Science of the Total Environment 468–469 (2014) 757–763

Contents lists available at ScienceDirect

Science of the Total Environment

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

Effects of rainfall events on the occurrence and detection efficiency of viruses in river water impacted by combined sewer overflows

Akihiko Hata [a,*], Hiroyuki Katayama [a], Keisuke Kojima [a], Shoichi Sano [a], Ikuro Kasuga [a], Masaaki Kitajima [b], Hiroaki Furumai [c]

[a] Department of Urban Engineering, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
[b] Department of Soil, Water and Environmental Science, The University of Arizona, 1117 E. Lowell St., Tucson, AZ 85721, USA
[c] Research Center for Water Environment Technology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

HIGHLIGHTS

- Concentration of viruses in river water was higher during rainfall-affected periods.
- SS level was negatively correlated to recovery of virus concentration.
- Efficiencies of PCR and nucleic acid extraction were low for dry weather samples.
- Water quality parameters did not correlate to either PCR or extraction efficiency.
- Aichi virus was highly abundant in the water samples.

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form 27 August 2013
Accepted 27 August 2013
Available online 23 September 2013

Editor: Damia Barcelo

Keywords:
Enteric viruses
Rainfall event
Suspended solid
Organic indicator
Virus detection efficiency

ABSTRACT

Rainfall events can introduce large amount of microbial contaminants including human enteric viruses into surface water by intermittent discharges from combined sewer overflows (CSOs). The present study aimed to investigate the effect of rainfall events on viral loads in surface waters impacted by CSO and the reliability of molecular methods for detection of enteric viruses. The reliability of virus detection in the samples was assessed by using process controls for virus concentration, nucleic acid extraction and reverse transcription (RT)-quantitative PCR (qPCR) steps, which allowed accurate estimation of virus detection efficiencies. Recovery efficiencies of poliovirus in river water samples collected during rainfall events (<10%) were lower than those during dry weather conditions (>10%). The $\log_{10}$-transformed virus concentration efficiency was negatively correlated with suspended solid concentration ($r^2 = 0.86$) that increased significantly during rainfall events. Efficiencies of DNA extraction and qPCR steps determined with adenovirus type 5 and a primer sharing control, respectively, were lower in dry weather. However, no clear relationship was observed between organic water quality parameters and efficiencies of these two steps. Observed concentrations of indigenous enteric adenoviruses, GII-noroviruses, enteroviruses, and Aichi viruses increased during rainfall events even though the virus concentration efficiency was presumed to be lower than in dry weather. The present study highlights the importance of using appropriate process controls to evaluate accurately the concentration of water borne enteric viruses in natural waters impacted by wastewater discharge, stormwater, and CSOs.

1. Introduction

Rainfall events generate combined sewer overflows (CSOs) that introduce multiple sewage-borne contaminants such as microbial pathogens, suspended solids (SS), organic contaminants, chemical compounds and heavy metals into aquatic environments (Baun et al., 2006; Rajal et al., 2007; Gasperi et al., 2008). Compared to most chemicals that cause the long-term health effects, the temporal increase of microbial pathogens in natural waters is a major concern due to their acute health outcomes in human upon each exposure event. Previous epidemiological investigation and quantitative microbial risk

Abbreviations: Ad5, adenovirus type 5; AiV, Aichi virus; BGM, buffalo green monkey; CSO, combined sewer overflow; DOC, dissolved organic carbon; EAdV, enteric adenovirus; EV, enterovirus; F-phage, F-specific coliphage; MNV, Murine norovirus; NoV, norovirus; PSC, primer sharing control; PV, poliovirus; qPCR, quantitative PCR; RT, reverse transcription; SS, suspended solids; SUVA, specific UV absorbance; TC, total coliforms; TOC, total organic carbon; UV_{254}, 254 nm ultraviolet.

* Corresponding author. Tel.: +81 3 5841 6242; fax: +81 3 5841 6244.
E-mail address: hata@env.t.u-tokyo.ac.jp (A. Hata).

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http://dx.doi.org/10.1016/j.scitotenv.2013.08.093

assessments have demonstrated the impact of rainfall events on the incidence of waterborne infectious diseases (Le Guyader et al., 2006; Ashbolt et al., 2010). Since enteric viruses are highly stable in aquatic environments, they can be transported over long distances with stormwater runoff after rainfall events. To understand the actual impact of rainfall events on viral loads in aquatic environments, it is important to quantify viruses in natural waters, stormwater, and CSOs in an appropriate manner.

Several pathogenic viruses can be found in sewage-impacted aquatic environments. Among them, caliciviruses (noroviruses and sapoviruses) are the leading cause of viral gastroenteritis (Clark and McKendrick, 2004). Some other viruses such as adenoviruses and enteroviruses (EVs) are known to be predominant, suggesting that detection of them from water is useful to monitor the presence of viral contamination (Wong et al., 2012). More recently, emerging viruses such as Aichi viruses (AiVs) and torque teno viruses have been detected in wastewater and river water in Japan (Haramoto et al., 2005, 2008; Kitajima et al., 2011), however, there is little information available on the distribution and prevalence of these viruses in aquatic environments. Therefore, it is important to investigate the occurrence and behavior of these viruses in environmental waters.

