvania, Florida, California, Minnesota, and Wyoming. Local environmental health programs in these areas inspect public and semipublic pools periodically to determine compliance with health regulations. A total of 22, 131 pool inspections from these locations were combined to form one data set containing information about code violations. From these inspections, 21, 561 violations of pool codes were noted. Water-chemistry accounted for 38.7% of violations, and filtration and recirculation system violations represented 38.6% of the total. It was found that the highest percentage of total violations attributable to pH infractions occurred in child wading pools where 8% had coincident free chlorine and pH violations (MMWR, 2003). Wading pools may host babies in diapers and because of the small volume of water in wading pools, there is a smaller ratio of chlorinated water to occupant mass. It has been demonstrated that swimmers themselves have a negative influence on the bacterial and chemical quality of bathing waters (Hanes and Fossa, 1970; Gerba, 2000), and that occupant load is a factor level of water quality where the water supply is finite or has limited opportunity for exchange (Fattal *et al.* 1991).

Recreational Waterborne Disease Outbreaks Over Time

Figure 6 shows outbreaks over time by etiologic agent for 46 outbreak reports. The two coxsackievirus outbreaks were excluded from this analysis because the number of outbreaks was too low to show a trend, though it is worth noting that the most recent report of a recreational waterborne outbreak of coxsackievirus was in 1974, over 30 years

ago. There has been a change in the etiologies of reported outbreaks over the last five decades. From the 1950s through the 1960s the majority of outbreaks reported were adenovirus outbreaks; in fact, only one other outbreak was reported to have occurred during this time period, an outbreak of hepatitis A among boy scouts (Byran *et al.* 1974). There have only been three outbreaks of adenovirus reported since 1990, and the remaining 10 outbreaks were reported prior to 1990. At the same time, reports of norovirus infection have been increasing. Since 1990, there have been 14 documented recreational waterborne norovirus outbreaks, while only three were reported prior to 1990. Testing for noroviruses became possible in the 1970s, and since then reports of norovirus outbreaks have been increasing. In the 1990s and the current decade, norovirus and echovirus outbreaks have been reported more than other etiologies. Norovirus outbreaks were reported more than any other kind of outbreak since 2000 (n = 11). There are probably numerous reasons for these changes, some of which reflect actual changes in disease incidence over time as new illnesses emerge, and some of which reflect reporting biases, changes and improvements in our ability to detect viruses in the environment, and trends in public health interests that influence attention to and reporting of certain diseases over others.

Changes in the total morbidity contributed by each type of virus during the last 20 years compared to > 20 years ago are displayed in Figure 7. Forty-seven outbreaks were included in this analysis. The large echovirus outbreak in Romania in 1999 was excluded on the basis that it was not comparable with the other outbreaks, as it was not a focal outbreak, but an ongoing state wide outbreak that became endemic in nature. In this

analysis, morbidity was assessed as total number of cases reported for each etiology during the designated time period. Adenovirus and norovirus have contributed the most morbidity with 1,648 and 1,419 cases respectively reported. While adenovirus morbidity has decreased over the last 20 years, both norovirus and echovirus have contributed more morbidity in the last 20 years than in years prior to that.

Conclusions and Research Needs

Epidemiological studies have shown that swimmers have higher rates of disease than non-swimmers, especially gastrointestinal, respiratory, and eye-ear-nose infection. Children have the highest rate of gastrointestinal illness and a greater overall increased risk of illness when compared to adults. Some epidemiological studies have shown that there is a correlation between level of contamination of recreational water and increased rates of disease among swimmers (Cabelli *et al.* 1982). The present review has supported the observations and conclusions drawn from epidemiological studies. In the outbreaks reviewed, children were the primarily affected population in over half (54%). Also, disinfection was an important factor in outbreak causation; 68% of outbreak reports reviewed included mention of inadequate chlorination or other disinfection. Disinfection failure due to equipment malfunction was found to have caused some outbreaks (Caldwell *et al.*, 1974; Martone et al, 1980; Turner *et al.*, 1987; Papapetropoulou *et al.* 1996; Harley *et al.*, 2001; CDC, 2004). Additionally, several outbreak investigations showed that head immersion is an important factor in increased risk of illness, indicating that there is a behavioral component to disease transmission among swimmers (Seyfried

et al., 1985; Parkin *et al.* 2002). A marked increase in the number of echovirus outbreaks reported was noted to have occurred over approximately the last ten years. Whether this is the result of increased detection and reporting or an actual increase in incidence of echovirus is unclear and warrants further investigation. Sixty-seven percent of these outbreaks occurred in swimming pools, suggesting that assessment of current disinfection guidelines and their efficacy for echovirus may be needed.

Non-uniform water quality and chlorination/disinfection standards may contribute to waterborne recreational disease outbreaks, since regulations are determined by localities and may not be sufficient to control the spread of disease. The lack of required reporting for recreational waterborne disease outbreaks makes surveillance efforts more difficult, and may bias or obscure observations made from those outbreaks that are reported. Training of pool maintenance personnel may also play an important role in reducing outbreaks, since it may reduce inadequate disinfection due to operator error and equipment malfunction. Castor and Beach (2004) have recently made several recommendations for the prevention and control of disease transmission in swimming venues. They recommend the redesign of aquatic facilities, increased governmental oversight of swimming pool maintenance, and training of staff and education of the public regarding healthy swimming habits. Additionally they recommend that high risk groups, such as the elderly and infirmed and pregnant women, be made aware of their increased risk of illness from swimming, even in apparently adequately disinfected swimming waters. This review of recreational waterborne viral disease outbreaks supports those recommendations.

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Table 1
Viruses Shown Epidemiologically to Cause Recreational Waterborne Disease Outbreaks

Virus	Illness(es)
Adenoviruses	conjunctivitis, gastroenteritis, respiratory disease, pharyngoconjunctival fever
Coxsackieviruses	meningitis, pharyngitis, conjunctivitis, encephalitis
Echoviruses	gastroenteritis, encephalitis, meningitis
Hepatitis A virus	hepatitis
Noroviruses	gastroenteritis
Astroviruses	gastroenteritis

Table 2
Adenovirus Outbreaks (n = 13)

Year	Location	Source	Number Ill	Type	Reference	Author
1951	Colorado	pool	206	"APC" virus	Am J Ophthalmol, 1953	Cockburn TA
1954	Canada	pool	112	"APC" virus	Can Med Assoc J, 1955	Ormsby HL et al.
1955	Sweden	lake	125	3	Acta Paediatr, 1957	Kjellén L et al.
1959	Japan	pool	124	3	Kyushu J Med Sci, 1961	Kaji M et al.
1960	Japan	pool	48	3	Kyushu J Med Sci, 1961	Kaji M et al.
1966	Washington	pool	26	3	Arch Environ Health, 1968	Foy HM et al.
1973	Kansas	pool	44	7	Am J Epidemiol, 1974	Caldwell GG et al.
1977	Georgia	pool	105	3	Am J Epidemiol, 1977	Martone WJ et al.
1977	Georgia	pool	72	4	J Infect Dis, 1979	D'Angelo LJ et al.
1982	Oklahoma	pool	77	7a	South Med J, 1987	Turner M et al.
1991	North Carolina	pond	595	3	MMWR-SS, 1993	Moore AC et al.
1995	Greece	pool	80	unknown	J Infect, 1998	Papapetropoulou M et al.
2000	Australia	pool	34	3	Commun Dis Intell, 2000	Harley D et al.

Table 3
Coxsackievirus Outbreaks (n = 2)

Year	Location	Source	Number Ill	Type	Reference	Author
1972	Vermont	lake	21	B5	JAMA, 1973	Hawley HB et al.
1974	France	lake	5	A16	JAMA, 1974	Denis FA et al.

Table 4
Echovirus Outbreaks (n = 9)

Year	Location	Source	Number Ill	Type	Reference	Author
1992	Ireland	pool	46	30	J Public Health Med, 1994	Kee F et al.
1997	Italy	pool	68	30	Int J Infect Dis, 2006	Faustini A et al.
1998	Italy	pool	unknown	30	J Clin Microbiol, 2002	Manzara S et al.
1999	Romania	water/body contact	5,000	4, 7, and 30	MMWR, 2000	CDC
2000	Italy	pool	unknown	30	J Clin Microbiol, 2002	Manzara S et al.
2001	South Africa	pool	90	3	Epidemiol Infect, 2005	Yeats J et al.
2001	Germany	pool, lake, pond	215	13 and 30	Epidemiol Infect, 2005	Hauri AM et al.
2003	Connecticut	pool	36	9	MMWR, 2004	CDC
2004	Siberia	swimming/reservoir	294	unknown	ITAR-TASS News Agency, 2004	Manenkov, V

Table 5
Hepatitis A Outbreaks (n =4)

Year	Location	Source	Number Ill	Reference	Author
1969	South Carolina	lake	14	Am J Epidemiol, 1974	Bryan JA et al.
1979	Hungary	thermal pool/spa	56	IAWQ Health Related Micro, 1991	Solt
1989	Louisiana	pool	20	J Infect Dis, 1992	Mahoney FJ
1997	Australia	pool	6	CDI, 1997	Tallis G et al.

Table 6
Norovirus Outbreaks (n = 20)

Year	Location	Source	Number Ill	Reference	Author
1977	Ohio	pool	229	Am J Epidemiol, 1982	Kappus KD et al.
1979	Michigan	lake	49	Am J Epidemiol, 1982	Koopman JS et al.
1987	North Dakota	pool	48	J Environ Health, 1987	Holmes SE et al.
1994	England	lake	7	J Med Virol, 1997	Gray JJ et al.
1996	Idaho	lake	55	MMWR-SS, 1998	Levy DA, et al.
1998	Ohio	lake	30	MMWR-SS, 2000	Barwick RS et al.
1998	Wisconsin	lake	18	MMWR-SS, 2000	Barwick RS et al.
1999	Idaho	hot springs	25	MMWR-SS, 2002	Lee SH et al
1999	New York	lake	168	MMWR-SS, 2002	Lee SH et al
2001	*Finland*	*pool*	*242*	*Epidemiol Infect, 2004*	*Maunula L et al.**
2001	Minnesota	lake	40	MMWR-SS, 2004	Yoder JS et al.
2002	The Netherlands	fountain	100	J Infect Dis, 2004	Hoebe CJ et al.
2002	Arizona	river rafting	130	unpublished	unpublished
2002	Minnesota	pool	36	MMWR-SS, 2004	Yoder JS et al.
2002	Minnesota	lake	11	MMWR-SS, 2004	Yoder JS et al.
2002	Wisconsin	pool	15	MMWR-SS, 2004	Yoder JS et al.
2002	Wisconsin	lake	44	MMWR-SS, 2004	Yoder JS et al.
2003	Arizona	river rafting	22	unpublished	unpublished
2004	Oregon	lake	150	http://www.oregonnews.com	popular media
2004	Vermont	pool	53	MMWR, 2004	Blevins LZ et al.

