Nested-PCR and TaqMan real-time quantitative PCR assays for human adenoviruses in environmental waters

Wen-Chien Huang, Yi-Pen Chou, Po-Min Kao, Tsui-Kang Hsu, Hung-Chang Su, Ying-Ning Ho, Yi-Chun Yang and Bing-Mu Hsu

ABSTRACT

Human adenovirus (HAdV) infections can occur throughout the year. Cases of HAdV-associated respiratory disease have been more common in the late winter, spring, and early summer. In this study, to provide viral pollution data for further epidemiological studies and governmental actions, the presence of HAdV in the aquatic environment was quantitatively surveyed in the summer. This study was conducted to compare the efficiencies of nested-PCR (polymerase chain reaction) and qPCR (quantitative PCR) for detecting HAdV in environmental waters. A total of 73 water samples were collected from Puzi River in Taiwan and subjected to virus concentration methods. In the results, qPCR had much better efficiency for specifying the pathogen in river sample. HAdV41 was detected most frequently in the river water sample (10.9%). The estimated HAdV concentrations ranged between $6.75 \times 10^2$ and $2.04 \times 10^9$ genome copies/L. Significant difference was also found in heterotrophic plate counts, conductivity, water temperature, and water turbidity between presence/absence of HAdV. HAdV in the Puzi River may pose a significant health risk.

Key words | conductivity, human adenovirus, heterotrophic plate count, qPCR, river water

INTRODUCTION

Water quality, and hence human health, is affected by the presence of pathogenic microorganisms derived from sewage discharged into the aquatic environment. Among these pathogens there are many types of viruses infecting humans. Groundwater and river waters have been inevitably and frequently contaminated with human enteric viruses by the discharge of industrial and untreated domestic wastewater (Jean 1999; Jean et al. 2006). The presence of human enteric viruses in the aquatic environments is a potential human health problem because of their low infectivity doses (Ward & Akin 1984). Several studies confirm not only that infectious waterborne diseases are a primary cause of morbidity and mortality world-wide, but also that both the incidence and spectrum of many waterborne diseases are increasing (WHO 2003).

Human adenoviruses (HAdVs) are medium-sized (90–100 nm) members of the genus Mastadenovirus in the Adenoviridae family, which comprises at least 52 types classified into seven species (A–G) (Jiang 2006; Jones et al. 2007; Wold & Horwitz 2007; Robinson et al. 2011; Walsh et al. 2011; Human Adenovirus Working Group 2014). They have a double stranded linear DNA genome and a non-enveloped (without an outer lipid bilayer) icosahedral nucleocapsid...
that has fiber-like projections from each of its 12 vertices (Stewart et al. 1993). The most common HAdV infections in children are linked to serotypes 1, 2 and 5 (species C) (Brandt et al. 1998). Some serotypes such as 40 and 41 of species F are unique in being responsible for most cases of HAdV-associated gastroenteritis in children. Species A adenoviruses (serotypes 12 and 31) cause hemorrhagic cystitis in children while species B are known to cause conjunctivitis and childhood respiratory diseases (Wold & Horwitz 2007).

HAdVs are usually more frequently identified than other viruses in water samples worldwide (Haramoto et al. 2010); thus they have been suggested as indicators of virus contamination of human origin in the aquatic environments (Pina et al. 1998; Bofill-Mas et al. 2000; Albinana-Gimenez et al. 2009a). Outbreaks of HAdV-associated respiratory and gastrocolic disease have been more common in the late winter, spring, and early summer; however, HAdV infections can occur throughout the year (http://www.cdc.gov/adenovirus/hcp/index.html). It has recently increased in many regions, including Europe, Asia and America (Li et al. 2005; Kajon et al. 2010; Wyn-Jones et al. 2011; Chhabra et al. 2013). In previous studies, HAdV has been reported in sewage, sea water, river water, swimming pool, bathing water, drinking water and other recreational water (Papapetropoulou & Vantarakis 1995; Bofill-Mas et al. 2000; Jiang 2006; Sinclair et al. 2009; Albinana-Gimenez et al. 2009a; Dong et al. 2010; Haramoto et al. 2010). Also, an increase in the yearly prevalence of cases of HAdV-associated gastroenteritis has been reported in Taiwan (Lu et al. 2009; Tsou et al. 2012).

