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Human Adenoviruses in Water: Occurrence and Health Implications: A Critical Review†

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Adenoviruses are important human pathogens that are responsible for both enteric illnesses and respiratory and eye infections. Recently, these viruses have been found to be prevalent in rivers, coastal waters, swimming pool waters, and drinking water supplies worldwide. United Sates Environmental Protection Agency (USEPA) listed adenovirus as one of nine microorganisms on the Contamination Candidate List for drinking water because their survival characteristic during water treatment is not yet fully understood. Adenoviruses have been found to be significantly more stable than fecal indicator bacteria and other enteric viruses during UV treatment. Adenovirus infection may be caused by consumption of contaminated water or inhalation of aerosolized droplets during water recreation. The goal of this review is to summarize the state of technology for adenovirus detection in natural and drinking waters and the human health risk imposed by this emerging pathogen. The occurrence of these viruses in natural and treated waters is summarized from worldwide reports.

The World Health Organization (WHO) reports 1.8 million people die each year from diarrheal diseases, of which 90% are children under 5. Over 88% of the diarrheal disease is waterborne or water-related (http://www.who.int/water_sanitation_health/publications/facts2004/en/index.html). Throughout human history, the safety of drinking and recreation water has been an ongoing challenge. Dr Lee Jong-wook, Director-General of WHO, stated “Water and sanitation is one of the primary drivers of public health.”

Waterborne disease is not restricted to developing countries. The outbreak of cryptosporidiosis in 1993 in Milwaukee, Wisconsin, provides a good example (*1*). More recent bacterial outbreaks have involved *E. coli* O157:H7, the most serious of which occurred in Walkerton, Ontario Canada in the spring of 2000 and resulted in six deaths and over 2300 cases (Bruce–Grey–Owen Sound Health Unit, 2000, http://enve.coe.drexel.edu/outbreaks/WalkertonReportOct2000/REPORT_Oct00.PDF). Morris and Levine (*2*) attempted to estimate the annual waterborne disease burden in the U.S. and indicated that a significantly greater number of people suffer from a mild to moderate waterborne infection each year than statistical reports indicate. Payment (*3*, *4*) agrees that if their figures are overestimated, both the health and economic burden are considerable for an industrialized society and a developing nation.

To reduce human health risk from waterborne and water-related illness, water quality standards are established by WHO and are adapted by most nations worldwide. Monitoring programs are conducted locally to meet water quality criteria. Total coliform, fecal coliform (or *E. coli*), and enterococcus are the most commonly used indicators for microbial water quality and human health risk assessment. However, the adequacy of these indicator bacteria to indicate the occurrence and concentration of human viruses and protozoa cysts has been questioned in recent years, because of their morphological and physiochemical differences from indicator bacteria. Viruses are significantly smaller, which can escape the filtration barriers designed to remove bacteria. Viruses and protozoa cysts have also been shown to be more resistant to wastewater treatment process and environmental degradation conditions than traditional indicator bacteria (see reviews (*5–7*). To prevent these potential human health threats, the U.S. has recently amended the Safe Drinking Water Act, which requires the Environmental Protection Agency (USEPA) to publish a list of unregulated contaminants and contaminant groups every 5 years that are known or anticipated to occur in public water systems, and which may require regulation. This list, the Drinking Water Contaminant Candidate List, commonly referred to as the CCL, will provide the basis for a mandated EPA decision to regulate (or not) at least five new contaminants every 5 years. Human adenovirus (Ad), one of the nine microbes on the current CCL, is considered an emerging contaminant in drinking water. These viruses are more resistant to UV disinfection than other human enteric viruses in almost all cases (*8*). They have also been found in river and coastal waters, swimming pools, and finished drinking waters worldwide (Table 4–6). Health outcomes attributed to Ads infection include both enteric related illnesses and respiratory system and eye infections (*9*). Adenovirus infection also emerges as a fatal outcome for immunocompromised patients and organ and bone marrow transplant recipients (*10*).