* Astroviruses also isolated during this outbreak

Table 7
Relative numbers of viral vs. bacterial/parasitic recreational waterborne gastrointestinal disease* outbreaks reported to Centers for Disease Control and the percent of these outbreaks that are viral for the years 1989-2002

Year	Viral Gastroenteritis	Bacterial/Protozoal Gastroenteritis**	% Viral
1989	1	9	11
1990	0	12	0
1991	1	9	11
1992	0	2	0
1993	0	8	0
1994	0	6	0
1995	0	9	0
1996	1	8	12
1997	0	3	0
1998	2	10	20
1999	2	11	18
2000	1	16	6
2001	1	9	11
2002	4	9	44

*Excludes acute gastroentestinal illness of unknown etiology
**Non-dermatologic, and excluding *N. fowleri,* Pontiac Fever, and Legionnaire's Disease

Figure 1
Outbreaks by etiologic agent, n = 48 (frequency) percentage

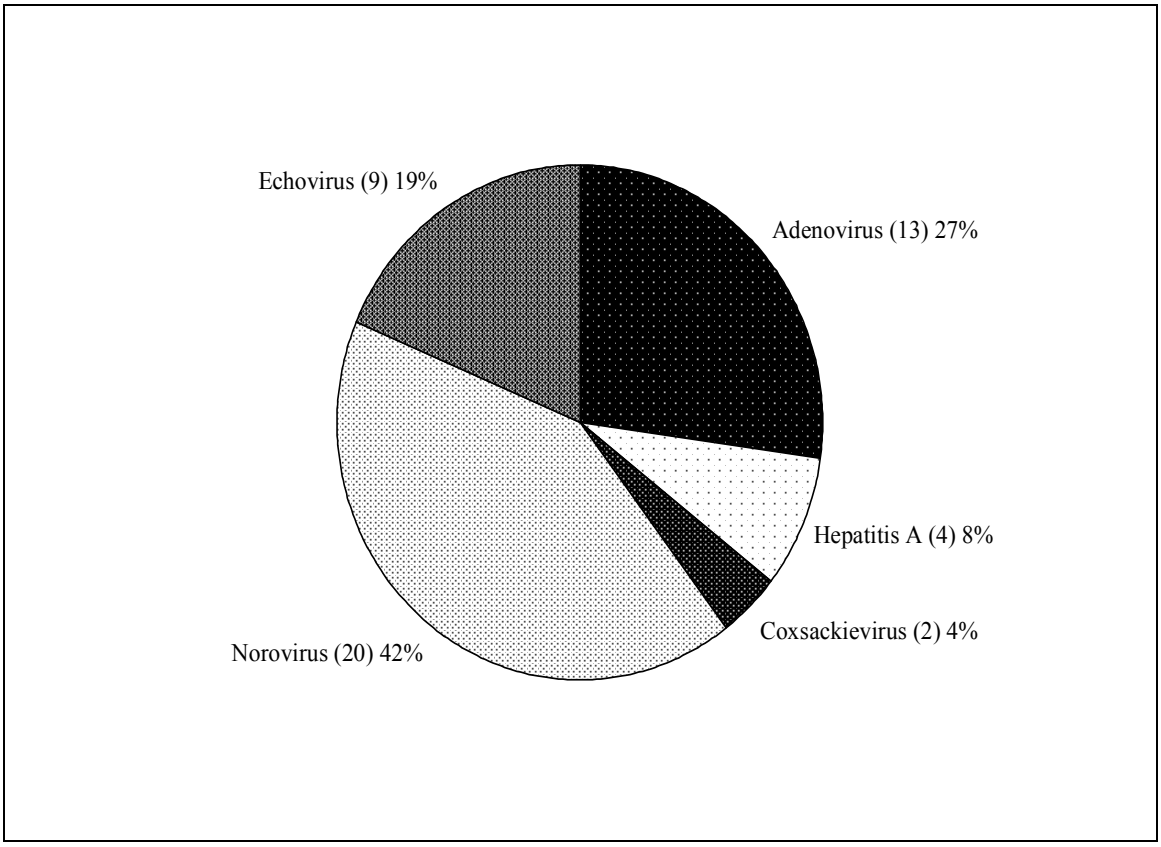


Figure 2
Outbreaks by type of recreational water, n = 48 (frequency) percentage

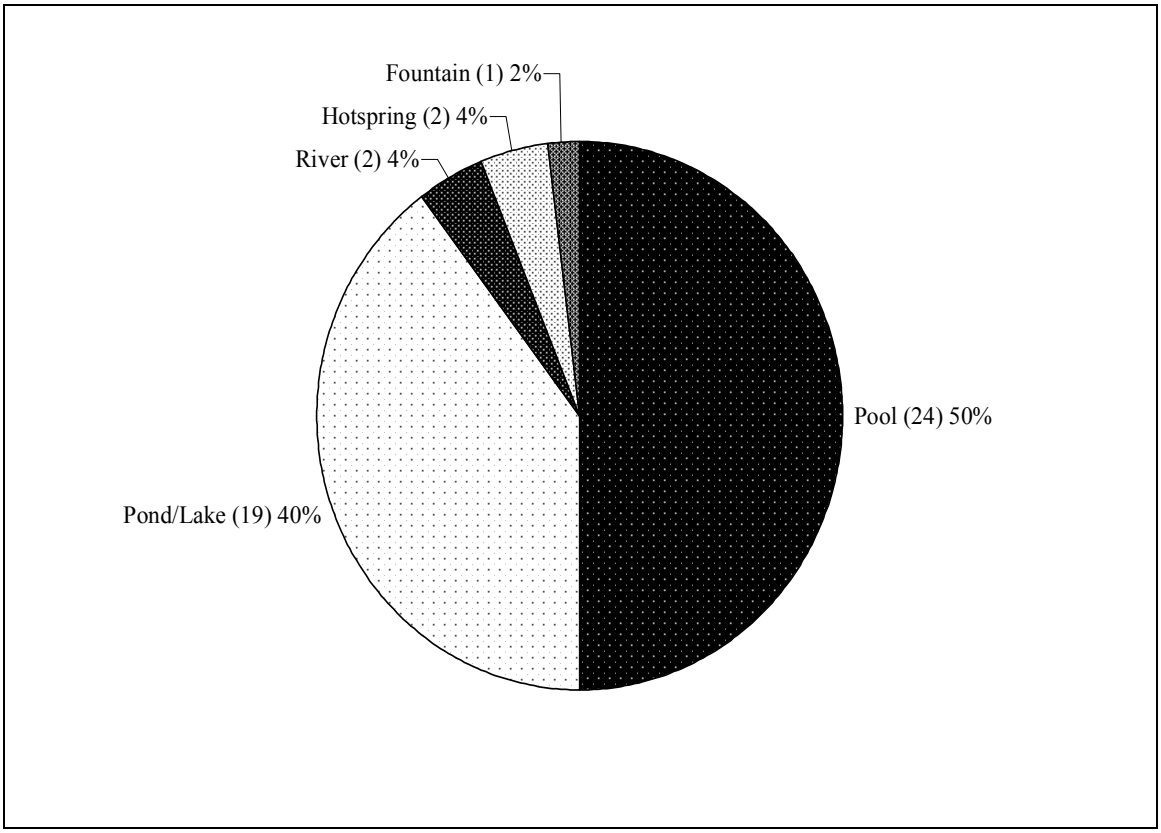


Figure 3
Frequency and Percentage of outbreaks affecting primarily children vs. outbreaks affecting all ages vs. outbreaks where age demographic is unknown, n = 48

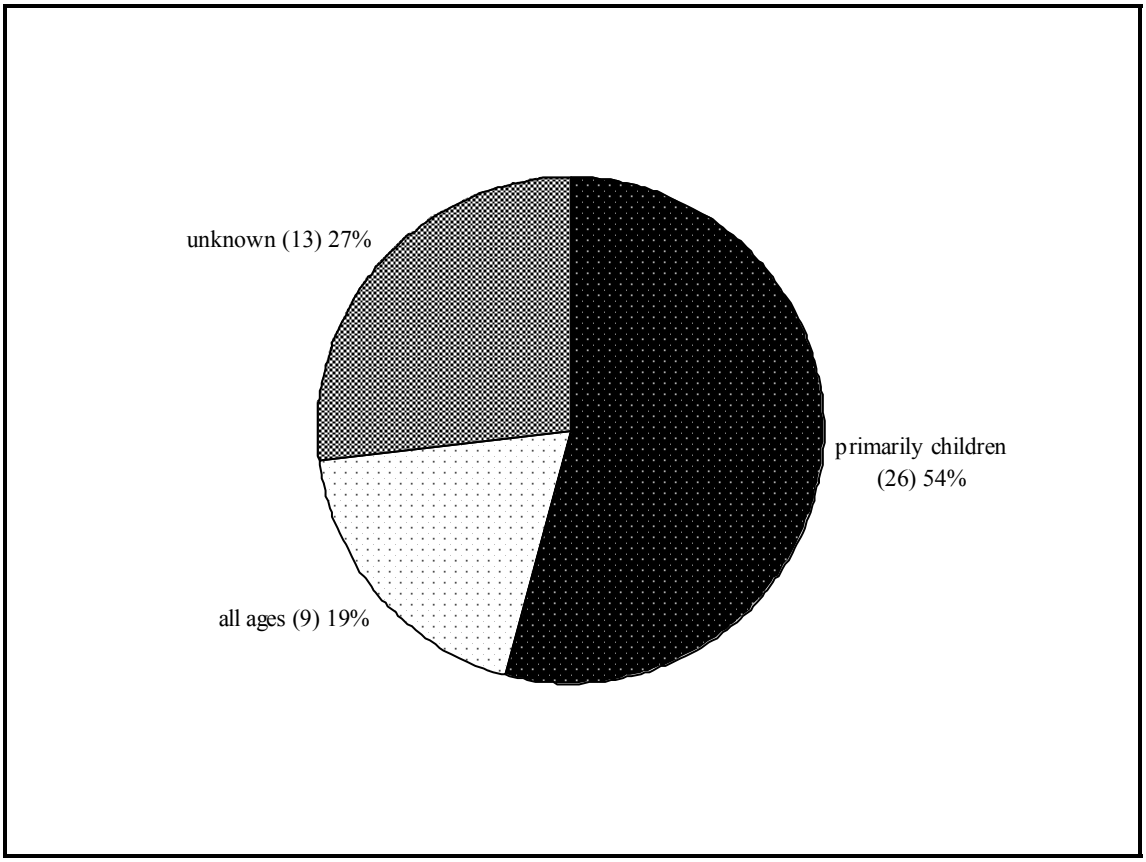


Figure 4
Presence or absence of bacterial indicators of fecal contamination in source water (frequency) percent, n = 48