The main objective of this study was to identify and quantify HAdV in water samples collected during summer months (July–September) in Taiwan. The efficiencies of the conventional PCR (polymerase chain reaction) and qPCR (quantitative PCR) were compared. In addition, the correlation between HAdVs and various water quality parameters in the river were investigated.

MATERIALS AND METHODS

Collection of river water samples and water quality measurements

A total of 73 river water samples were collected from the Puzi River (23°28’N, 120°13’E) in mid-southern Taiwan (Figure 1), from August 2012 and July and September 2013. Adenoviruses circulate year-round in Taiwan, and several community outbreaks have been reported to Taiwan CDC (https://www.cdc.gov.tw/english/index.aspx). Between May and September, 2011, a large community outbreak of HAdV in Taiwan was detected by the nationwide surveillance system (Tsou et al. 2012). Therefore, these warm weather months (July, August, and September) were chosen in this study. The Puzi River corridor lies within a classic subtropical monsoon region located
near the Tropic of Cancer (23°5’N). It flows through Chiayi County. It has a total length of 75.9 km and a watershed area of 427 km², a mean river flow of 16 m³/s, a maximum peak flow of 2,660 m³/s, a lowest flow of 0.06 m³/s, and nine tributaries. It is mainly used for agricultural irrigation, animal husbandry, fish farming, public water supply, and recreational water activities. Over 420 thousand people live in the rivershed. People live in the rivershed. Water samples were obtained at 30 cm depth. For each sampling, a 1.3 L water sample was collected in a 1 L polypropylene bottle and 0.3 L sampling bags (Nasco Whirl-Pak, USA), placed on ice and delivered to the laboratory within 6 h of collection. The 1 L sample was used for viral analysis, while the 0.3 L sample was used for microbiological analysis. Microbiological analysis was conducted as soon as the samples arrived.

The physico-chemical water quality parameters, including pH value, water temperature, conductivity, salinity, total dissolved solids (TDS) and oxidation–reduction potential (ORP) were recorded real-time by a portable multi-parameter water quality meter (HI9828, Hanna Instruments Inc., USA). Water turbidity was measured using a ratio turbidimeter (Waterproof Portable TN100, Eutech Instruments Pte Ltd, Singapore). For the microbiological water quality parameters, total coliform was measured by membrane filtration and using a differential medium as described in Standard Methods (Method 9222 B) (APHA 2012). Heterotrophic bacteria were cultured on the m-(HPC) heterotrophic plate count agar base by the spread plate method (Method 9215C) (APHA 2012).

**Virus concentration and viral DNA extraction**

The concentration of virus was carried out using a modification of the standard method described in APHA (2012). One liter of water sample was filtered through 0.45 μm pore size, 47 mm diameter GN-6 Metricel® Mixed Cellulose Esters Sterile Packages Membrane Disc Filters (Pall, USA). Sample conditioning was performed as needed (such as addition of cation with 0.05 M MgCl₂ and pH control of 3.5), and the sample aliquots were filtered using a vacuum pump. After filtration, the membranes were scraped, and the collected material was washed with 50 mL of phosphate-buffered saline (PBS; 7.5 mM Na₂HPO₄, 5.3 mM NaH₂PO₄, 108 mM NaCl, pH 7.2). The resulting solution was then transferred into 50 mL conical centrifuge tubes and centrifuged at 2,600 x g for 30 min (KUBOTA-Model 2420 Compact Tabletop Centrifuge, Japan). After removing the top 45 mL, the remaining 5 mL pellet was resuspended with 5 mL PBS. The resulting 10 mL volume was centrifuged at 16,300 x g for 15 min, and the pellet was finally resuspended in 1 mL of PBS at 4°C for total DNA extraction. The recovery efficiency for viruses in aquatic environment was investigated in an earlier study by our study team (Hsu et al. 2007). The no-charged cellulose membrane gave higher recovery efficiency than charged membranes. Virus in the water may or may not be attached to other particles. Consequently, sample recovery rate for virus may not be adequately assessed through virus addition. Theoretically, a molecular sieve (50 kDa, UFC905096, EMD Millpore Corp., USA) can separate virus from liquid effectively. The defect of the method is small capacity admission for water sample analysis. Assuming a recovery rate of 100% for molecular sieve method (in the ideal interception and detachment situation), the relative recovery rate for the method used in this study was calculated and the results were between 45 and 69%. Viral DNA extraction was done with the concentrated pellet (1 mL) using the MagPurix Bacterial DNA Extraction Kit ZP02006 and automated DNA extraction by MagPurix 12 s Automated Nucleic Acid Purification System (Zinexts Life Science Corp., Taiwan) according to the manufacturer’s specifications. The resulting solution (final volume: 100 μL) was analyzed for the presence of HAdVs by nested-PCR and qPCR.

