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Field evaluation of an improved cell line for the detection of human adenoviruses in environmental samples

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A B S T R A C T

Human enteric adenoviruses (HAdVs) are commonly detected in waters contaminated with human fecal material and persistent in the environment. Detecting infectious enteric HAdVs is limited by the difficulty of growing them in cell cultures. Recently, an improved cell line (293 CMV) has been described, which enhanced the propagation of enteric HAdVs (Kim et al., 2010. Appl. Environ. Microbiol. 76, 2509). The present study evaluated the transactivated 293 CMV cell line for detecting enteric HAdVs from field samples, which is an important step in demonstrating the usefulness of the improved cell line for water monitoring programs. Field samples consisted of the following: concentrated sewage samples (from 1 L) collected from three different wastewater treatment plants (WWTPs) and concentrated raw source water samples (from 20 L) collected from six water treatment plants (WTPs). Infectious HAdVs were detected using a combined cell culture/mRNA RT-PCR assay. Concentrated samples were assayed, in parallel, using the standard (STD) G293 and 293 CMV cell lines. Viral replication was determined by measuring viral mRNA and viral DNA levels during infection. Infectious HAdVs were successfully detected from environmental samples using the new transactivated and standard cell lines. Infectivity assays of concentrated sewage samples demonstrated higher viral mRNA expression ($p = 0.02$) and viral DNA concentrations ($p = 0.02$) in the transactivated 293 CMV than in the G293 cell line. Although not statistically significant, infectious HAdVs were detected in more raw water samples using the 293 CMV cells (8 of 18) than in the STD G293 cells (4 of 18). However, when results of the source water samples were pooled, the number of flasks positive using the 293 CMV cells was significantly greater than those using the G293 cells ($p = 0.01$). Overall, the results of the present study demonstrate the effectiveness of the new transactivated 293 CMV cell line for improved propagation and detection of HAdVs from environmental samples.

1. Introduction

Human adenoviruses (HAdVs) are non-enveloped, linear dsDNA viruses of the *Adenoviridae* family. Adenoviruses are widely present in ambient waters, persist over long periods of time in the water environment (Enriquez et al., 1995; He and Jiang, 2005), and are extremely resistant to inactivation by UV (Ko et al., 2005; Thurston-Enriquez et al., 2003). HAdV abundance and persistence presents challenges for disinfection by water treatment plants (WTPs) and they have been detected consistently in disinfected sewage (Rodriguez et al., 2008; Sedmak et al., 2005; Simmons et al., 2011). HAdVs have also been detected frequently in drinking water, surface water, groundwater, and recreational waters (Aslan et al., 2011; Bofill-Mas et al., 2010; Keswick et al., 1984; van Heerden et al., 2005; Wyn-Jones et al., 2011). After rotaviruses, enteric HAdV (40 and 41) are the leading causes of childhood diarrhea and most abundant HAdV species detected in surface waters and sewage (Mena and Gerba, 2009). For the above reasons, HAdVs are one of the microbiological agents included in the United States Environmental Protection Agency's drinking water contaminant candidate list (CCL) (Unites States Environmental Protection Agency, 2010). Considering the public health impact and ongoing concern, it is important to develop methods that detect and quantify infectious HAdVs found in environmental samples, such as sewage and water, which pose health risks from waterborne exposures.

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The detection of HAdVs from environmental samples is usually performed by PCR or cell culture methods (Jiang, 2006). Although direct PCR is a rapid and robust approach for virus detection, the infectivity of detected viruses is uncertain and difficult to establish from PCR results (Richards, 1999). Detecting enteric adenoviruses by cell culture infectivity is usually problematic due to the inconsistent onset of viral induced cytopathic effect (CPE). Furthermore, detection using the PLC/PRF/5 cell line could take 10 to 20 days and several cell passages may be required before viral presence becomes apparent (Thurston-Enriquez et al., 2003). The combined use of cell culture with PCR has been used for detecting adenovirus. This combined method can be used for detecting the presence of viral genome (CC–PCR) or for detecting viral mRNA (CC Reverse transcription PCR, Ko et al., 2003). Recently, a stable cell line was developed to promote the propagation of enteric HAdVs. This cell line consistently expressed high levels of viral transactivating IE1 protein of cytomegalovirus (CMV) (Kim et al., 2010). The CMV viral transactivated protein can activate and stimulate viral genes and transcription factors from enteric HAdVs, which aid to increase viral mRNA levels and promote propagation of these fastidious human adenoviruses in cell culture (Kim et al., 2010). This cell line has only been tested with laboratory reference strains and clinical isolates, and its utility in detecting HAdVs from environmental samples has not yet been determined.

The goal of this study was to test the feasibility of using the improved cell line (293 CMV) for detecting enteric HAdVs, using environmental sewage and water samples collected from different surface water and wastewater sources. Detection of enteric adenovirus was compared between the standard (G293) and the improved transactivated (293 CMV) cell lines. For this study, the replication of HAdV in the cell line was determined by measuring the production of viral mRNA and determining the levels of viral DNA; therefore, the results are representative of what could be expected when using this improved cell line for either a cell culture–qPCR or a cell culture mRNA RT qPCR approach. To our knowledge, this is the first report using this improved cell line for detecting infectious adenovirus from environmental water samples.