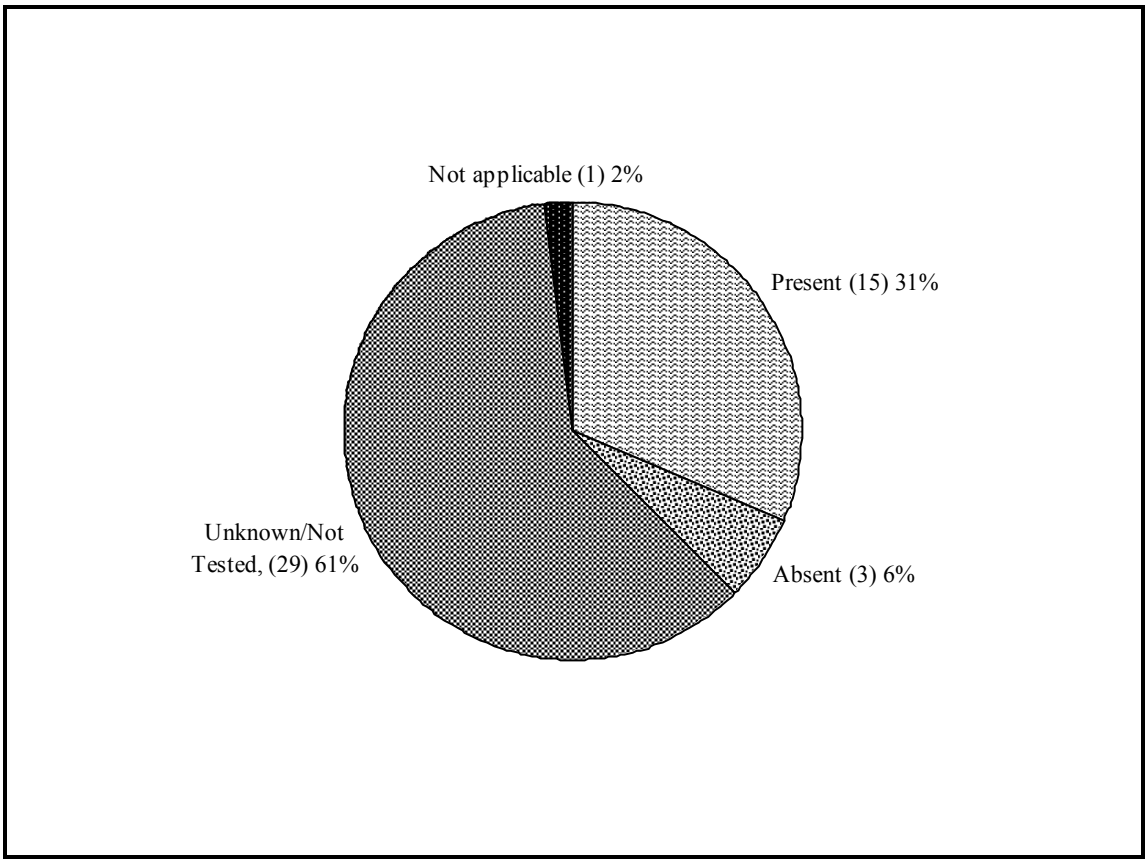
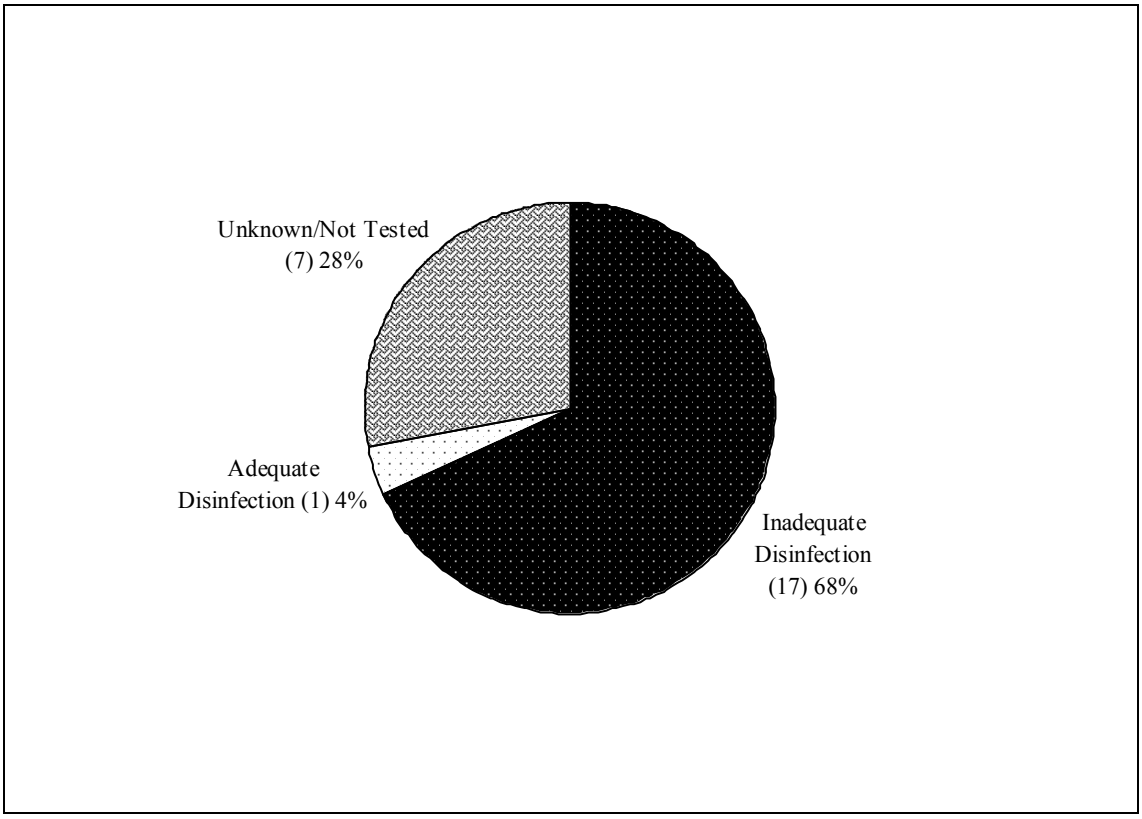
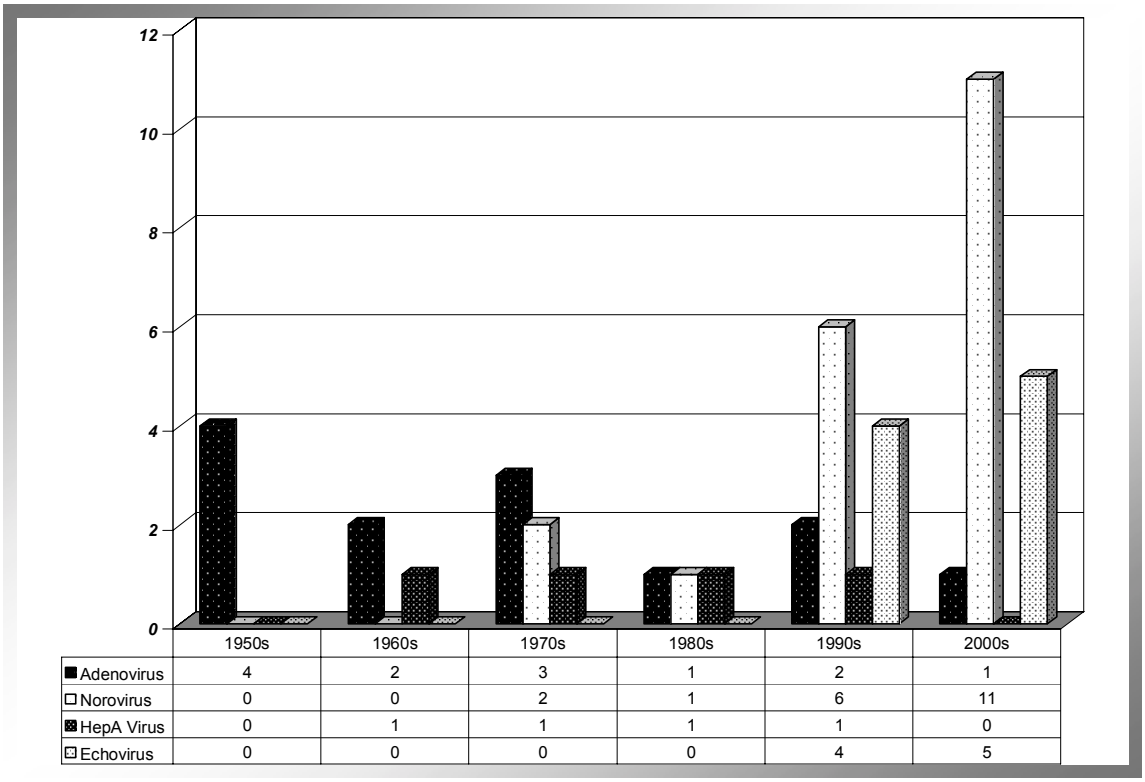


Figure 5
Frequency and percentage of outbreaks where inadequate disinfection* could be demonstrated vs. adequate disinfection vs. unknown or untested disinfectant levels
n = 25 (only swimming pool outbreaks included)



* Inadequate disinfection is defined as absent or insufficient level of free chlorine (<3ppm), chlorination or disinfection equipment failure, or inadequate method of disinfection such as improper use of hydrogen peroxide and/or UV.

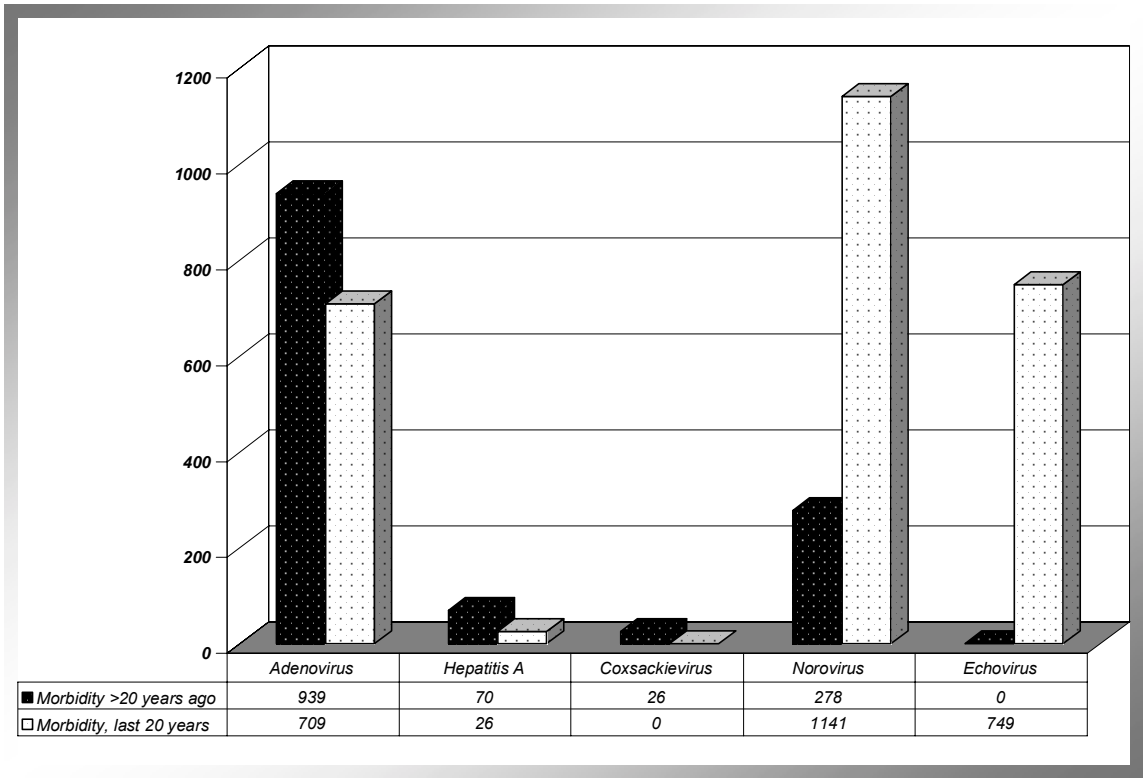
Figure 6
Outbreaks over time by etiologic agent, n = 46*



	1950s	1960s	1970s	1980s	1990s	2000s
Adenovirus	4	2	3	1	2	1
Norovirus	0	0	2	1	6	11
HepA Virus	0	1	1	1	1	0
Echovirus	0	0	0	0	4	5

* The two coxsackievirus outbreaks were excluded from this analysis.