**HAdVs nested-PCR for detection and typing**

Nested-PCR was employed for the typification of HAdV-positive samples. Aliquots of 100 μL of the extracted nucleic acid and their respective 10-fold dilution were used, using primers Hex1deg and Hex2deg for the first round of amplification and primers neHex3deg and neHex4deg for the second round in each test (Allard et al. 2001). The nested-PCR solution was prepared with 2 μL of the DNA templates together with the nested-PCR mixture to make a total volume of 25 μL. The nested-PCR mixture included 5 μL Fast-Run Taq Master Mix with Dye, 1 μL each of the forward and reverse primers (0.4 μM), and 16 μL PCR-grade water. The cycling conditions were as follows: initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 5 min. The amplicons were resolved by electrophoresis through a 2% agarose gel stained with ethidium bromide (0.5 μg/mL, 10 min) and subsequently visualized by UV transillumination.
For HAdV typing, the nested-PCR products were sequenced. The nested-PCR products were cut out of the gel and purified and then sequenced by MB Mission Biotech, Taiwan. All nucleotide sequences were compared with those available in the National Center for Biotechnology Information (NCBI) GenBank databases using the PubMed NCBI BLAST program analysis. Phylogenetic analysis (construction/test neighbor-joining tree) was performed using MEGA software version 6.06 (Mega Software, USA), with a generation of 1,000 bootstrapped data sets.

Quantification of HAdVs by TaqMan real-time qPCR

The concentrations of HAdV in the river water samples were estimated by using qPCR with a TaqMan probe. Quantitative detection was performed using an ABI StepOne™ Real-Time PCR System (Applied Biosystems, Singapore). The generic primers and TaqMan probe used for quantification of HAdVs were forward primer JTVX-F, reverse primer JTVX-R and probe JTVX-P (Jothikumar et al. 2005). This JTVX-P assay amplifies 96 bp of hexon gene. Aliquots of 3 μL of DNA sample were mixed with 17 μL of reaction buffer containing 10 μL of Fast-Run Taq Master Mix with Dye, 0.8 μL of forward and reverse primer (0.4 μM), 0.8 μL of TaqMan probe (0.4 μM), and 4.6 μL of PCR-grade water. The qPCR running program was 5 min at 95 °C, followed by 40 cycles at 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 20 s, with a final step for 30 s at 40 °C. The samples and standards were each run at least in duplicate. All PCR runs included a negative control reaction mixture (PCR-grade water without template) and a positive control reaction mixture (HAdV serotype 41).

qPCR standard curve for HAdVs

The yT&A cloning vector kit (Yeasterm Biotech Corporation, Taiwan) was used to determine the HAdV hexon gene copy number. HAdV serotype 41 was used. Recombinant plasmid DNA was purified in duplicate using a HiYield™ plasmid mini kit (Real Biotech Corporation, Taiwan). Following purification, the concentration of plasmid DNA was determined using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, USA). The number of construct copies in the plasmid solution was calculated based on plasmid and insert sizes. In order to obtain a standard curve, a standard sample of HAdV-plasmid DNA (5.11 × 10^{10} gene copies/μL) was diluted by serial 10-fold dilution (in a range from 10^9 to 10^{10} copies per PCR).

Statistical analysis

The Mann–Whitney U-test was used to compare associations between physico-chemical water quality parameters and HAdVs positive and negative samples. The statistical software used was STATISTICA® version 6.0 (StatSoft, Inc., USA).

RESULTS AND DISCUSSION

Presence of HAdVs in river water samples

This study has evaluated the applicability of molecular methods to detect HAdVs in river water samples. The results of detection of HAdVs are summarized in Table 1. A total of 12 samples (detection rate 16.4%, 12/73) and 18 samples (detection rate 24.6%, 18/73) showed positive results for HAdVs by nested-PCR and qPCR, respectively. HAdV was detected in 22/73 (30.1%) samples using either the nested-nested-PCR or qPCR. Only eight samples were positive by both methods. According to confirmed nucleotide sequencing results, HAdV was detected in nine (12.3%) of the 73 samples. Among them, 5.8% (2/34) and 17.9% (7/39) corresponded to HAdV results for 2012 and 2013, respectively. In this study, the detection rate (12.3%) of HAdV in river water was similar to some reports in the USA, South Africa, France, Germany and Australia (Cox et al. 1993; Castignolles et al. 1998; Choi & Jiang 2005; van Heerden et al. 2005). However, the detection rate of HAdV in the aquatic environments may be influenced by detection methods, water types, water sources, climatic conditions and geographical areas.