2. Materials and methods

2.1. Adenovirus type 41 stock

The adenovirus type 41 stock (ATCC, VR-930) was propagated using the G293 host cells in 150 cm^2 tissue culture flasks and purified by PEG as described by Rodríguez et al. (2013). The viral titer was determined using the mRNA qRT-PCR method described by Rodríguez et al. (2013).

2.2. Sewage samples

Sewage samples were collected from three wastewater treatment plants (WWTP) in North Carolina. One liter was collected and concentrated, using organic flocculation, and polyethylene glycol precipitation (PEG) to a final volume of 5 mL as described by Rodríguez et al. (2013). To kill any bacteria and remove soluble impurities, 5 mL of chloroform was added to the sample and mixed to emulsify by inverting the tubes vertically for one minute. The samples were centrifuged (3000 × *g*, 20 min, 4 °C). The supernatant was recovered, without disturbing the thin film layer above the chloroform, yielding a final sample volume of 5 mL. The supernatant was stored at −80 °C and later assayed for viruses.

2.3. Source water sample collection and concentration

Samples were collected from six geographically distinct water treatment plants, within the United States, during three different sample times (September 2009, November 2009, and February 2010) per plant, yielding 18 samples. Each facility collected 24 L of raw source water. Samples were stored overnight at 4 °C before shipping, by next day air express, to our laboratory. The temperature of the samples was recorded using a data logger system and never surpassed 7 °C. Upon arrival at the UNC laboratory, 20 L samples were concentrated using hollow fiber ultrafiltration (HFUF), polyethylene glycol precipitation (PEG), and ultracentrifugation procedures in succession to a final volume of approximately 5 mL. The concentrated samples were stored in −80 °C for future cell culture analysis. Twenty-liter volumes of source water were initially concentrated using tangential flow HFUF with a Hemoflow F80A sterile ultra-filter cartridge (molecular weight cut-off 15,000 to 20,000). The source water was re-circulated using a peristaltic pump at pressure between 15–25 psi until the recirculating water volume was emptied in the cubic polyethylene container holding the initial sample. To recover any viruses attached to the filter, the viruses were eluted by adding 250 mL of HFUF eluting solution (1XPBS/1% Laureth-12) consisting of 10 g/L of Laureth-12 and 50 μL antifoam-A per 1 L of PBS (pH 7.4). The resulting volume of retentate was approximately 200–300 mL. The retentate was concentrated further using PEG precipitation at a final concentration of 9% of PEG 8000 (Fisher, Cat no. 233-1, or equivalent) and 0.3 M NaCl; the sample was separated into 150 mL volumes using 250 mL conical centrifuge bottles. The bottles were placed on an orbital shaker and allowed to shake overnight (200 rpm, 4 °C). The next day, the bottles were centrifuged (5400 × *g*, 1 h, 4 °C). The supernatant was decanted and pellets were resuspended using 15 mL of PBS (pH 7.5). The resulting water sample concentrates were combined and stored in a 50 mL polypropylene centrifuge tube. To kill any bacteria and to remove certain soluble impurities, 5 mL of chloroform was added to the concentrated sample from step 2 (PEG precipitation) and mixed to emulsify by inverting the tubes in vertical movement for one minute. The samples were centrifuged (3000 × *g*, 20 min, 4 °C), the supernatant was recovered from the top without disturbing the thin film layer above the chloroform, and the volumes were recorded. The supernatant was stored at −80 °C or ultracentrifugation immediately followed. The volume of the supernatant recovered after chloroform extraction (in the previous step) was adjusted to 50 mL by adding PBS (pH 7.5). The sample was transferred to a 75 mL ultracentrifuge tube and centrifuged (100,000 × *g*, 4 h, 4 °C). Ultracentrifugation separated the viruses from the buffer and further reduced the sample volume to 5 mL. The supernatant was immediately decanted and the pellet was resuspended in 5 mL of PBS (pH 7.5). The concentrated samples were aliquoted in four 1.2 mL tubes and stored at −80 °C for subsequent virus infectivity assay.

2.4. Cell culture infections and detection of adenovirus mRNA and DNA during cell culture infectivity assays

The infectivity assays were performed as described by Rodríguez et al. (2013). A 1.5 mL inoculum was produced by diluting 350 μL of concentrated sample with 1050 μL complete MEM medium, without serum and containing 10 μg kanamicin, 50 μg gentamicin, and 20 μg nystatin per mL of medium. The use of media in the inocula reduced cell detachment from their substrate after inoculation. After one hour of incubation at 37 °C for viral adsorption, the inoculum was removed, 6 mL of complete MEM medium with 2% fetal bovine serum was added to each flask, and the cell cultures were incubated at 37 °C in a water-jacked CO_2 incubator.

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