Figure 7
Morbidity by etiology; last 20 years vs. > 20 years ago, n = 47*



	Adenovirus	Hepatitis A	Coxsackievirus	Norovirus	Echovirus
■ Morbidity >20 years ago	939	70	26	278	0
□ Morbidity, last 20 years	709	26	0	1141	749

* The large echovirus outbreak in Romania in 1999 was excluded on the basis that it was not comparable with the other outbreaks, as it was not a focal outbreak, but an ongoing state wide outbreak that became endemic in nature.

APPENDIX B:

ROLE OF FOMITE CONTAMINATION DURING AN OUTBREAK OF NOROVIRUS ON HOUSEBOATS

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Abstract

An outbreak of suspected norovirus gastroenteritis among three consecutive groups of houseboaters on a large recreational lake in Arizona was investigated to assess the role of fomite contamination, and to provide recommendations for prevention of future outbreaks. Interior boat surfaces were sampled for norovirus using transport swabs. Onboard toilet reservoirs were swabbed as a surrogate for stool samples from ill participants, since none were available, and onboard potable water supplies were sampled for norovirus. All samples were analyzed using RT-PCR with primers specific for human norovirus. Widespread fomite contamination was documented in the houseboats; 83% (5/6) of bathroom surface samples, 40% (2/5) of kitchen surface samples, and 100% (3/3) of doorknob samples were positive for the presence of norovirus. Samples of onboard potable water supplies were all negative. One of the participants on the first boating trip arrived already displaying symptoms of gastrointestinal illness prior to boarding the boat. This investigation demonstrates the potential role of widespread fomite contamination in outbreaks in confined spaces. To prevent or minimize future outbreaks in confined spaces, the adoption of practices such as surface disinfection and the utilization of methods to identify and exclude those with gastroenteritis from trips or activities in confined spaces, where others may become infected, are recommended.

Key Words

norovirus; outbreak; recreation; water; lake; fomite

Introduction

Noroviruses are the most common etiological agent of gastroenteritis in the United States, causing an estimated 23 million cases per year (Mead *et al*., 1999). These viruses have been implicated in numerous outbreaks, especially in confined spaces such as cruise ships (Isakbaeva *et al.,* 2005; McEvoy *et al*. 1996; Koo *et al*., 1993), hospitals and nursing homes (Verbelen *et al,*. 2004; Fretz *et al*. 2003; Khanna *et al*., 2003; Mcall and Smithson 2002; Ward *et al.,* 1999), and hotels (Cheesebrough *et al*., 2000; Mark *et al.* 2000; Dippold *et al*., 2003; Love *et al.,* 2002). Noroviruses are transmitted through the fecal-oral route, person to person, and via inhalation and subsequent ingestion of aerosolized vomitus. Food and waterborne outbreaks are common, while fomite contamination in norovirus outbreaks has become increasingly identified as an important factor in the transmission (Cheesebrough *et al*., 2000; Evans *et al*., 2002). Noroviruses are quite resistant to environmental stresses. They can withstand cleaning with detergent (Barker *et al*., 2004) and repeated vacuuming (Cheesebrough *et al*., 1997), and they are detectable after exposure to 5000 ppm chlorine (Barker *et al*. 2004). Noroviruses are also heat resistant; a less than 1 $\log_{10}$ reduction in PCR units of norovirus by exposure to 100°C for up to one minute, and 37°C for up to 168 hours, was determined by Duizer *et al*. (2004). Freezing or exposure to low temperatures (<4°C) only serves to preserve the virus.

Noroviruses cause acute onset of projectile vomiting and diarrhea, sometimes with low grade fever, headache, and malaise (Dolin *et al*., 1972). Symptoms are usually self-limited, lasting 24 to 72 hours. The incubation period is usually 24 to 48 hours, but

onset of symptoms as soon as 10 hours after exposure has been reported (Heymann 2004). Noroviruses are extremely contagious, and though the infectious dose (ID_{50}) is unknown, it is thought to be very low, perhaps as low as 10 to 100 viral particles. Outbreak attack rates are usually around 50%, but range from around 30% to over 80%, depending on the population affected, the vehicle of transmission, and the setting of the outbreak (Castro *et al*., 2004; Prato *et al.,* 2004; Lynn *et al*., 2004). Norovirus gastroenteritis causes rapid dehydration, which is of particular concern among the elderly and very young, as well as those who are engaged in physically demanding activities which hasten dehydration, such as during outbreaks among soldiers during deployment (Matson 2005). There is no specific therapy available for norovirus infection, but symptomatic treatment such as IV fluids and antiemetics can be helpful. There are no known non-human reservoirs for human norovirus, and no long-term immunity is gained from infection (Parrino *et al*., 1977).

Death from norovirus infection can occur, most frequently as a result of dehydration, but occasionally from aspiration of vomitus; Centers for Disease Control (CDC) estimates that there are around 300 deaths per year in the US from norovirus infection (Mead *et al*., 1999). In addition to the mortality caused by this virus, its morbidity is significant and causes an estimated 50,000 hospitalizations a year in the US (Mead *et al*., 1999). This level of morbidity undoubtedly has a great economic impact. In a case control study conducted in Switzerland, of 126 patients who visited a medical practitioner for treatment of norovirus, 56% (71/126) reported missing an average of 4.3 days of work due to their illness (range 0.5-14 days) (Fretz *et al*., 2005).

Noroviruses are members of the family *Caliciviridae* and the genus *Norovirus*. They are 27-32 nm single-stranded nonenveloped RNA viruses (Heymann 2004). There are four currently recognized genogroups (GI-GV) which are further divided into at least 22 genetic clusters (Vinje *et al*. 2004). Genogroups I and II cause the majority of illness in humans, but genogroup IV also contains some human genotypes. Noroviruses have not yet successfully been grown in cell culture (Duizer *et al*., 2004). As a result, it is more difficult to assess properties of noroviruses such as sensitivity to different methods of disinfection or survival under differing environmental conditions. Diagnosis of and testing for noroviruses is now commonly accomplished by reverse transcriptase polymerase chain reaction (RT-PCR) (Vinje *et al*., 2003), which can be used to test stool, vomitus, food, water, or samples from environmental surfaces.

This study was conducted to investigate an outbreak of suspected norovirus gastroenteritis among three consecutive groups of houseboaters on a large recreational lake in Arizona, and to assess the role of fomite contamination during the outbreak. An additional goal was to provide information for prevention or minimization of future outbreaks.

Outbreak Description

Participants in three consecutive 5-night educational boating trips became ill with symptoms of nausea and vomiting between May 9 and May 29, 2004. Of the 54 total participants, the US Public Health Service was able to contact twenty-seven (50%) for interview, and 20 of those interviewed (74%) fit the case definition of vomiting and/or diarrhea with onset between May 9 and May 30 (Table I). The boating trips took place

on a large recreational lake in northern Arizona. Participants were all senior citizens taking part in educational trips organized in conjunction with a local community college. The college rented four 52-foot houseboats from a local vendor. During the first trip, one boat was exchanged with the vendor due to mechanical problems. Then the new boat was also exchanged, again due to mechanical problems. In total, six boats were used during the first trip, however the four boats in use by the end of the first trip were the four boats used for the following two trips. The first trip began on May 9. The subsequent investigation revealed that one of the participants on this trip arrived displaying symptoms of gastroenteritis, and this participant appears to have been the index case for the outbreak. Two days after the start of the first trip, other participants on the trip began to become ill. The next trip began on May 16, and the same four boats were used. Participants on that trip began to show symptoms of gastroenteritis one day later on May 17. The final trip began on May 23, again the same four boats were used, and participants on that trip began to show symptoms of gastroenteritis on May 24 (Figure 1). Among those interviewed, the illness attack rate by trip ranged from 50% to 86%, with an overall attack rate of 74%. If those interviewed are representative of the group that participated in the trips, up to 40 illnesses may have resulted from this outbreak.

Environmental Investigation

The source of drinking water for trip participants was large onboard tanks that had been filled with tap water prior to departure. The houseboats were equipped with onboard kitchen and bathroom facilities. The boats were casually cleaned during the trips by the participants (i.e. rags were used to wipe counter tops and surfaces), but surfaces

were not disinfected. Between trips, and after the first outbreak of illness, instructors from the community college wiped down surfaces such as vinyl tablecloths, doorknobs, vinyl mattresses, food containers, and other hard surfaces with a diluted bleach solution of unknown concentration. The boats were returned to the vendor for refill of the potable water tanks and emptying of the waste holding tanks between each trip.

While there is not a record of which passengers were on which boats, the groups frequently interacted and shared meals. The trip participants prepared their own meals, and lunch and dinner were often communal with all of the boats participating in food preparation. This high level of interaction between participants on different boats, especially food preparation and sharing, could explain transfer of the virus from boat to boat.

Outbreak Investigation

Virus Sample Collection: On June 1, 2004, after the last trip was completed, but before the boats were cleaned, samples were collected. Random samples representative of bathroom, kitchen, and frequent-touch surfaces, were collected (n = 14) by swabbing surfaces with transport collection swabs. Swab samples from waste holding tanks were also collected (n = 6) as a surrogate for stool samples from ill participants, since no stool samples were available for testing. Drinking water samples were collected from each of two potable water tanks by pumping the water through positively charged filters for collection and concentration of virus.

Participant Interviews: When a phone number was provided, trip participants were contacted by telephone for voluntary interview. Interviews were conducted with 27 of 54 total participants (50%), 20 of whom reported becoming ill during their trip. Thirteen of those interviewed (65%) reported experiencing diarrhea and vomiting, five participants reported vomiting only, and two reported diarrhea only. The average duration of illness was 2.8 days (range <1 – 7 days). The mean age of those interviewed was 68.2 years (range 50-85). Four of the ill participants (15%) reported that they had sought treatment at area hospitals (mean age 81.2 years, range 75-85). The symptoms, duration of illness, and incubation period as evidenced by the time from departure to first illness on each trip, were all consistent with norovirus. Illnesses were reported on all four boats and during all trips. The attack rate for each trip was 86%, 50%, and 74%, respectively. The houseboat vendor did not report any illnesses among their staff.

Methods

Virus Collection from Surfaces: Fomite samples were collected using microbiological specimen collection and transport swabs (BD Diagnostic Systems, Sparks, MD). Flat surfaces were swabbed in a 100 cm^2 area, while objects such as handles and knobs were swabbed in their entirety. Swabs were placed in sealable plastic bags for transport overnight to the laboratory on ice.