Reverse transcription (RT)-PCR after virus concentration and nucleic acid extraction is widely used for detection of viruses in environmental water samples. Each detection step has been optimized for relatively clean water samples; however, the reliability of each step can be greatly affected by the complexity of water components that increase during rainfall events such as turbidity, SS, and various organic compounds (Haramoto et al., 2007; Karim et al., 2009; Victoria et al., 2009; Fong et al., 2010; Sima et al., 2011). SS in the original water samples reduce the virus recovery efficiency during the virus concentration (adsorption–elution) procedure (Sobsey and Glass, 1984; Guttman-Bass and Catalano-Sherman, 1985; Rajal et al., 2007; Kuo et al., 2010) while organic compounds (i.e., humic acids) are known to interfere with molecular detection processes including nucleic acid extraction and PCR amplification (Hamza et al., 2009; Hata et al., 2011). To overcome these problems, pre-filtration of the sample before primary concentration (i.e., membrane filtration) and the addition of RT-PCR inhibition suppressors such as bovine serum albumin and T4 gene 32 protein have been applied (Kreader, 1996; Gregory et al., 2006). However, the quantitative relationship between water quality and virus detection efficiency has not been well understood, and therefore, the optimal condition for the virus detection process remains unknown. Consequently, the increase in the concentration of human pathogenic viruses after rainfall events has not been evaluated consistently due to inaccuracies associated with the interferences. In order to avoid underestimation of virus occurrence, it is important to assess the reliability of each detection step by including process controls especially for highly polluted environmental waters (Lambertini et al., 2008; Honjo et al., 2010; Zakhour et al., 2010; Hata et al., 2011; Sima et al., 2011; Gibson and Schwab, 2011).

In the present study, we evaluated the effect of rainfall on water quality and how the water quality affects the reliability of each virus detection step from primary concentration to quantitative PCR (qPCR) detection. The water matrices included in this study consisted of river water samples collected during rainfall events and dry weather conditions. The river receives wastewater discharges and is also impacted by stormwater and CSOs during rainfall events.

2. Materials and methods

2.1. Viruses

Poliovirus type 1 (PV, LSc 2ab Sabin strain), human adenovirus type 5 (Ad5) and murine norovirus (MNV, S7-PP3 strain) were used as process controls for virus concentration, DNA extraction and RNA extraction, respectively (Table 1). PV was propagated in buffalo green monkey (BGM) kidney cells. Ad5 and MNV were kindly provided by Dr. M. Ito (Kyoto City Institute of Health and Environmental Sciences, Kyoto, Japan) and Dr. Y. Tohya (Nihon University, Kanagawa, Japan), respectively. These viruses were propagated in HEp-2 (ATCC CCL-23) and RAW 264.7 (ATCC TIB-71) cell lines (American Type Culture Collection, Manassas, VA), respectively.

Table 1
Process controls employed in this study.

Process controls	Step for efficiency monitoring of
PV	Virus concentration
Ad5	DNA extraction
MNV	RNA extraction
PSC-DNA	qPCR for EAdVs
PSC-RNA	RT-qPCR for GI-, GII-NoVs, AiVs

2.2. Collection of water samples

River water samples were collected from the Kanda River (24.6 km total length and 105 km^2 catchment area with 1.7 million habitants in the catchment basin), which is a tidal influenced river that flows through Tokyo metropolitan area, Japan. Approximately 90% of the river flow originates from treated wastewater effluent during dry weather while stormwater runoff and associated CSOs dominate after heavy rainfall events. A total of 29 grab samples (3.6 L each) were collected at low tide every 24 h between September 18 and October 17, 2009 at a station located downstream of the wastewater discharge sites. Samples were taken using a plastic bucket (5 L), dispensed into two of pre-sterilized polyethylene containers (1.8 L) and kept under refrigeration (<10 °C) for up to 24 h prior to water quality analysis and virus concentration.

During the study period, heavy rainfall events occurred around the study area from 29th September to 8th October and on 14th October (total precipitation of 281 mm and 16 mm, respectively). In this study, "rainfall-affected sampling periods" were defined as the day when the rainfall event occurred and one day after precipitation, according to a previous study (Richardson et al., 2009). The samples that were collected during rainfall-affected periods were classified as group W (wet weather) samples and the rest of the samples were classified as group D (dry weather) samples. Among 29 samples collected, 13 and 16 samples were classified as groups W and D, respectively.

2.3. Concentration of river water samples

Viruses in river water samples were concentrated by an adsorption–elution method using an electronegative filter (Katayama et al., 2002). Briefly, 2.5 M $MgCl_2$ was added to the sample to obtain a final concentration of 25 mM. Then, 1000 mL of the sample was passed through an HA electronegative filter (0.45 μm pore size, 90 mm diameter, Millipore, Tokyo, Japan). Subsequently, the filter was rinsed with 200 mL of 0.5 mM H_2SO_4 (pH 3.0) followed by elution of the viruses from the membrane filter using 10 mL of 1.0 mM NaOH (pH 10.8). The filtrate was recovered as a primary concentrate in a tube containing 50 μL of 100 mM H_2SO_4 and 100 μL of 100× Tris–EDTA Buffer (Wako, Osaka, Japan). The concentrate was subjected to a further concentration using the Centriprep YM-50 (Millipore) to obtain approximately 650 μL of final concentrate.

In order to evaluate virus concentration efficiency, 2 μL of PV (6.7×10^4 PFU and 4.4×10^7 copies determined by plaque assay and RT-qPCR, respectively) was spiked into the 1000 mL of sample before the concentration (Table 1). The spiked PV in the concentrate after adsorption–elution method was evaluated by RT-qPCR and a plaque assay using BGM cells (Haramoto et al., 2007).