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Effective concentration, recovery, and detection of infectious adenoviruses from environmental waters

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A B S T R A C T

Current application of fecal indicator bacteria (FIB) as an indicator of sewage contamination has its limitation and other alternative indicator systems are needed for enhanced monitoring of recreational water quality. To explore the potential use of human enteric viruses (HEV) as a potential indicator, human adenovirus type 7 (HAdV-7) was tested in this study as a model virus. Through spiked HAdV-7 into the sample seawater, different procedures for viral concentration and elution were comparatively analyzed in a side-by-side fashion. Nearly 80% infectious viruses were recovered from the spiked seawater with the use of a magnetic stirring method and 1.0 mM NaOH as elution buffer. A viral plaque assay was established with optimized conditions for the detection and quantification of infectious HAdV and used to determine the stability of HAdV-7 in seawater and a possible correlation for HAdV detection between the viral infectivity assay and PCR. Findings from this study suggest it is possible to concentrate and recover infectious HAdV from environmental waters effectively with optimized laboratory conditions, which warrants the future test and establishment of using HEV as an alternative indicator of water quality.

1. Introduction

Fecal contamination is the main cause of waterborne illness, and is of public health concern due to the widespread negative health impact. The number of viral disease outbreaks from recreational waters has increased over recent years (Jofre and Blanch, 2010), which indicates the need of improved monitoring of water quality regularly. In the United States, fecal indicator bacteria (FIB) such as *Escherichia coli* and enterococci are currently being used to monitor water quality, as established and enforced by the EPA since 1986 (Dufour and Ballentine, 1986; Turbow et al., 2003; Choi and Jiang, 2005). There are many benefits of using the FIB as indicators including lower costs, simple and rapid procedures, and standardization. However there are still some limitations that deem this system insufficient. There are sources other than human feces that give rise to FIB, and the presence of FIB does not necessarily correlate with the presence of human pathogens particularly enteric viruses (Lees, 2000; Noble and Fuhrman, 2001; Boehm et al., 2003). It has been frequently documented the outbreaks of viral-related illnesses among waters that were free of FIB or met necessary water quality criteria (Bosch et al., 1998; Papapetropoulou and Vantarakis, 1998; Hauri et al., 2005; Sinclair et al., 2009; Okoh et al., 2010). Thus, water quality criteria rely on FIB for assessments might not reflect true health risk.

Human enteric viruses are currently being tested as alternative indicators for enhanced water quality monitoring, and the European Union regulation has already listed enteroviruses as a parameter for governing water quality (Jiang et al., 2001; Fong and Lipp, 2005; Center and Warrenton, 2007; Tong et al., 2011). As a major causal agent of waterborne diseases, human enteric viral pathogens are able to remain infectious for long periods of time with low doses while being transmitted between hosts under various environments, including sewage treatment plant discharge, ground water, and infected persons (Fong and Lipp, 2005). Because of the specific host range, HEV do not multiply in aquatic environments after being shed from the affected host.

Previous studies have suggested HAdV to be a potential indicator pathogen since it is one of the most common enteroviruses in the environment that can often cause waterborne diseases (Jiang and Chu, 2004; Walters and Field, 2009; Soller et al., 2010). Also HAdV is the only enteric virus with a double-stranded DNA genome and thus they are relatively more stable and persistent in aquatic environments compared to most enteric viruses possessing an RNA genome (Pina et al., 1998; Thurston-Enriquez et al., 2003; Fong and

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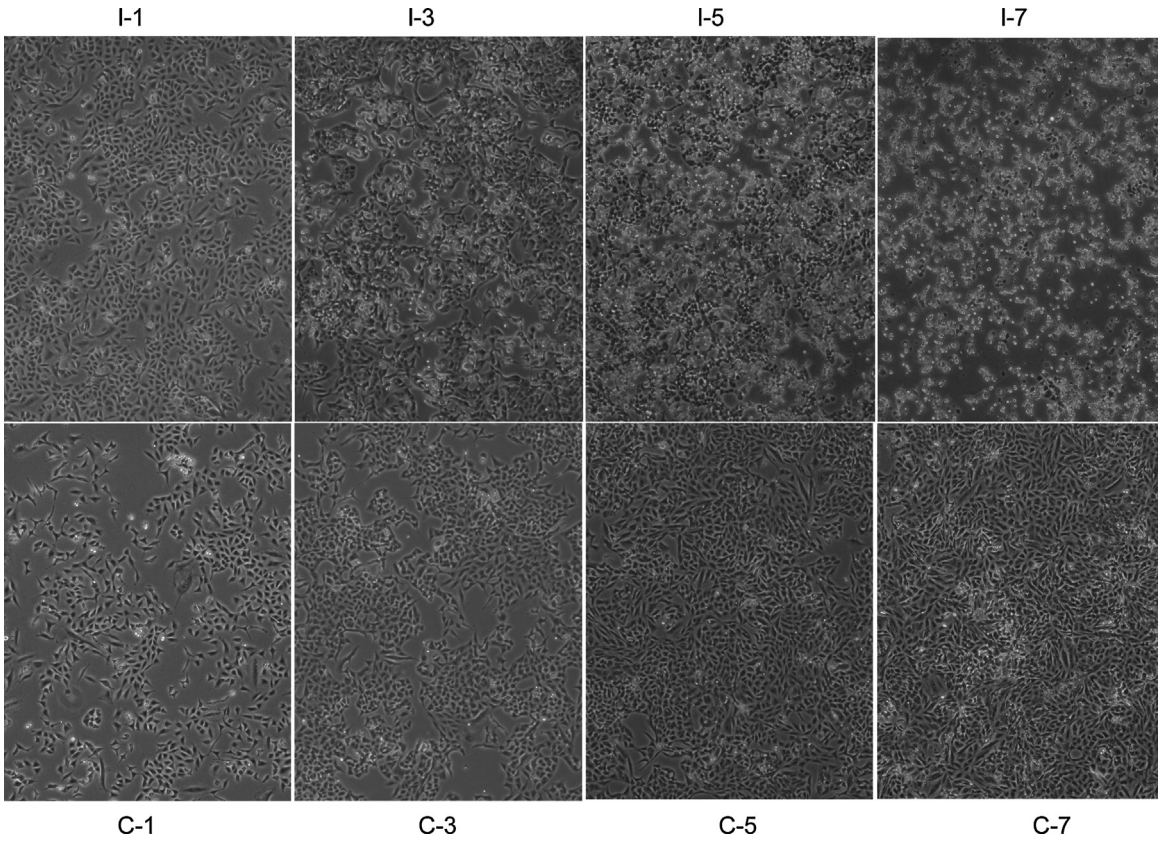