Virus Concentration from Water: The general procedures for the concentration of enteric viruses used in this study are described in Method 9510 in Standard Methods for the Examination of Water and Wastewater, 20^{th} Edition (1998). Two potable water samples, one 78.7 liter sample and one 50.0 liter sample, were collected from two of the

boats by pumping the water through Virosorb™ 1-MDS Cartridge Filers (CUNO, Meriden, CT) in plastic filter housings. Noroviruses were concentrated by adsorption onto the positively charged filters, as previously described (Sobsey and Glass 1984). Cartridge filters were stored in sealable plastic bags and transported on ice overnight to the laboratory. One liter of 3% beef extract (pH 9.5) was used to elute virus from each filter. The pH of the eluate was then lowered to 3.5 using 1N HCl to flocculate the beef extract, and the solution was stirred slowly for 30 minutes on a stir plate. The solution was then centrifuged, and the supernatant discarded. The pellet was suspended in 20 ml of 0.15M sodium phosphate, and then filter sterilized. Concentrated samples were stored at -20ºC for further processing.

RNA Extraction: Viral RNA was purified from the samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA), and the Mini Spin Protocol was followed with the following minor modifications: the total sample volume was doubled to 280µl, and a double elution using two consecutive 40µl volumes of Buffer AVE was performed. The purified RNA samples were stored at -20ºC.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR): RT-PCR was performed on the purified RNA using the QIAGEN OneStep RT-PCR Kit (QIAGEN, Valencia, CA). The primers MJV12 (5'-TAY CAY TAT GAT GCH GAY TA-3') and RegA (5'-CTC RTC ATC ICC ATA RAA IGA-3') (Vinje *et al*., 2004), which are modified JV12/JV13 primers (Vinje *et al*., 2003), that are specific for human norovirus GI and GII polymerase region A were used. A 10µl volume of purified RNA template and a final concentration of 1µM of each primer were used in a total reaction volume of

50µl. Thermal cycling conditions were as follows: reverse transcription of viral RNA for 60 min @ 42ºC; activation of Taq polymerase for 15 min @ 95ºC; 40 cycles: 30 sec @94ºC, 30 sec @50ºC, 30 sec @72ºC; and final extension for 10 min @ 72ºC. RNase-free water negative controls and known positive norovirus controls were run concurrently with the unknown samples. RT-PCR product was visualized using an ethidium bromide stained 2% agarose gel run in 0.5X TBE buffer. Norovirus positive RT-PCR product was purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA), and then sent to the University of Arizona DNA Sequencing Laboratory. Sequences were compared to known sequences in the National Center for Biotechnology Information's nucleotide-nucleotide BLAST database for confirmation of positive samples as human norovirus, though the genogroup and strain were not determined. All amplicons reported as norovirus positive were confirmed as human noroviruses by sequencing.

Results

The results of norovirus RT-PCR testing of swab samples from boat surfaces are shown in Table II. Samples taken from randomly selected representative surfaces in kitchen, bathroom, and frequent-touch areas of the boats sampled were positive for the presence of norovirus. Six swab samples were taken from bathroom surfaces, including toilet lids and faucet handles, on two of the affected boats. Five of these samples (83%) were positive for norovirus. Five kitchen surfaces, that included sink and refrigerator surfaces, were tested on three different boats. Two of five kitchen surface samples (40%) were positive for norovirus. Door handles were sampled on three boats, and all of these were positive for norovirus. Each boat's onboard toilet reservoir, including the two boats

that were exchanged during the first trip, was swabbed, and four of six (67%) were positive for norovirus; however, strong oxidizers used to control odor were used in these reservoirs and were visible as a blue liquid on the sample swabs. It is possible that these chemicals may inhibit RT-PCR and therefore interfered with our ability to detect norovirus using RT-PCR. Although not directly relevant to fomite contamination, the toilet reservoir samples were essentially stool sample surrogates, and had we not found norovirus on the houseboat surfaces these samples would have served to help determine if the etiologic agent of the outbreak was a norovirus. Both potable water samples were negative for the presence of norovirus by RT-PCR.

Discussion

This investigation demonstrated widespread fomite contamination in the houseboats, while samples of drinking water were negative for the virus. It was also learned that one of the participants on the first boating trip arrived displaying symptoms of gastrointestinal illness prior to boarding the boat, and this participant likely spread the virus to subsequent groups who used the same houseboat via fomite contamination and other modes of transmission.

It is likely that multiple modes of transmission were responsible for spreading norovirus during this outbreak. Due to the close quarters, shared bathrooms, and shared meals, it is presumable that person-to-person and foodborne transmission and environmental contamination all played a role. Negative results from the testing of the drinking water supply and the identification of an index case support the idea that this outbreak was not waterborne. Although drinking water samples were collected from only

two of the four boats, the source of water used to fill the onboard reservoirs was the same for all four boats. The detection of norovirus on fomites throughout the boats illustrates how the virus can be spread extensively in close quarters. It is also interesting to note that viral contamination did not remain confined to the bathrooms, but extended throughout the boat. Even passengers who are careful about hygiene in the bathroom may be complacent in areas where viral contamination is perceived more unexpected, such as the kitchen, where, in this investigation, 40% of kitchen surfaces tested were positive for norovirus.

The participants on these trips were an older population with an average age of 68.2 years, and the older age of the participants may account for the high rates of infection (74% overall attack rate), since the elderly are more susceptible to norovirus (Gerba and Rose 1996). The subpopulation of participants that went to the hospital (n = 4) for treatment was older still (average age = 81.2 years) than the general population of participants. This may be explained by an increase in illness severity with increased age. Because passengers on consecutive trips became ill when they boarded the same boats, it is likely that environmental contamination played a role in sustaining the outbreak from trip to trip. Isakbaeva *et al*. (2005) described a sustained outbreak of norovirus on a cruise ship in which passengers on six consecutive cruises became ill despite a rigorous 1-week sanitation of the ship following the second cruise. Genetic sequencing was used to show that the same strain of norovirus was detectable before and after the 1-week sanitation effort. The authors concluded that these findings suggest environmental

contamination may have helped to perpetuate the outbreak, but they also point out that infected crewmembers could have been a reservoir for the infection.

Extensive spread of norovirus following a vomiting incident in a confined area has been reported (Marks *et al.,* 2000). Aerosolization of vomitus may have been in part responsible for spread of norovirus in this outbreak, since the houseboats were small (52 ft.). Barker *et al*. (2004) showed that cleaning norovirus contaminated surfaces with a detergent did not eliminate the virus. In fact, when the same cloth was subsequently used to wipe a clean surface, the virus was transferred to that surface and to the hands of the person holding the cloth. In the houseboat outbreak, casual cleaning without disinfection could have served to further spread the virus throughout the boats.

Extensive environmental contamination and sustained illness has also been reported in a hotel setting in which cases of norovirus were reported over a 5-month period from January to May 1996, despite efforts to clean the hotel with detergents (Cheesebrough *et al*., 2000). Norovirus was detected in 24% of samples from "frequently handled objects" such as phones and door handles, in 20% of samples from "soft furnishings" such as cushions and curtains, and in 75% of carpet samples where no known vomiting incident had occurred. The hotel outbreak has several factors in common with the outbreak described here. The hotel guests, like the participants in the houseboat trips, were elderly. Like the houseboat trips, there was a rapid turnover of guests, with new groups of hotel patrons arriving every 3 or 4 days.

Noroviruses cannot as yet be grown in cell culture. For this reason, studies about their survival in the environment are limited because there is no cell line in which to

determine infectivity. There is evidence that noroviruses are resistant to environmental conditions and remain infectious on environmental surfaces for extended periods of time such as several days to over a week (Cheesebrough *et al*., 1997). RT-PCR is commonly used to detect noroviruses, but the method does not discriminate between infectious and inactivated viruses. Consequently this study and similar studies are limited by the inability to ascertain the infectivity of virus detected in the environment. However, the likelihood of naked RNA remaining undegraded in the environment is small, which suggests that virus detected by RT-PCR may closely reflect the true presence of infectious virus (Barker *et al*., 2004). This study is also limited by the small number of samples collected. Future studies that aspire to determine fomite transmission during an outbreak would benefit from a larger number of samples obtained from individual fomites so that those surfaces that may play a pivotal role in transmission could be identified.

Fomite contamination has long been recognized as having an important role in the transmission of viruses. The importance of this route of transmission in the epidemiology of noroviruses is not well understood, but cumulative evidence of prolonged outbreaks originating from contamination of confined spaces, as well as indications that the virus survives for days to weeks on surfaces, indicates that fomite contamination plays a particularly important role in the transmission of this virus and the perpetuation of outbreaks over time. It may be worth investigating the seemingly disproportionate role of fomite transmission in norovirus gastroenteritis verses other types of gastroenteritis, as this could help to focus prevention or clean-up efforts. In this outbreak, and others in

confined spaces, disinfection of all hard surfaces with a 10% bleach solution is recommended to minimize spread of the virus via exposure to contaminated surfaces. Non-bleachable surfaces, such as carpeting, blankets, and upholstered furniture pose a problem for disinfection and may harbor virus for some time (Cheesebrough *et al*., 2000; Cheesebrough *et al*., 1997).

Education of the public regarding norovirus transmission in confined spaces may aid people in making important decisions about excluding themselves from activities and trips when they are ill. This outbreak among houseboaters was likely attributable to a participant who arrived ill with gastroenteritis at the start of the first trip. People who opt to participate in trips and activities when they are ill with gastrointestinal symptoms may not realize that they have the potential to make others ill, cause widespread outbreaks of disease, or cause economic harm to businesses. This outbreak may have been minimized or prevented if the index case had been excluded from the trip based on evidence of symptoms of norovirus gastroenteritis, or if the index case had excluded himself on the basis of those symptoms. Screening mechanisms could be developed to minimize participation by ill consumers, but reimbursements and incentives for self-exclusion must be sufficient to make participation in these programs worthwhile for those who may be reluctant to reveal their illness for fear of disrupting their planned vacation or excursion.

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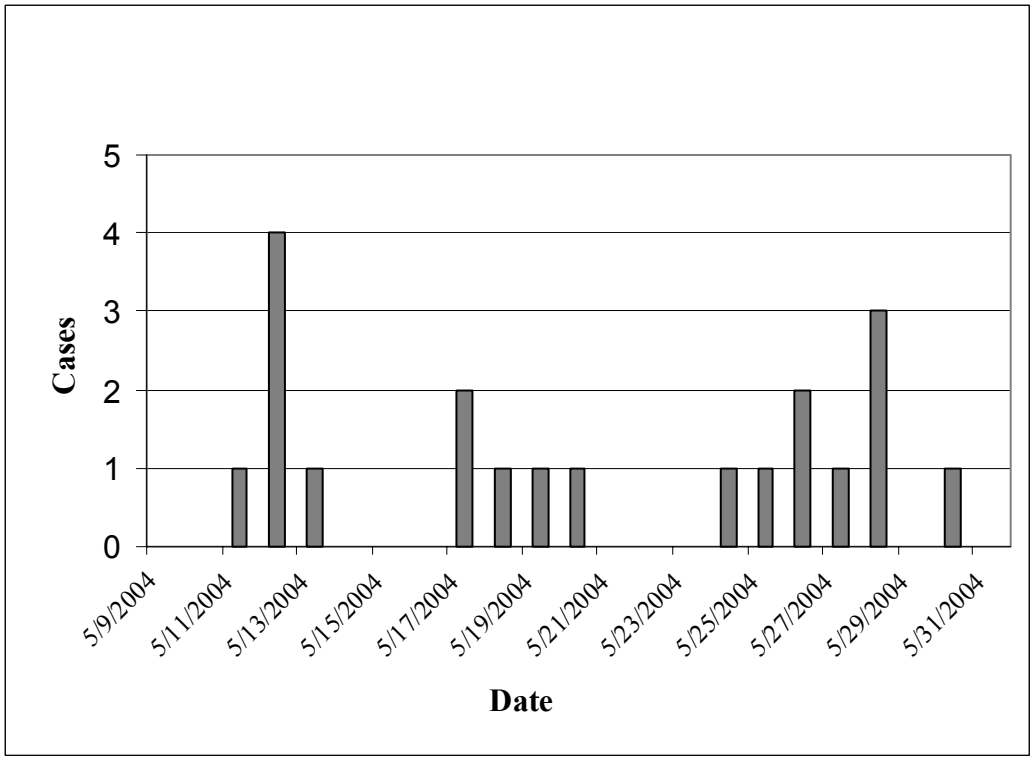

