Science of the Total Environment 472 (2014) 757–766

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Occurrence of human enteric viruses at freshwater beaches during swimming season and its link to water inflow

Chang Soo Lee [a,1,2], Cheonghoon Lee [a,2], Jason Marion [a,3], Qiuhong Wang [b], Linda Saif [b], Jiyoung Lee [a,c,]*

[a] College of Public Health, Division of Environmental Health Sciences, The Ohio State University, Columbus, OH 43210, USA
[b] Food Animal Health Research Program, Ohio Agricultural Research and Development Center, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH 44691, USA
[c] Department of Food Sciences & Technology, The Ohio State University, Columbus, OH 43210, USA

## HIGHLIGHTS

- Human adenovirus, enterovirus and norovirus were monitored using qPCR assays at freshwater beaches during the swimming season.
- Human adenovirus (40%) and enterovirus (17%) were detected, but norovirus was not detected.
- Enteric virus densities exhibited no relationships with densities of fecal indicators or culture-independent genetic markers.
- Densities of human enterovirus were correlated with water inflow rates into reservoirs of freshwater beaches.

## ARTICLE INFO

*Article history:*
Received 15 July 2013
Received in revised form 9 November 2013
Accepted 18 November 2013
Available online xxxx

*Keywords:*
Freshwater beach
Adenovirus
Enterovirus
Norovirus
Fecal indicators
Water quality

## ABSTRACT

Human enteric viruses are significant etiological agents for many recreational waterborne illnesses. The occurrence and density of human enteric viruses such as human adenovirus (HAdV), human enterovirus (HEnV), and human norovirus genogroups I/II (HNoV GI/GII) were investigated using quantitative real-time PCR (qPCR) at freshwater beaches along with monitoring fecal indicators and environmental parameters. During the 2009 swimming season, water samples were collected from three inland freshwater beaches in Ohio, USA. Of the total samples, 40% (26/65) and 17% (11/65) were positive for HAdV and HEnV respectively, but HNoV GI/GII were not detected. There was no significant association among the detected human enteric viruses (HAdV and HEnV) and fecal bacteria indicators (*Escherichia coli* and *Bacteroides*) by Spearman correlation and principal component analyses. Logistic regression analysis also revealed that the odds of finding HAdV or HEnV was not influenced by levels of fecal bacteria indicators. However, there was a 14-fold increase in the odds of HEnV detection for each 1-log increase in daily water inflow ($m^3/s$) into freshwater beach reservoirs (adjusted odds ratio = 14.2; 95% confidence interval = 1.19–171). In summary, the viral occurrence at the freshwater beaches was not readily explained by the levels of fecal bacteria indicators, but appeared to be more related to water reservoir inflows. These results suggest that hydrological data must be considered in future epidemiology efforts aimed at characterizing beach water safety.

## 1. Introduction

The number of recreational waterborne disease outbreaks in the United States (US) has increased substantially over the past thirty years according to reports from state health departments and the nationwide Waterborne Disease and Outbreak Surveillance System reports (Yoder et al., 2008). Viral agents were identified in the etiology of 7% of the outbreaks (Craun et al., 2005) and were also suspected in 29% of the outbreaks caused by unknown etiological agents (Dziuban et al., 2006; Yoder et al., 2004). These unidentified outbreaks share similar disease patterns as those caused by human enteric viruses (Sinclair et al., 2009; Yeh et al., 2009). Furthermore, among all viral outbreaks in recreational water, 50% occurred in fresh waters (Craun et al., 2005), including freshwater beaches that provide important recreational services in the US. For example, the Ohio Department of Natural Resources estimated that about 5 million visitors were engaged in swimming or water skiing in Ohio's state park waters in 2009 (Ohio Department of Natural Resources, 2010).