Detection differences between nested-PCR and qPCR for HAdVs were found in this study. The lengths of the amplicons obtained by qPCR (very short template) could explain a better performance when using this method in relation to PCR used for amplifying the polymerase region of HAdV. It should not be ruled out that qPCR might overestimate genome counts due to nonspecific signals near the detection limits. Studies have shown that different PCR protocols and primer sets may result in varying quantification and detection results (Bofill-Mas et al. 2006; Prado et al. 2011). Furthermore, qPCR is not always more sensitive than conventional PCR (Bastien et al. 2008), and assessment of these methods is needed for detecting different types of viruses in environmental water samples. On the other hand, PCR inhibition may have been a factor. In comparison, the detection rates of nested-PCR are consistently lower than qPCR outcomes. The detection rate of nested-PCR is lower...
than qPCR in the river samples possibly because the primers with higher A/T ratio were more sensitive to PCR inhibitors in receiving waters. Nevertheless, PCR has been considered a useful tool in aquatic environmental virology studies, especially because of its specificity and sensitivity to detect a few viral genomic copies in several environmental matrices (Girones et al. 2010).

Quantification of HAdVs in river water

The concentration of HAdV detected is expressed as genome copies per liter, and where present the results are shown in Table 1. The concentration of HAdV in the river water ranged from $6.75 \times 10^2$ to $2.04 \times 10^9$ copies/L (mean: $2.13 \times 10^8$ copies/L), which were different to that found in previous studies. The HAdV concentration quantified in river water is higher than the concentration observed in various river water samples in Japan ($7 \times 10^3$ to $1.38 \times 10^5$ copies/L) (Haramoto et al. 2010; Kishida et al. 2015), Spain ($1 \times 10^1$ to $7.17 \times 10^6$ copies/L) (Albinana-Gimenez et al. 2013a; Albinana-Gimenez et al. 2013b; Girones et al. 2015; Calgua et al. 2014), France ($4.57 \times 10^3$ to $1 \times 10^5$ copies/L) (Castignolles et al. 1998; Ogorzaly et al. 2009), USA ($1 \times 10^2$ to $1 \times 10^4$ copies/L) (Choi & Jiang 2005), South Africa ($3 \times 10^1$ to $2.53 \times 10^4$ copies/L) (van Heerden et al. 2004; van Heerden et al. 2005), New Zealand ($1.7 \times 10^1$ to