Figure 1: Date of onset of illness reported by those interviewed (n = 20)

Table I:
Total number of participants, number interviewed, and % of those interviewed who reported illness, by sex and trip date

Trip	Sex	# Participants	# Interviewed	# Ill	% Ill
5/9/2004	Male	7	3	3	100
	Female	9	4	3	75
	Total	16	7	6	86
5/16/2004	Male	4	2	2	100
	Female	9	6	2	33
	Total	13	8	4	50
5/23/2004	Male	7	0	0	N/A
	Female	18	12	10	83
	Total	25	12	10	83
Total	Male	18	5	5	100
	Female	36	22	15	68
	Total	54	27	20	74

Table II:
Norovirus RT- PCR Swab Sample Results (n = 20) by Surface Type and Boat #

Bathroom Surfaces		**Kitchen Surfaces**		**Door Handles**		**Toilet Reservoirs**	
Boat #		Boat #		Boat #		Boat #	
1	+	1	+	1	+	1	+
	-	3	-	2	+	2	-
3	+		-	3	+	3	+
	+	4	+			4	-
	+		-			5	+
	+					6	+
Percent Positive = **83%**		Percent Positive = **40%**		Percent Positive = **100%**		Percent Positive = **67%**	

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APPENDIX C:

RECURRING OUTBREAKS OF NOROVIRUS AMONG RAFTERS ON THE COLORADO RIVER

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Abstract

Every year over 22,000 people raft the Colorado River through the Grand Canyon in Arizona. Since 1994, over 400 rafters in six separate outbreaks have become ill with norovirus while rafting on this stretch of the river. An analysis of the water quality along the Colorado River during the 2004 rafting season was conducted. Water from the nearby Glen Canyon Dam Wastewater Treatment Plant and the Lee's Ferry launch site was also examined during the 2003 and 2004 rafting seasons. Parameters examined include: norovirus by reverse transcriptase polymerase chain reaction (RT-PCR), coliforms, *Escherichia coli*, temperature, turbidity, and pH. Stool samples from ill rafters and composite stool samples from onboard toilet-cans were tested by RT-PCR for the presence of norovirus during the 2003 and 2004 season outbreaks. The history and epidemiology of norovirus outbreaks among rafters on this portion of the Colorado River was also reviewed, and historical Colorado River water quality data is presented and examined. Potential sources of norovirus outbreaks among rafters include drinking contaminated river water, consuming contaminated food stuffs, rafter importation of the virus and subsequent person to person spread, and contaminated campsites or equipment. It is likely outbreaks are the result of more than one source of norovirus, though the exact source remains unknown for several outbreaks. Because rafters are exposed to extreme heat and experience intense physical exertion, they are at high risk of becoming quickly dehydrated once they are infected with norovirus; six rafters ill with norovirus have been evacuated by helicopter from the canyon. No norovirus was detected in the Colorado River during the 2004 field sampling. Norovirus has been detected in the Glen Canyon

Dam Wastewater Treatment Plant on two occasions, and inactivation of the virus prior to discharge of treated water into the Colorado River may be inadequate. Overall water quality in the Colorado River is historically high, with most test samples in compliance with guidelines for recreational waters (<200 fecal coliform/100ml). Occasionally samples have exceeded these limits, but the occurrence of high numbers of *E. coli* is sporadic and does not indicate specific ongoing sources of contamination, rather it points to localized random events. Recommended preventive measures include carrying in drinking water and the use of water filters that remove viral particles from the water; however it is important to note that because of the possibility of rafter importation of the virus or consumption of contaminated food, this intervention could reduce but not eliminate the frequency of norovirus outbreaks among river rafters. The disinfection of boats and equipment between trips, as well as use of ethanol-based hand sanitizers and careful vomitus disposal are also advised.

Key Words: norovirus, river rafters, recreational water, outbreak, Colorado River

Introduction

Norovirus is the most common cause of gastroenteritis in the United States, accounting for an estimated 23,000,000 cases of gastroenteritis per year (Mead *et al*., 1999). The virus is spread through the fecal-oral route, as well as by aerosolized vomitus and fomite contamination, and can be transmitted by eating contaminated foods, drinking or accidentally swallowing contaminated water, contact with contaminated surfaces, or via close contact with an ill person. Norovirus causes the abrupt onset of nausea, vomiting and diarrhea. The incubation time for the virus is 12 to 48 hours, and it usually lasts one to three days, though occasionally severe cases last longer. This virus is highly contagious. Outbreaks among vulnerable populations such as the elderly or infirmed can have devastating consequences, including death (Lopman *et al*., 2003), while outbreaks among other populations, such as cruise ship passengers (CDC, 2002), are extremely unpleasant and of economic importance to businesses. In addition to causing millions of cases of gastroenteritis, the virus is also a public health burden and the cause of an estimated 50,000 hospitalizations and 300 deaths per year in the United States (Mead *et al*., 1999).

Many people from all over the world travel to Arizona to visit the Grand Canyon National Park, and over 22,000 people a year raft the 386 K (240 mile) stretch of the Colorado River that flows through the Grand Canyon (National Park Service, 2005). This portion of the river is downstream of Lake Powell, a recreational lake, and the Glen Canyon Dam which forms Lake Powell. Consequently, all flow into the Colorado River beyond the Glen Canyon Dam is controlled by dam releases. Rafting is a popular

activity, and accordingly reservations to raft the river can be difficult to obtain and private rafting companies that conduct organized trips with river guides can be costly, averaging two to three thousand dollars. The trips vary in length and in amenities, but the average trip takes about 1 week to 10 days, and is about 225 miles long. Rafters travel either in motorized rafts or oar paddled rafts and camp on beaches along the river. During the 1972 and 1979 rafting seasons, outbreaks of gastroenteritis among rafters prompted investigation. An outbreak of the enteric pathogen *Shigella sonnei* among rafters was reported (Merson, 1974). Due to the number of people utilizing the river, and in an effort to reduce the spread of disease, the NPS mandated in 1978 that all human fecal waste be carried out, and not buried on beaches or disposed of in the river. Despite this mandate, outbreaks of gastroenteritis still occur among rafters. Since 1994, over 400 rafters in six separate outbreaks have become ill with norovirus (Table 1). While there was a span of four years between the 1994 outbreak and the next outbreak in 1998, outbreaks have occurred consecutively for the last four rafting seasons (2002-2005).

Norovirus illness among those participating in recreational activities has been previously reported. In 1994, 7 of 11 canoeists became ill with norovirus after exposure to contaminated recreational lake water (Gray *et al*., 1997). Swallowing water and eating food before getting changed out of clothing worn while canoeing were associated with increased risk of infection ($p < 0.02$, for both exposures). In May and June of 1999, an outbreak of norovirus gastroenteritis occurred among long-distance hikers on the Appalachian Trail between Catawba and Troutville, Virginia (Peipins *et al*., 2002). People who consumed food prepared at the general store in Catawba were almost twice

as likely to become ill as those who did not. Tests of water from taps in the store revealed fecal contamination, but were negative for norovirus. However, stool and serum samples from hikers were positive for norovirus. The authors conclude that poor sanitation on the trail, scarce water supplies, and crowding may have contributed to increased risk of norovirus among long distance hikers. A waterborne outbreak of norovirus among snowmobilers occurred in Wyoming in 2001 (Anderson *et al*., 2003). The snowmobilers were consuming well water, which was later confirmed to contain norovirus. At least 35 cases resulted from this outbreak. In 2002, an outbreak of norovirus resulted from primary school children who had played in a recreational water fountain (Hoebe *et al*., 2004), and in 2004 a large outbreak of norovirus occurred among visitors and workers at Yellowstone National Park (billingsgazette.com). Norovirus outbreaks have also frequently been associated with swimming in contaminated recreational water (CDC, 2004; Maunula *et al*., 2004).

The present study is an investigation of the water quality along the Colorado River between Glen Canyon Dam and the convergence of the Colorado River with Diamond Creek, near Peach Springs, Arizona, including specifically testing for the presence of human norovirus by reverse transcriptase polymerase chain reaction (RT-PCR). Additional parameters examined include: coliforms and *E. coli* most probable number per 100/ml (MPN), temperature, turbidity, and pH. Potential sources of norovirus contamination in the Colorado River were also assessed for norovirus content, including Glen Canyon Dam Wastewater Treatment Plant, Page, Arizona Wastewater Treatment Plant, and Wahweap Wastewater Treatment Plant. Four field studies were

conducted for sample collection: July, 2003; August, 2003; May, 2004; July, 2004. Stool samples from ill patients and composite stool samples from on-board toilet-cans were analyzed for the presence of human norovirus during the 2003 and 2004 outbreaks. The history and epidemiology of norovirus outbreaks among rafters on the Colorado River was reviewed, and historical Colorado River water quality data was examined.

Methods

Virus Concentration from Water: The general procedures for the concentration of enteric viruses used in this study are described in Method 9510 in Standard Methods for the Examination of Water and Wastewater, 20th Edition (1998). Water samples were collected by pumping the water through Virosorb™ 1-MDS Cartridge Filers (CUNO, Meriden, CT) in plastic filter housings. Noroviruses were concentrated by adsorption onto the positively charged filters, as previously described (Sobsey and Glass, 1984). Cartridge filters were stored in sealable plastic bags and transported on ice overnight (when possible) to the laboratory. One liter of 3% beef extract (pH 9.5) was used to elute virus from each filter. The pH of the eluate was then lowered to 3.5 using 1N HCl to flocculate the beef extract, and the solution was stirred slowly for 30 minutes on a stir plate. The solution was then centrifuged, and the supernatant discarded. The pellet was suspended in 20 ml of 0.15M sodium phosphate, and then filtered through a 0.2µm pore sized filter to remove bacteria. Concentrated samples were stored at -20ºC for further processing. This method has been proven effective for the concentration and purification of noroviruses (Schwab KJ, *et al*., 1995).

Virus Concentration from Stool: Noroviruses were concentrated from stool by suspending 1 gram of stool in 7ml phosphate buffered saline. Suspensions were then vortexed for 60 seconds, and centrifuged for 30 minutes. The supernatant was removed and aliquoted for storage at -20°C until further processing.

RNA Extraction: Viral RNA was purified from the samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA), and the Mini Spin Protocol was followed with the following minor modifications: the total sample volume was doubled to 280µl, and a double elution using two consecutive 40µl volumes of Buffer AVE was performed. The purified RNA samples were stored at -20ºC.