Biology and Epidemiology of Human Adenoviruses

Adenoviruses are members of the *Adenoviridae* family, which comprises five genera and infects hosts across the broad spectrum of vertebrates (*11–13*). Human Ad, genus *Mastadenovirus*, is a double stranded DNA virus; it has a non-eneveloped icosahedral shell with fiber-like projections from each of the 12 vertices (*14*). The shell is approximately 90–100 nm in diameter. Its linear double-stranded DNA of about 35 kilobases in most serotypes encodes more than 30 structural and non-structural proteins (*15*). The first of over 51 currently recognized distinct Ad serotypes was first isolated in 1953 from human adenoid tissue and named for the tissue of origin (*16*). The 51 serotypes are divided into six species based on their hemeagglutination properties, their oncogenic potential in rodents and DNA homology or GC content of their DNA. There is some clinical significance to the classification scheme, as certain organ specificity and disease patterns appear to cluster within the six subgroups (Table 1).

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TABLE 1. Serotype Classification of Human Adenoviruses

subgroup (species)	serotype	sites of infection
A	12, 18, 31	gastrointestinal tract
B	3, 7, 16, 21, 50 (B1) 11, 14, 34, 35, (B2)	lung, urinary tract
C	1, 2, 5, 6	upper respiratory tract
D	8, 9, 10, 13, 15, 17, 19, 22, 23, 24, 26, 27, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51	eye, gastrointestinal tract
E	4	respiratory tract
F	40, 41	gastrointestinal tract

Adenovirus infection results in a broad spectrum of clinical presentation. Although many Ads can replicate in the intestine and shed in feces, Ad40 and Ad41 of subgroup F are unique in being responsible for most cases of Ad-associated gastroenteritis in children. Adenovirus serotypes 1, 2, and 5 are responsible for 5−10% of childhood respiratory diseases, which are mostly self-limiting (*17*). These viruses also cause conjunctivitis in healthy adults (*18*) and certain serotypes can cause keratitis (*19*). Haemorrhagic cystitis by Ad11 may occur in normal hosts (*20*) but this self-limiting disease can be more serious in immunocompromised patients.

After a primary infection, lifelong immunity is conferred for the specific causative serotype. Hence adults are more susceptible to infections by Ad3, Ad4, and Ad7 that are less commonly seen in children (*21*). After acute infection, some Ad types (1, 2, and 5) may be shed in stool for months to years, and this is probably responsible for the endemic spread to other susceptible groups via the fecal-oral route. Inhalation of aerosolized droplets contaminated with Ad has also been shown to be an important pathway for Ad infection (*22*). The recovered patients can serve as carriers of the viruses for extended periods of time. Alternately, some infections may persist in a latent phase in tissues such as tonsils, adenoids, intestine, and the urinary tract for life (*23*).

Outbreaks of Ad infection have been described in day care centers, hospitals, military quarters (*24*), and swimming pools. The first water-related outbreak of Ad infection was reported during the summer of 1977 (*25*). An outbreak of pharyngoconjunctival fever (PCF) occurred at a private recreational facility in Georgia. A total of 72 cases were attributed to Ad4. The virus was also recovered from water obtained from the swimming pool at the facility on different dates (*25*). Serotype 7a and 3 were also reported to associate with swimming pool related Ads outbreaks (*26*−*28*). The more recent water-related outbreak is reported from Greece in July 1995. At least 80 athletes participating in a swimming contest displayed symptoms of pharyngoconjuctivitis, with the predominant symptoms being high fever, sore throat, conjuctivitis, headache, and abdominal pain. Adenoviruses were detected in swimming pool water by nested polymerase chain reaction (PCR) amplification (*29*), but the serotype of the virus was not identified.

The only waterborne enteric disease outbreak that is attributed to Ads was reported in a Finnish municipality in April 1994. Some 1500−3000 people had symptomatic acute gastroenteritis. Laboratory findings confirmed Ads, norovirus, small round viruses (SRV), and group A and C rotaviruses as causative agents. Norovirus was considered the main cause of the outbreak (*30*). Thus, the role of Ads in the outbreak is inconclusive since enteric Ads infections are mostly asymptomatic among adults due to endemic illness cases and establishment of immunity to this virus. They may manifest as day care center and hospital outbreaks due to the high number of susceptible population in these environments.