In monitoring these recreational freshwater beaches, fecal indicators such as *Escherichia coli* have been used (U.S. Environmental Protection Agency, 1986), since the epidemiology studies of the 1980s demonstrated

---

[*] Corresponding author at: College of Public Health, Division of Environmental Health Sciences, The Ohio State University, Columbus, OH 43210, USA. Tel.: +1 614 292 5546; fax: +1 614 293 7710.
*E-mail address:* jlee@cph.osu.edu (J. Lee).
[1] Present address: Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea.
[2] Contributed equally to this work.
[3] Present address: Department of Environmental Health Science, Eastern Kentucky University, USA.

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.11.088

significant positive associations between the incidence of recreational waterborne illnesses among freshwater swimmers and culturable *E. coli* and enterococci densities (Dufour, 1984). With the recent advent of polymerase chain reaction (PCR) techniques, quantitative real-time PCR (qPCR) assays targeting multiple host-specific genetic markers have become more accessible thereby enabling rapid quantification of fecal contamination levels, identification of likely fecal contamination sources, and enhancing prediction of gastrointestinal illness in environments (Simpson et al., 2002; Wade et al., 2008). For example, fecal bacterial genetic markers such as gyrB (Lee and Lee, 2010), g-Bfra (Matsuki et al., 2002), and tetQ (Nikolich et al., 1994) are regarded as useful alternative genetic indicators for fecal contamination due to their abundance in feces or human/animal-impacted waters (Converse et al., 2009; Layton et al., 2006) as their higher die-off rates in the environment enable improved explanation of more recent fecal contamination episodes than *E. coli* or enterococci (Converse et al., 2009). This toolbox approach is gaining significance within the microbial source tracking community because it integrates multiple markers to assess water quality and contamination sources (McQuaig et al., 2012; Noble et al., 2003). However, at beaches with diffuse nonpoint source fecal contamination, these bacterial markers and culture-based measures of fecal indicator bacteria were relatively ineffective for predicting human illness (Colford et al., 2007). It is noteworthy that the likely source of fecal contamination in these Mission Bay, California beach studies (Colford et al., 2007) was avian (Gruber et al., 2005) and few, if any studies, have evaluated recreational water illnesses and rapid measures of water quality (qPCR) in freshwater environments with non-point source contamination dominated by human inputs.

Human enteric viruses can be present in water regardless of fecal indicator bacteria densities, thereby presenting a significant limitation for predicting virus-associated waterborne disease risks by currently used fecal indicators (Contreras-Coll et al., 2002; Hauri et al., 2005; Wong et al., 2009). Human enteric viruses not only cause asymptomatic or mild infections in humans, but can be the origin of more serious diseases or outcomes, particularly in vulnerable populations such as young children (Wong et al., 2009). Of the enteric viruses, human adenovirus (HAdV), enterovirus (HEnV), and norovirus (HNoV) are the significant etiologic agents of conjunctivitis, pharyngitis, and gastroenteritis among recreational water users (Sinclair et al., 2009). It is now understood that HAdV is composed of many serotypes classified into 7 subgenera (Jones et al., 2007), which have been recognized as important etiologic agents responsible for respiratory illnesses, conjunctivitis and gastroenteritis (Jiang, 2006). HEnV is also a significant viral agent of concern for beach users as the temporal pattern of HEnV outbreaks corresponds with the US swimming season (summer through early autumn) (Fong and Lipp, 2005). Among the five genogroups of norovirus, genotypes I and II (HNoV GI and GII) were focused upon in this study because they have been reported as the most frequently detected viral types in water (Wyn-Jones et al., 2010) and are the most common causes of outbreaks for nonbacterial gastroenteritis (Noel et al., 1999; Verhoef et al., 2010).