Reverse Transcriptase-Polymerase Chain Reaction: RT-PCR was performed on the purified RNA using the QIAGEN OneStep RT-PCR Kit (QIAGEN, Valencia, CA). The primers MJV12 (5'-TAY CAY TAT GAT GCH GAY TA-3') and RegA (5'-CTC RTC ATC ICC ATA RAA IGA-3') (Vinje *et al*., 2004), which are modified JV12/JV13 primers (Vinje *et al*., 2003), that are specific for human norovirus GI and GII polymerase region A were used. A 10µl volume of purified RNA template and a final concentration of 1µM of each primer were used in a total reaction volume of 50µl. Thermal cycling conditions were as follows: reverse transcription of viral RNA for 60 min @ 42ºC; activation of Taq polymerase for 15 min @ 95ºC; 40 cycles: 30 sec @94ºC, 30 sec @50ºC, 30 sec @72ºC; and final extension for 10 min @ 72ºC. RNase-free water negative controls and known positive norovirus controls were run concurrently with the unknown samples. RT-PCR product was visualized using an ethidium bromide stained 2% agarose gel run in 0.5X TBE buffer. Norovirus positive RT-PCR product was

purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA), and then sent to the University of Arizona DNA Sequencing Laboratory. Sequences were compared to known sequences in the National Center for Biotechnology Information's nucleotide-nucleotide BLAST database for confirmation of positive samples as human norovirus, though the genogroup and strain were not determined. All amplicons reported as norovirus positive were confirmed as human noroviruses by sequencing.

Additional Analytes: In addition to testing for norovirus, the following water quality parameters were tested using the methods shown: pH (Waterproof pHTestr 3+ Double Junction, Oakton Instruments, Vernon Hills, IL), turbidity (DRT-15CE Portable Turbidimeter, HF Scientific, inc., Fort Myers, FL), temperature (Waterproof pHTestr 3+ Double Junction 550, Oakton Instruments, Vernon Hills, IL), *E. coli* and total coliform bacteria (IDEXX, Colilert®, Westbrook, Maine).

Results

Results from the 2003 field sampling of possible sources of norovirus contamination in the Colorado River are shown in Table 2. Samples taken from Glen Canyon Dam Wastewater Treatment Plant, Page, Arizona Wastewater Treatment Plant, and Wahweap Wastewater Treatment Plant were all negative for the presence of human noroviruses genogroups I and II by RT-PCR specific for the detection of human norovirus belonging to both genogroups I and II. An additional sample, taken from the drinking water tap at Lee's Ferry boat launch was also negative for noroviruses. In May 2004, samples of water were taken from six locations along the Colorado River: Lee's

Ferry (mile 0), Vasey's Paradise (mile 31.9), Eminence Break (mile 44.0), Nankoweap Beach (mile 52.2), and Bright Angel Creek (mile 87.8). All samples were negative for the presence of norovirus (Table 3). In July 2004, samples were collected from the Glen Canyon Wastewater Treatment Plant and from the Colorado River at Lee's Ferry (Table 4). The sample collected from Lee's Ferry was negative for noroviruses, but did contain >23 coliforms (MPN/100ml). Of two samples collected from the secondary clarifier at the Glen Canyon Dam Wastewater Treatment Plant, one was positive for human norovirus, and one was negative. Two samples of treatment plant effluent were collected after disinfection, and both showed the presence of coliforms and *E. coli*, though levels were within regulations for recreational water (<200 fecal coliform/100ml).

Historical water quality data from points along the Colorado River is shown in Table 5. From the years 1995 through 1998, *E. coli.* (MPN/100ml) was sampled twice during each rafting season. These studies reveal that overall water quality in the Colorado River is historically high, with only 6/64 test samples (9.4%) above compliance with guidelines for recreational waters (<200 fecal coliform/100ml). Upon examination of the six occasions when the limits were exceeded, the contamination appears sporadic, and does not indicate specific ongoing sources of contamination in one or several areas, rather it points to localized random events. In Table 6, the conventional names and corresponding river miles for each sampling site are provided.

An overview of outbreaks of norovirus or suspected norovirus among river rafters from 1994-2005 is shown in Table 1. Of the six outbreaks reported, four have occurred in the consecutive previous four years. Outbreaks have begun in May, July, August, and

September, and have lasted from just under two weeks in duration to almost two months. The river mile that the first case of each outbreak became ill varies from outbreak to outbreak. Sometimes, such as the 1998, 2002, and 2003 outbreaks, the first illness occurred during the first day of the trip, within 10 to 16 miles downriver. During the 1994, 2004, and 2005 outbreaks, the first case was reported to have occurred several days into the trip and much farther downriver (20-136 miles). In some cases, the source of the outbreak was speculated to be the river water, while some outbreaks are of unknown cause. The 2005 outbreak was epidemiologically linked to contaminated sliced deli meat. Some outbreaks have involved only one river rafting company, while others have involved several; the 1994 outbreak affected 15 companies, the 2002 affected nine, and the 2005 affected 13. The number of individual trips affected varies from one in 1998 and 2004 to 17 in 2002. During both the 2003 and 2005 outbreaks, helicopter evacuation of affected rafters was necessary. All outbreaks except one from 1998 to present were confirmed via the presence of norovirus in stool samples from individual rafters or from composite stool samples taken from on-board toilets. Additionally, positive norovirus samples were obtained from the river water at Lee's Ferry and the Glen Canyon Dam Wastewater Treatment Plant during the 2002 outbreak. During the 2005 outbreak, epidemiological evidence pointed toward sliced deli meat as a contaminated food source, and norovirus was detected on the meat samples (personal communication, Dr. Lee-Ann Jaykus).

In September, 2003 six composite stool samples from one trip with ill passengers were analyzed for norovirus; one sample tested positive. In October, 2003 stool samples

from three individual rafters were tested for norovirus, and two of the three were positive. From the June 2004 outbreak, only two composite stool samples were tested and both were negative.

Discussion

Potential sources of norovirus outbreaks among rafters include drinking contaminated river water, consuming contaminated food stuffs, rafter importation of the virus and subsequent person to person spread, and contaminated campsites or equipment. Water samples from along the Colorado River and historical water quality data indicate that there is not an ongoing high level of contamination in the river; norovirus has only been isolated directly from the river on one occasion, in 2002 when the Centers for Disease Control isolated norovirus from river water sampled at Lee's Ferry (Higgins, 2002). Though *E. coli* was detected, its detection follows no geographical pattern implicating a particular source of contamination, such as a tributary. Rafter importation of norovirus may certainly be a contributing factor in some outbreaks, especially those where the first case became ill during the first day of the trip, since the average incubation period for norovirus is 24 hours, when rafters become ill quickly, it is reasonable to assume they may have been infected prior to boarding the raft. Travelers are exposed to illness at airports, hotels, restaurants, etc., during long trips from all over the US and from all over the world to arrive at Lee's Ferry. They may arrive at the start of the trip having been exposed to the virus, but not yet showing symptoms. In an article published in *The Oregonian* in 2004, one rafter recounts her experiences arriving at Lee's Ferry on the date of the launch of her trip. She relays that she was "violently ill" prior to

launch and suspected that she was infected with norovirus, but "tried to keep a low profile while a Park Service ranger inspected (their) equipment". Despite being aware of a large outbreak of norovirus on the river the year before, she did not want to get "tossed from the trip before (they) had even set out." The author writes, "I was struggling into my dry-suit at the put-in… too weak to pop my head through the neck gasket without help. I had been throwing up all night." Once one person on a trip becomes ill, spread to trip-mates occurs readily via aerosolization of vomitus, food contamination, fomite contamination, person to person spread, and contamination of the river and campsites.

Treated wastewater is discharged into the Colorado River 15 miles upstream of Lee's Ferry where most rafting trips launch. The treatment plant is located inside of the Glen Canyon Dam, and was implicated by the Centers for Disease Control in the 2002 norovirus outbreak among rafters. The source of the sewage treated at the plant is the Carl B. Hayden Visitor Center and the facilities operation for the dam itself. The plant uses UV light as its means of disinfection, and uses no chemical disinfection. Viruses tend to be fairly UV resistant, requiring high doses of UV to become inactivated, so this treatment may not be adequate to inactivate norovirus. Enteric viruses, such as adenoviruses have been shown to be UV-resistant at doses effective at meeting wastewater regulations for total coliform (Thompson *et al*., 2003).

Fomite contamination in norovirus outbreaks has been recognized as an important factor in the transmission of the disease in closed populations or confined spaces (Cheesebrough *et al*., 2000; Evans *et al*., 2002). Noroviruses are quite resistant to environmental stresses; they can withstand cleaning with detergent, and they are

detectable after exposure to 5000 ppm chlorine on fomites (Barker *et al*., 2004). Noroviruses are also heat resistant and can survive a temperature of 37°C for up to 168 hours (Duizer *et al*., 2004). Also, feline calicivirus, a surrogate for norovirus, has been shown to survive for up to three days on telephone buttons and receivers and one to two days on a computer mouse (Clay, *et al*., 2006). Given the ability of this virus to survive on fomites in the environment, fomite contamination may play an important role in transmission of norovirus among river rafters, as commonly shared items such as drinking container spigots or serve ware handles are handled by everyone on the trip.

Several factors exacerbate disease severity on the river. The rafters are frequently exposed to high heat during the rafting season when temperatures in the canyon often exceed 100°F. At the same time, river rafting is a physically demanding activity, especially on non-motorized trips. Rafters are not screened for health problems prior to departure. The age range of rafters is 12 to 82 years (NPS). Risk groups for complicated norovirus infections have been identified and they include the elderly, the immunosuppressed, and those who have cardiovascular disease (Mattner *et al*., 2006). Both high temperatures and physical exertion hasten dehydration and increase disease severity. At the same time, sanitation is limited on the river. The inability to stop the trip while ill rafters recover leads to vomiting and defecation accidents in or on the rafts, resulting in a presumably high level of environmental contamination. During the throws of illness, rafters must sometimes vomit or defecate directly into the river. Since the river is also the source for drinking, cooking, and wash water, this is problematic. Due to the depth of the canyon, the rafters are geographically isolated and medical care is

unavailable without evacuation by helicopter. Six rafters have been evacuated due to norovirus infections. Normal outbreak control measures are difficult or impossible to apply during an outbreak among river rafters. For example, those who are ill cannot be effectively isolated from those who are well.

Rafters are exposed to river water when it is used as the primary source of drinking water, but also from accidental ingestion, bathing and washing, and sometimes brushing teeth. Water filtration devices most commonly used by the rafters do not remove viruses from water, so chemical treatment of the water after filtering is necessary to ensure the water is safe for drinking. The chemical disinfection step is, however, sometimes omitted by those who object on principle to chemical water treatment or ingestion of chemically treated water. Point of use (POU) devices capable of removing viruses are commercially available (Gerba and Naranjo, 2000) and have been recently shown to remove human noroviruses (Jones, unpublished). The use of these devices would eliminate the need for chemical treatment and increase compliance with water treatment goals, and may help reduce the number of new outbreaks, or lessen the number of those who become ill in the advent of an outbreak.