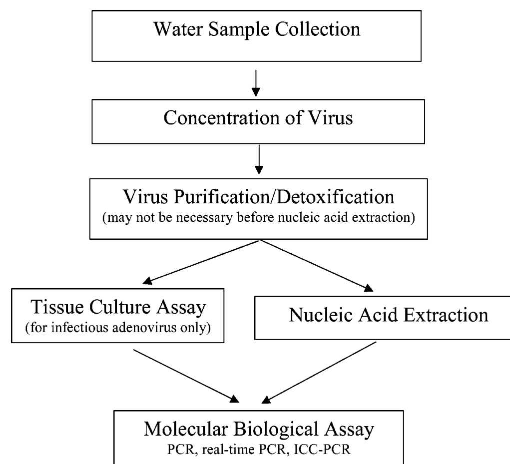

FIGURE 1. Flow chart of adenovirus detection for aquatic samples.

Detection of Adenoviruses in the Aquatic Environment

In addition to supporting the mandated development of drinking water regulations, CCL is intended to be the source of priority contaminants for the EPA's drinking water program as a whole, including research, monitoring, and guidance development. Current virus monitoring methods, according to USEPA Information Collection Rule (ICR), are based on the total culturable virus assay enumerated by most-probable-number (TCVA-MPN) on buffalo green monkey kidney (BGMK) cells. However, the adequacy of this method for assaying human Ads in water requires review and assessment.

Techniques that are applicable for Ads detection in aquatic environments are not significantly different from those used for clinical diagnosis. In fact, most of the methods for environmental virology have been adapted from their clinical applications. The challenge that is unique to environmental virologists is the generally low concentration of target viruses in aquatic samples. A viral concentration step is necessary before target detection can be achieved. In addition, the sensitivity of the test is critical to identify the target. Electron microscopy and commercial enzyme immunoassays (EIA) are sensitive enough to detect the large number of viruses in acute-phase stool specimens (approximately 10^6 virus particles/g). However, these techniques are not sensitive enough to detect low numbers of viral particles in large volumes of water.

Another challenge for environmental virologists is the complexity of the environmental matrices. Toxic metals, humic, and fulvic acid associated with sewage, river, and coastal waters can be either toxic for tissue culture cell lines or inhibitory for PCR reactions. Thus, sample purification is necessary after concentration to reduce the cytotoxic or inhibitory effect. However, there is a trade off between multiple purification steps needed to remove toxicity/inhibitors and the recovery rate of target viral particles or viral nucleic acid. Figure 1 shows generic steps involved for detection of Ads in aquatic samples. The concentration and purification procedures required for Ads are not different from other enteric viruses assay procedures in water samples. Information on these procedures can be found in recent reviews by Griffin et al. (*31*) and Lipp et al. (*32*), therefore, they will not be repeated here. Below, only the detection techniques that are specific to Ads are reviewed.

Tissue-Culture-Based Assay. Tissue culture assay is the only USEPA approved method for virus monitoring in the aquatic samples and the only method for virus detection before the age of molecular biology and PCR technology. Adenoviruses can be cultured and isolated using multiple

TABLE 2. Comparison of the Susceptibility of Tissue Culture Cell Lines for Detection of Adenoviruses

reports	cell lines	sample source and serotypes	results
Brown and Petric 1986 (*88*)	293 and BGMK	clinical samples	293 more sensitive
Witt and Bousquet 1988 (*89*)	A549, KB, Chang's conjunctiva, 293 and HeLa	purified Ad40 and Ad41	A549 and KB most efficient
Bryden 1992 (*90*)	Vero, BSC-1 and HEp-2	clinical samples	HEp-2 most efficient
Grabow et al..1992, 1993 (*91*, *92*)	Graham 293, A549, KB, Chang conjunctival and PLC/PRF/5	Ad 40 and 41	PLC/PRF/5 most efficient
Tani et al. 1992 (*80*)	HeLa, Vero, MA-104, RD-18s, HEp-2	river water samples	HEp-2 most efficient
Pinto et al..1994, 1995 (*93*, *94*)	Caco-2 and HEp-2	Ad5, 40, 41 isolates and freshwater samples	caco-2 more sensitive
Greening et al. 2002 (*57*)	A549 and BGMK	aquatic samples	A549 performed better
Lee et al. 2004 (*39*)	A549 and BGMK	aquatic samples	A549 performed better

cell lines including BGMK, Caco-2, HeLa, Hep-2, KB (human oral epidermoid), A549, PLC/PRF/5, and 293 human embryonal kidney (HEK). However, the efficiency of replication on tissue cultures varies with serotypes. The enteric Ad serotype 40 and 41 are very slow growing and produce little or no cytopathic effect (CPE) on most cell lines. Ad40 cannot be passaged in HeLa cells. Mautner et al. (*33*) showed that only cell lines expressing Ad E1A and that supply Ad E1B functions are permissive for Ad40. HEK Graham 293 is a permanent line of HEK transformed by sheared human Ad type 5 (Ad5) DNA. The cells express the transformed genes of Ad5 including E1A and E1B functions (*34*). For this reason, Graham 293 cells may be used for propagation of Ad40 and Ad41.