### Table 1 | River water results for the detection of HAdVs by nested-PCR and qPCR

<table>
<thead>
<tr>
<th>Samples (n = 34)</th>
<th>PCR</th>
<th>qPCR (copies/L)</th>
<th>Species/serotype</th>
<th>Samples (n = 39)</th>
<th>PCR</th>
<th>qPCR (copies/L)</th>
<th>Species/serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR01–05</td>
<td>–</td>
<td>–</td>
<td></td>
<td>PR35</td>
<td>+</td>
<td>$3.02 \times 10^8$</td>
<td>HAdV F/41</td>
</tr>
<tr>
<td>PR06</td>
<td>+</td>
<td>–</td>
<td>HAdV A/12</td>
<td>PR36</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR07</td>
<td>+</td>
<td>–</td>
<td>HAdv F/41</td>
<td>PR37</td>
<td>+</td>
<td>$1.09 \times 10^8$</td>
<td>HAdV F/41</td>
</tr>
<tr>
<td>PR08</td>
<td>–</td>
<td>$1.38 \times 10^5$</td>
<td></td>
<td>PR38</td>
<td>+</td>
<td>$2.04 \times 10^9$</td>
<td>HAdV F/41</td>
</tr>
<tr>
<td>PR09–11</td>
<td>–</td>
<td>–</td>
<td></td>
<td>PR39</td>
<td>+</td>
<td>$9.68 \times 10^7$</td>
<td>HAdV F/41</td>
</tr>
<tr>
<td>PR12</td>
<td>–</td>
<td>$2.94 \times 10^3$</td>
<td></td>
<td>PR40</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR13</td>
<td>–</td>
<td>$6.95 \times 10^2$</td>
<td></td>
<td>PR41</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR14–15</td>
<td>–</td>
<td>–</td>
<td></td>
<td>PR42</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR16</td>
<td>–</td>
<td>$6.75 \times 10^2$</td>
<td></td>
<td>PR43</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR17</td>
<td>–</td>
<td>$1.25 \times 10^5$</td>
<td></td>
<td>PR44</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR18</td>
<td>–</td>
<td>$1.12 \times 10^5$</td>
<td></td>
<td>PR45</td>
<td>+</td>
<td>$5.28 \times 10^7$</td>
<td>HAdV F/41</td>
</tr>
<tr>
<td>PR19–21</td>
<td>–</td>
<td>–</td>
<td></td>
<td>PR46</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR22</td>
<td>+</td>
<td>–</td>
<td>PAdV C/5a</td>
<td>PR47</td>
<td>+</td>
<td>$3.76 \times 10^7$</td>
<td>HAdV F/41</td>
</tr>
<tr>
<td>PR23</td>
<td>–</td>
<td>–</td>
<td></td>
<td>PR48</td>
<td>–</td>
<td>$1.07 \times 10^3$</td>
<td></td>
</tr>
<tr>
<td>PR24</td>
<td>–</td>
<td>$3.00 \times 10^3$</td>
<td></td>
<td>PR49</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>PR25</td>
<td>–</td>
<td>$7.42 \times 10^2$</td>
<td></td>
<td>PR50</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR26</td>
<td>–</td>
<td>$2.71 \times 10^5$</td>
<td></td>
<td>PR51</td>
<td>+</td>
<td>$9.42 \times 10^7$</td>
<td>HAdV F/41</td>
</tr>
<tr>
<td>PR27</td>
<td>+</td>
<td>$1.12 \times 10^5$</td>
<td>PAdV C/5</td>
<td>PR52</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR28–31</td>
<td>–</td>
<td>–</td>
<td></td>
<td>PR53</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR32</td>
<td>+</td>
<td>–</td>
<td>PAdV C/5</td>
<td>PR54</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>PR33–34</td>
<td>–</td>
<td>–</td>
<td></td>
<td>PR55–73</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>% Positive</td>
<td>5/34 (14.7%)</td>
<td>10/34 (29.4%)</td>
<td></td>
<td>7/39 (17.9%)</td>
<td>8/39 (20.5%)</td>
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<td></td>
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<tr>
<td>% Positiveb</td>
<td>14/34 (41.1%)</td>
<td></td>
<td></td>
<td>8/39 (20.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Totalc</td>
<td>22/73 (30.1%)</td>
<td></td>
<td></td>
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<tr>
<td>% Totald</td>
<td>9/73 (12.3%)</td>
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</table>

*PAdV: Porcine adenovirus.

bTotal frequency of detection using PCR and/or qPCR.

cTotal frequency of detection using PCR and/or qPCR for all positive samples.

dHAdV confirmed by nucleotide sequencing.
The presence of high HAdV concentrations found in the river water samples suggests that this river is contaminated with more than point sources.

For sampling time results, the concentrations of HAdVs in 2013 (range: 1.07 × 10^3 to 2.04 × 10^3 copies/L and mean: 4.87 × 10^8 copies/L) were higher than those in 2012 (range: 6.75 × 10^2 to 3.00 × 10^3 copies/L and mean: 1.58 × 10^5 copies/L). Results at seven sampling sites were extremely high in July and September. All sampling sites are located near point pollution sources (Puzi hospital, Puzi city and Chiayi city), making it difficult to explain the phenomenon of unexpected high concentration. The HAdV level in the river water may be affected by untreated sewage, and the bacterial indicators may be diluted after typhoon hits. In southern Taiwan, rainfall mainly occurs in summer with frequent thunderstorms and occasional typhoon hits. The Puzi rivershed receives an average of about 1,855 mm of rainfall, which is primarily attributed to plum rain in late spring (May and June) and to typhoons between July and September. River flow volumes in the dry season (October to April) and rainy season (May to September) vary greatly, from 0.2 to 1,100 m^3/s. According to the Central Weather Bureau typhoon database of Taiwan (http://www.cwb.gov.tw/eng/index.htm), the number of typhoon hits recorded in Taiwan in August 2012 was twice the number in July and September of 2013. Further, the amount of monthly rainfall recorded in August (699.8 mm) 2012 was higher than in July (336.7 mm) and September (134.9 mm) 2013. The decreased rainfall and the low water flow might in part explain the high HAdV concentration obtained in the Puzi River water. On the other hand, the qPCR results do not indicate the infectivity status. Besides monitoring the microbial status of Puzi River, it may also be helpful to compare the monitoring outcomes with epidemiological data in future studies.