Fig. 1. Photomicrographs of HAdV infection of A549 cells seeded in TC −25 cm^2 flasks. I-1, I-3, I-5, and I-7 showing HAdV-7 infected cultures at days 1, 3, 5, and 7 post infection respectively; and C-1, C-3, C-5, and C-7 showing uninfected cultures at days 1, 3, 5, and 7 as controls. Viral induced CPE became visible in I-3, more extensive in I-5, and affected entire flask in I-7. Original magnification at 100×.

Lipp, 2005). HAdV has also been previously found in polluted waters and implicated in some disease outbreaks associated with swimming pools and other recreational water exposures (Hlavsa et al., 2011). Aslan et al. (2011) has recently shown that there is a strong association between HAdV and the sources of human fecal pollution in the Great Lakes and Chicago areas of the United States.

To explore the potential use of HAdV as a possible indicator of water quality, our laboratory has recently developed a sensitive molecular method for effective detection of HAdV from environmental waters (Tong and Lu, 2011). However, since such detection method do not reflect the presence or absence of infectious viruses, and also because little is known regarding effective concentration and recovery of infectious HAdV from environmental waters, there is an urgent need to develop effective methods for enhanced recovery and detection of infectious HEV from environmental waters. To meet the present need for the potential use of HAdV as an alternative indicator, this study aims to test and develop optimized laboratory conditions for the effective concentration and recovery of infectious human adenoviruses from environmental waters.

2. Methods

2.1. Cell line

A549 cell line derived from Human lung carcinoma (adenocarcinomic alveolar basal epithelial) was obtained from ATCC (ATCC, Manassas, VA, Cat No. CCL-xxx) and employed for propagation, detection, and isolation of adenovirus in this study. A549 cells were cultured and maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 4.5 g/L glucose, L-glutamine, and sodium pyruvate and mixed with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and incubated at 37 °C with 5% CO_2. All cell culture tests were conducted inside a cell culture biosafety hood (SterilGARD III Advance and The Baker Company, Sanford, ME) equipped with a UV sterilization lamp for sterilization.

2.2. Human adenovirus

A laboratory strain of human adenovirus type 7 (HAdV-7) available in this laboratory was employed as a model of human enteric viruses (HEV) to test and establish optimized laboratory conditions in this study. All the viral isolation, propagation, and quantification were performed in a cell culture biosafety hood (Labconco Purifier Class II Biosafety Cabinet Delta Series, Labconco, MO).

Adenovirus stocks used in this study were prepared by infecting A549 cells. In brief, A549 cells at their exponential growth phase were harvested using a trypsin-EDTA solution and individual cell suspensions were seeded into a TC-75 cm^2 flask. After a 24-h incubation, cell monolayers formed in the flask were infected with 1 mL of adenovirus at multiplicity of infection (MOI) of 0.1. Infected cultures were incubated at 37 °C and then transferred into an −80 °C freezer when 90% of the cells showed cytopathic effects (CPE) at post infection days 8–10. The flask was removed from the freezer and completely thawed at room temperature (23 ± 2 °C), then stored in −80 °C freezer again. After 2 more freeze–thaw cycles, the culture medium was collected from the flask and cell debris was removed through centrifugation at 3000 rpm (Beckman) for 5 min. Recovered supernatant was transferred into 1.5-mL centrifuge tubes at 0.5 mL/vial, and aliquots of virus were stored in −80 °C and used as viral stock for this study. The infectious titer of the virus stock was determined by a viral plaque assay after the virus was stored for 48 h at −80 °C.