A number of studies have been done to evaluate the efficiency of various tissue culture cell lines in support of Ads replication. These reports are summarized in Table 2. The susceptibility of the cell lines may vary slightly depend on the sample source and the researcher performing the test, but the literature generally agrees that 293, A549, PLC/PRF/5, and Caco-2 performed better for Ads than BGMK, the USEPA recommended cell line for water monitoring. A review of current literature on Ads detection in aquatic environments indicates that BGMK was most often the tissue culture of choice (*35*−*39*). Thus, the occurrence of Ads in the aquatic environment may be underestimated using EPA ICR, especially for the fastidious Ad40 and 41.

In addition to the issue of susceptibility, one other shortcoming of tissue-culture-based detection is the long lag time required for conclusive results. To accelerate the speed of detection and confirmation, immunofluorescence staining with genus-specific monoclonal antibodies was used (*40*). Van Doornum et al. (*41*) reported detection of Ads in 567 clinical specimens with significantly shortened detection time for most samples. This technology was later developed into a commercial Ads diagnostic kit called Adenovirus Direct Immunofluorescence Assay (DFA) by Chemicon Corp. However, the immunofluoresecent based method has not been applied to aquatic samples.

Molecular-Biology-Based Assay. The advancement of genome based technologies, especially the application of PCR methods, for the detection of Ads has significantly shortened the detection time and improved the sensitivity of detection. Genomic methods target the unique and conservative gene sequences in the Ads genome. Gene sequences targeted so far include those coding for the hexon protein, fiber protein, and DNA polymerase of the virus. Many primers and probes for single step PCR, nested-PCR, multiplex PCR, and real-time PCR have been published in recent years. Table 3 lists PCR primers and probes that have been used for Ads detection in aquatic samples. Primers and probes designed and tested for clinical diagnoses, including for subtyping and serotyping (i.e., refs *42*−*46*), are also likely to work for detecting and quantifying Ads in water samples if adequate sample concentration and viral purification methods are applied.

Among all primers and probes reported, those designed by Allard et al. (*47*, *48*) for nested-PCR were the most widely used for environmental applications (*35*, *37*−*39*, *49*−*59*). Evaluation of these primers using 47 different Ad serotypes yielded positive detection for all types (*51*). However, there are mismatches of nucleotide sequences between these primers and different Ad serotypes in the NCBI database. Nearly 10 years later, Xu et al. (*42*) reported the design of degenerate primers for all Ad serotypes, which was later adapted by Ko et al. (*60*) for environmental applications. Likewise, other degenerate primers original tested for clinical applications (i.e. refs *61*, *62*) were successfully adapted and applied to the environmental samples (*63*, *64*). All primers for Ads detection in aquatic samples target the Ad hexon gene because of the conservative nature of this gene among diverse serotypes of Ads. The sensitivity of PCR is generally close to 1 plaque forming unit (PFU) or less based on seeding study (i.e., refs *47*, *48*, *52*). However, the sensitivity of the PCR can be significantly reduced in the presence of PCR inhibitors from the aquatic samples (*65*). Improvement in nucleic acid purification technology from environmental matrix is a universal challenge facing all environmental researchers.

Although the improved speed and sensitivity for Ads detection using PCR methods is a significant technological achievement, presence/absence information obtained from the traditional PCR assays alone often offers little or no-information on public health risk associated with exposure to contaminated waters. Information on the quantity and infectivity of viruses are critical for health risk assessment. The desire for quantitative technology has driven the development and application of quantitative real-time PCR (QPCR) in environmental samples. Four recent articles have reported the primers and probes that are applicable to the quantification of Ads in environmental samples. The primers and probes for QPCR are either partially based on previous published PCR primers (*64*, *66*, *67*), or are redesigned to capture specific serotype of Ad (*65*). The sensitivity of QPCR is shown to be similar to nested PCR or slightly lower (*65*). QPCR relay on a standard curve established using the known genome numbers to calibrate the unknown samples. Viral genome equivalence is generally orders of magnitude greater than PFU (*9*), thus the sensitivity of QPCR is generally in the range of 10−50 genome equivalence or 10^{-1} to 10^{-3} PFU (*67*). Standard curves are commonly established by seeding titrated genome copies into sterilized water and reagents, thus they are unequivocal to environmental samples where PCR inhibitors may be present. Jiang et al. (*65*) has shown that the QPCR efficiency reduces to 60% when seed in seawater and to 30% in secondary sewage effluent in comparison with seeding in sterilized water. Thus, the accuracy of QPCR for aquatic samples, as in PCR assay, is