Testing the river for noroviruses is problematic because the total volume of the river is so high that the virus becomes very dilute and difficult to detect. Using a reach averaged model of diurnal discharge wave propagation down the Colorado River generated by the US Geological Survey (Wiele and Smith, 1996), and monthly stream flow statistics for the Colorado River below Glen Canyon Dam, it can be approximated that on average there are 1.7×10^{11} liters of river water between Lee's Ferry and Diamond

Creek, in July, at any moment in time. This is a large volume of recreational water. At the same time, however, rafter exposure to this water is quite great. The average rafter may consume up to 4 liters of river water per day, and there may be 200 to 500 rafters on the river on any given day. This is an overall exposure of 800 to 2,000 liters of water per day. There are between 10^6 and 10^{11} noroviruses in just one gram of fecal matter (Lund and Lindqvist, 2004; Pang *et al*., 2004). At an average of 10^9 viruses per gram, an inoculum of 500g of norovirus containing stool into $1.7x10^{11}$ liters of water would result in a level of contamination of 3 viruses per liter, assuming even dispersion. Because of the volume of water consumed and the infectiousness of the virus, the level of contamination required to make one person ill on the Colorado River is small. At this time, no human dose-response data or human dose-response model fitting has been published for norovirus. Consequently, extrapolations and microbial risk assessments are difficult or impossible to perform for this virus. It is possible that norovirus is present in the Colorado River at such low levels that it is below the level of detection of current methodology, 3 viruses (PFU) per 1,000 liters (Sobsey and Glass, 1980); however, negative test results from repeated testing of the river do indicate that there is not an ongoing high level of norovirus contamination in the river. Dose-response data is available for many other enteric viruses, and the concentrations of organisms resulting in a 10^{-4} annual risk of infection has been shown to be quite low: 2.2×10^{-7} organisms per liter for rotavirus, 1.5×10^{-5} organisms per liter for polio 1, 6.8 X 10-5 organisms per liter for echovirus 12 (Regli *et al*., 1991).

Recommendations

To reduce the probability of infection among rafters, additional testing of point-of-use water filters that are effective at virus removal should be conducted. These devices would lessen or eliminate the need for chemical treatment of the river water, and could reduce incidence of infection. Continued examination of the epidemiology of norovirus outbreaks among rafters on the Colorado River is needed, because the source of most outbreaks is unknown. Epidemiological investigations may result in information about the source or sources of norovirus, and this information would be useful in prevention or control of future outbreaks. Continued analysis of Glen Canyon Dam Wastewater Treatment Plant, including evaluation of the UV disinfection method used there would be useful to assess if it is adequate to inactivate enteric viruses. The existing technologies are only aimed at meeting bacterial standards, and this may not be enough for inactivation of enteric viruses (Thompson *et al*., 2003). Due to the quantity of river water ingested, the number of rafters simultaneously on the river, and the highly infectious nature of this virus, even low levels of viral contamination could trigger an outbreak on the river. Ethanol solutions of 70% and 90% have been proven effective at killing 99% of feline calicivirus (a norovirus surrogate) within a contact time of just one minute (Malik *et al*, 2006). The use of 70% ethanol based hand-sanitizing gels is advised on rafting trips for use when clean water for hand washing is not available to minimize spread of the virus. Because noroviruses can withstand cleaning with detergents (Barker *et al*., 2004) and because of their potential for survival on fomites (Clay, *et al*., 2006), the disinfection of rafts and equipment between trips is advised. Also, vomitus has been

shown to contain approximately 10^7 viruses per vomiting incident (Lund and Lindqvist, 2004), and it should be considered infectious and disposed of in the same manner as fecal waste.

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Table 1.
Overview of outbreaks of norovirus or suspected norovirus among river rafters on the Colorado River, Arizona: 1994-2005

Year	Duration	Total Cases	Mile 1st Case	Day (approx.)	Suspect. Source	Number of Companies	Number of Trips	Evacuations	Norovirus Lab Conformation
1994	7/29-8/12	108	50	2-4	river water	15	16	0	unconfirmed
1998	7/20-8/1	12	16	1	unknown	1	1	0	PCR positive stool samples
2002	5/29-6/14	>130	<10	1	river water	9	17	0	PCR positive stool samples, river water at Lee's Ferry, and Glen Canyon Dam WWTP water
2003	9/2-10/25	39	16	1	unknown	1	3	4	PCR positive stool samples
2004	5/26-6/9	8	136	4-7	unknown	1	1	0	unconfirmed
2005	8/19-9/23	136	20	2	sliced deli meat	5	13	2	PCR positive stool samples and meat

PCR = polymerase chain reaction
WWTP = wastewater treatment plant

Table 2.
Norovirus reverse transcriptase polymerase chain reaction (RT-PCR) results from sampling of possible sources of norovirus contamination in the Colorado River: July and August, 2003*

Date	Site	Sample Type	Disinfectant Type	pH	Turbidity (NTU)	Norovirus (PCR)
7/18/03	Page, AZ WWTP	effluent	chemical	7.2	2.8	negative
7/18/03	Glen Can. Dam WWTP	effluent	UV only	7.6	4.3	negative
7/18/03	Lee's Ferry	drinking water tap	N/A	7.8	0.7	negative
8/6/03	Wahweap WWTP	effluent	chemical	8.8	N/D	negative

**E. coli*/total coliforms not tested for on these dates
WWTP = wastewater treatment plant
PCR = polymerase chain reaction

Table 3.
Norovirus RT-PCR results from Colorado River water quality testing, May, 2004

Date	Site	River Mile	Liters Filtered*	Norovirus (PCR)	Temp (°C)	pH	Turbidity (NTU)
5/11/2004	Lee's Ferry	0	375.9	negative	10	8.2	1.15
5/12/2004	Vasey's Paradise	31.9	422.9	negative	16	9.0	1.64
5/12/2004	Eminence Break	44.0	378.0	negative	12	8.1	1.40
5/13/2004	Nankoweap Beach	52.2	400.0	negative	11	8.3	1.40
5/13/2004	Bright Angel Creek	87.8	378.0	negative	18	8.8	3.42

* Virosorb™ 1MDS cartridge filters were used for this analysis.
PCR = polymerase chain reaction

Table 4.
Norovirus RT-PCR results from Glen Canyon Dam Wastewater Treatment Plant and Lee's Ferry, July, 2004

Date	Site	Sample Type	Coliforms (MPN/100ml)	*E. coli* (MPN/100ml)	Norovirus (PCR)	Temp (°C)	pH	Turbidity (NTU)
7/29/04	Glen Can. Dam WWTP	aeration tank	>23	>23	N/D	N/D	7.3	416.0
7/29/04	Glen Can. Dam WWTP	secondary clarifier	>23	>23	negative	N/D	7.3	12.2
7/29/04	Glen Can. Dam WWTP	post UV treatment	>23	>23	N/D	N/D	7.6	7.1
7/29/04	Lee's Ferry	Colorado River water	>23	<1.1	negative	N/D	7.9	0.9
7/30/04	Glen Can. Dam WWTP	secondary clarifier	>2,420	>2,420	positive	24	7.5	10.7
7/30/04	Glen Can. Dam WWTP	during UV treatment	>2,420	649	N/D	24	7.5	8.3
7/30/04	Glen Can. DamWWTP	post UV treatment	162	14	N/D	25	7.9	4.4

WWTP = wastewater treatment plant
N/D = test not done

Table 5.
Colorado River water quality monitoring 1995-1998: *E.coli* MPN/100ml by river mile and sampling month (early vs. late)

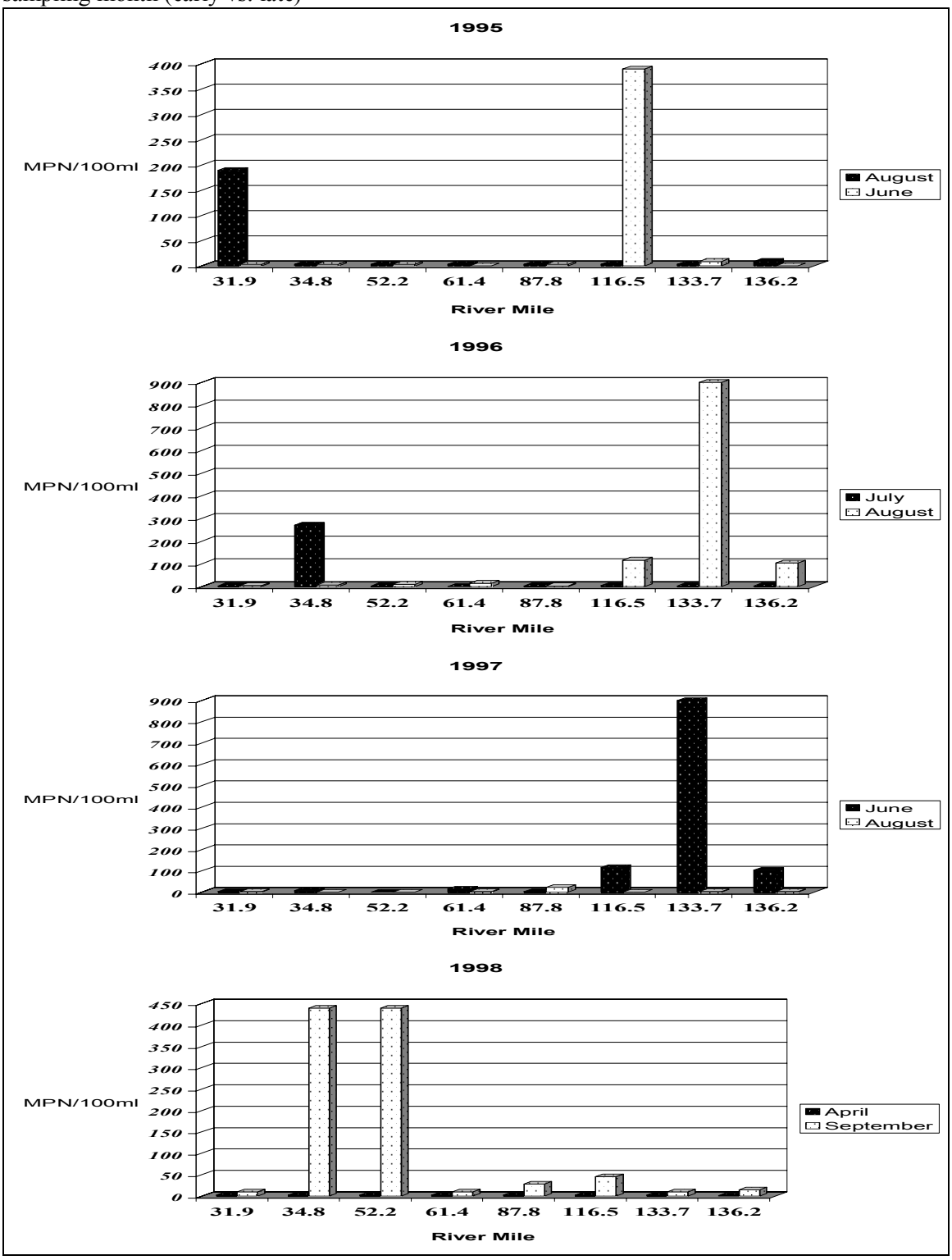

Table 6.
Conventional names and corresponding Colorado River mile for sampling sites

Name	River Mile
Lee's Ferry	0
Vasey's Paradise	31.9
Nautiloid Canyon	34.8
Nankoweap Camp	52.2
Conf. Little Colo. River	61.4
Bright Angel Creek	87.8
Elves Chasm	116.5
Tapeats Creek	133.7
Deer Creek	136.2