The surveillance reports (Dziuban et al., 2006; Yoder et al., 2004) and epidemiological evidence (Jiang, 2006; Sinclair et al., 2009) suggest that human enteric viruses can threaten public health after exposure to contaminated beach water. Despite this evidence, little is known regarding the contamination of human enteric viruses in freshwater beach environments and their correlations with other biological and environmental factors. In this study, qPCR-based molecular assays were used to determine the occurrence and densities of human enteric viruses (HAdV, HEnV, and HNoV GI/GII) and fecal bacteria genetic markers (gyrB, g-Bfra, and tetQ) in freshwater beaches in the state of Ohio (United States). Using the results of multiple viral and bacterial genetic markers, statistical associations were evaluated between viral, bacterial, and environmental parameters, ultimately providing useful information for managing microbial water quality in recreational freshwater environments.

## 2. Materials and methods

### 2.1. Sampling sites

This study was conducted at three public freshwater beaches in Ohio. East Fork Lake (39° 01′ 98″ N; 84° 14′ 32″ W) is a human-made 8.7 km$^2$ flood-control reservoir with a maximum depth of 33.4 m (Ohio Department of Natural Resources, 2003) impacted primarily by non-point pollution sources, with dominant watershed land uses being row crop agriculture (37%), light urban/residential (33%), and forestland (25%) (Marion et al., 2010). Delaware Lake (40° 37′ 15″ N; 83° 05′ 87″ W) is a human-made 3.9 km$^2$ reservoir with a maximum depth of 9.1 m constructed by the U.S. Army Corps of Engineers for flood control, water supply, recreation, and fish and wildlife management (Ohio Department of Natural Resources, 2008). Predominant land uses near the 3.9 km$^2$ Delaware Lake are cropland (66%), livestock pastures (15%), forestland (15%), and low/high density residential (2%) (Olentangy Watershed Alliance, 2007). Madison Lake (39° 86′ 98″ N; 83° 37′ 44″ W) is a 0.4 km$^2$ shallow lake with a maximum depth of 1.8 m in the Darby Plains of Madison County, in which the lake represents the majority of the county water acreage (1.2 km$^2$) (Ohio Department of Natural Resources, 2010). Agriculture is the major land use near Madison Lake, comprising 91% of Madison County, which largely includes the Madison Lake watershed area (Extension OSU, 2007).

### 2.2. Sample collection

A total of 65 water samples were collected two times per week from the three lakes during June 2009 through August 2009. All water samples (>2 L each) were collected during the daytime and were typically gathered in the afternoon between 12:00 and 18:00 (57%, 37/65). Samples were taken 0.3 m below the surface and at a depth of 1 m near the center of each lake using autoclaved 500 mL bottles (Nalgene, Rochester, NY). The number of people using the beach was approximately 200 at East Fork Lake during the sampling period; the number was based on a daily census of beach users at three time intervals (early afternoon, mid-afternoon, and late afternoon) the day of sampling. The samples at East Fork were collected on weekends; whereas the samples from Delaware and Madison lakes were collected during the week when relatively fewer beach users were observed. After collection, the samples were transported on ice to the laboratory.

### 2.3. Environmental parameters

Temperature, pH, and specific conductivity, and dissolved oxygen levels were measured at the time of sampling using a YSI 600XL multiprobe data sonde (Yellow Springs Instruments, Yellow Springs, OH). Hydrological data (reservoir inflow and precipitation) were obtained from the National Weather Service (http://www.erh.noaa.gov/iln/lcdpage.htm) and the Louisville District of the U.S. Army Corps of Engineers (http://www.lrl.usace.army.mil/wcds/dlbrpt.htm). Archived records of daily precipitation and reservoir inflow for the three lakes were extracted from these databases; however, inflow data for Madison Lake was not available as there is no flood control-related infrastructure at this reservoir. Precipitation and reservoir inflow data hyphenated with ‘0’, ‘24’, and ‘48’ were defined based on the records from the sampling day, a day before the sampling day, and the sum totals for precipitation or reservoir inflow for the accumulative past 2 days.

### 2.4. E. coli plate count

*E. coli* densities were determined within 6 h after transportation to the laboratory using EPA Method 1603 in triplicate (U.S. Environmental Protection Agency, 2002).