TABLE 3. Primers and Probes Used for Adenoviruses Detection in Water Samples

designation	oligonucleotide sequence (5′-3′)	assay type	target	references
hexAA1885	GCCGCAGTGGTCTTACATGCACATC	nested PCR	Ad hexon	*47*−*49*, *51*
hexAA1913	CAGCACGCCGCGGATGTCAAAGT			
nehexAA1893	GCCACCGAGACGTACTTCAGCCTG			
nehexAA1905	TTGTACGAGTACGCGGTATCCTCGCGGTC			
Ad1	TTCCCCATGGCICAYAACAC	Single step PCR	Ad Hexon	*42*, *60*, *67*
Ad2	CCCTGGTAKCCRATRTTGTA			
Ad1/Hex1	TTCCCCATGGCICAYAACAC	second step for nested PCR		
Hex3	AGGAACCARTCYTTRGTCAT			
AdC-E1AF	CCACCTACCCTTCACGAACT	RT-PCR for transcribed adenovirus	Ad2/5 E1A	*60*
AdC-E1AR	CTCGTGGCAGGTAAGATCG			
AdF-E1AF	GGGAACTGGGATGACAT	E1A DNA	Ad40/41 E1A	
AdF-E1AR	CCSTCTTCATAGCATTTC			
AD2	CCCTGGTAKCCRATRTTGTA	Real-time PCR	Ad Hexon	*67*
AD3	GACTCYTCWGTSAGYGGCC			
ADP	*FAM*-AACCAGTCYTTGGTCATGTTRCATTG-*TAMRA*			
f-AD157	ACCCACGATGTAACCACAGACA	real-time PCR	Ad40 Hexon	*65*
r-AD245	ACTTTGTAAGAGTAGGCGGTTTCC			
p-AD196	*FAM*-CTGCGCTTCGTGCCCGTCG-*TAMRA*			
ADHEX1F	AACACCTAYGASTACATGAAC	nested PCR	Ad Hexon	*61*, *6*3
ADHEX2R	KATGGGGTARAGCATGTT			
ADHEX2F	CCCMTTYAACCACCACCG			
ADHEX1R	ACATCCTTBCKGAAGTTCCA			
Hex1deg	GCCSCARTGGKCWTACATGCACATC	multiplex-PCR for detection and serotyping	Ad Hexon	*62*,*64*
Hex2deg	CAGCACSCCICGRATGTCAAA			
Hex3deg	GCCCGYGCMACIGAIACSTACTTC			
Hex4deg	CCYACRGCCAGIGTRWAICGMRCYTTGTA			
AQ1	GCCACGGTGGGGTTTCTAAACTT	real-time PCR	Ad Hexon	*45*, *64*, *66*
AQ2	GCCCCAGTGGTCTTACATGCACATC			
AP	*FAM*-TGCACCAGACCCGGGCTCAGGTACTCCGA-*TAMRA*			

largely relay on nucleic acid purification technology. Sometimes a simple dilution in sterilized water will improve the PCR sensitivity by diluting out the inhibitors. In the absence of 100% satisfying nucleic acid purification methods, the QPCR yields the conservative estimates of the quantity of Ads in the aquatic samples.