Pathogenic potential of identified HAdV serotypes and phylogenetic analyses

All samples tested positive by nested PCR were subjected to DNA sequencing for species identification. Statistical analysis of the results of the phylogenetic analysis obtained by neighbor-joining method showed that the bootstrap values were significant (>95%) as presented in Figure 2. In the phylogenetic tree, serotype reference strains and positive sample strains (97–100% nucleotide sequence identity) were classified into seven main clusters, referring to species A, F and Pig/C, especially for the species F, the most prevalent HAdV serotype 41 (n = 8) found in this study. The NCBI Genbank accession number of sample PR07, PR37, PR45, PR47 and PR51 of HAdV serotype 41 prototype is AB330122.1. Sample PR35 and PR38 prototype accession number is JX412913.1, and Sample PR59 is HQ005284.1. HAdV serotype 12 (HM209232.1) (species A) was only detected once (Sample PR06). However, three positive sample strains (PR22, PR27 and PR32) were identified as porcine adenovirus (PAdV) serotype 5 (species C), with 100% nucleotide sequence identity to the reference strain (AF289262.1).

Most PCR methods for virus detection and typing were designed for testing of human clinical samples, and only a few of them can be applied to environmental water samples without modifications and additional processing. The primer pairs (Hex1deg/Hex2deg and neHex3deg/neHex4deg) were originally designed for nested-PCR based detection of HAdVs for human clinical samples. However, animal adenovirus types do not exist in human clinical samples. On the other hand, environmental water bodies may contain a variety of different types of adenovirus. HAdVs and PAdVs are highly prevalent in river water with fecal contamination (Fong & Lipp 2005; Hundersha et al. 2009). According to previous reports, these primers for detection of non-HAdVs on PCR and qPCR cannot be excluded (Hartmann et al. 2013). Hence, these primers maybe match the nucleotide sequence of PAdV 5.

Relationships between HAdVs and water quality parameters

The Mann–Whitney U-test was used to determine the relationship between the water quality parameters and HAdV presence in river water samples. Results of the non-parametric tests are presented in Table 2. No significant differences were observed between the positive/negative samples of HAdVs with respect to total coliform, water temperature, pH value, water turbidity, ORP, TDS and salinity for both methods (PCR and qPCR). No significant differences were observed between samples that did and did not HAdV detected by PCR. However, there were significant differences (P < 0.05) between the HAdV positive and negative samples with respect to heterotrophic plate count (HPC) and conductivity for both PCR methods. In this study, the water sample low conductivity favored easier detection of HAdV from river waters for both methods.
The results supported previous findings that water sample conductivity affects virus detection and recovery in the aquatic environment (Dong et al. 2010). Furthermore, significant difference was observed when HAdVs were detected only using the qPCR. Significant differences were observed between presence/absence of water temperature and water turbidity for qPCR. The detection of HAdV was correlated with water turbidity, suggesting human and animal contamination coming from upper reaches after rainfall events. Viruses may be less stable in river waters in summer,
especially with higher water temperature. High water temperature can damage the virus capsid or nucleic acids, which might prevent adsorption of the virus to its host and may inactivate enzymes required for replication (Fong & Lipp 2005). On the other hand, the virus detection and concentration were independent of most indicator data such as HPC and water turbidity, which have been proposed as possible indicators of pollution by pathogenic microorganisms (Kishida et al. 2012).

CONCLUSIONS

In this study, PAabD and HAdV were found in the Puzi River water samples, suggesting that swine and humans were sources of viral pollution in river. The high prevalence and concentration of HAdV in the Puzi River water may pose a significant health risk. HAdVs showed statistical significance with HPC, conductivity, water temperature and water turbidity. HAdV may be proposed as possible indicators of microbial pollution by pathogenic microorganisms.

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CONFLICT OF INTEREST

All authors have agreed to publish the results.

REFERENCES


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