PCR methods, like many other genome-based techniques, report the presence of genetic material of the target organism, but offer no information on the viability and infectivity of the organism. Many argue that PCR-positive results have little or no public health significance in terms of the microbial quality of the water because viruses inactivated by chlorination have also been detectable by PCR (*68*). Choi et al. (*69*) has shown that the high genome prevalence of Ads detected by QPCR in Southern California urban rivers are non infectious on tissue culture. One possible approach that addresses this issue is integrated cell culture PCR (ICC-PCR). ICC-PCR combines the sensitivity of PCR and the ability of tissue culture to amplify infectious viral particle present in the water samples. Chapron et al. (*35*) reported detection of Ads in water combining BGMK tissue culture with nested-PCR, and showed significant improvement of sensitivity from the EPA-required TCVA-MPN method and shortened the detection time from 14 days to 3 days. Interestingly, the Ad detected in this study is Ad40, which is generally fastidious in BGMK cell line. Similar research conducted by Greening et al. (*57*) showed that using A549 to replace BGMK can further improve the sensitivity of ICC-PCR. Lee et al. (*70*) advocate use multiple cell lines for ICC-PCR to improve the spectrum of human viruses that are detectable by the method. To address the issue of potential carry over of non-infectious Ad viral DNA in the tissue culture, Ko et al. (*60*) developed a reverse transcription (RT)-PCR method that targets the mRNA transcribed during the tissue culture assay to demonstrate the occurrence of true infectious viruses.

Occurrence in The Aquatic Environment

Source of Human Adenoviruses. Although Ads infect a variety of animals, human Ads are highly specific to human. Examination of feces from domestic, agriculture, and wild life sources—including adult cattle, calve, sheep, pig, dog, horse, poultry, cat, kangaroo, wombat, pademelon, wallaby, possum, platypus, antechinus, wood duck, rat, wild pig, fox, rabbit, goat, deer, carp (*Cyprinus carpio*), and ferry cat (*Felis catus*)—were all negative for human Ads (*71*). Human waste has been the only known source of human Ads. Human Ads have been found in high concentrations in domestic sewage collected worldwide (*51*, *52*, *56*, *72*−*76*). The occurrence and concentration of human Ads in sewage also have little seasonal variability (*52*). Therefore, the occurrence of human Ads in the natural aquatic environment is likely due to the contamination with untreated or inefficiently treated human sewage.

Studies have shown that Ads and especially Ad40 and 41 survive longer than fecal indicator bacteria in sewage and the environment (*77*) and are very resistant to UV light (*8*). This increased UV resistance showed by Ads may be associated with the double stranded nature of their DNA genome, which if damaged, may be repaired by the host cell DNA-repair mechanisms (*78*). Thus, Ads may be discharged to the environment with sewage effluent that meet bacterial indicator standard. Chlorination seems to be the only effective method to disinfect Ads (*79*).

Occurrence in Fresh and Coastal Waters. Early detection of Ads in the aquatic environment was achieved by tissue culture and was reported in PFU (Table 4). For example, Tani et al. (*80*, *81*) reported the occurrence of Ads in the river of Nara Prefecture, Japan and showed that infectious Ads occurred at low level throughout the years. Grohmann et al. (*82*) showed that approximately 12% of the 202 river water, 9−24% of coastal water and 10−25% of the stormwater were contaminated with human viruses including Ads in Sydney, Australia.

The number of reports on the occurrence of Ads in river and coastal waters escalated over the recent years owing to the advent of PCR technology and the increased awareness of this emerging pathogen in the environment. These reports are summarized chronologically in Table 4. High frequencies

TABLE 4. Occurrence of Adenoviruses in River and Coastal Waters

reports	sample source	methods of detection	findings
Tani et al. 1992, 1995 (*80*, *81*)	river water, Japan	tissue culture	low level, year-around (0–25 PFU/liter)
Grohmann et al. 1993 (*82*)	river water, coastal water, and stormwater, Australia	tissue culture	12% of the 202 river water, 9–24% of coastal water and 10–25% of the stormwater were positive
Puig et al. 1994 (*51*)	river water, Spain	nested PCR	all 9 samples were positive
Genthe et al. 1995 (*95*)	surface water, South Africa	gene probe	up to 59% samples were positive for enteric adenoviruses
Pina et al. 1998 (*52*)	river and coastal water, Spain	nested PCR	65% of 23 river samples were positive, 7 of 9 coastal waters were positive
Vantarakis and Papapetropoulou 1998 (*53*)	bathing beach water, Greece	nested PCR	28% of 120 samples were positive
Castignolles et al. 1998 (*54*)	Seine River, France	Nested PCR	some estuary samples were positive
Chapron et al. 2000 (*35*)	surface water, U.S.	ICC-nested PCR	48% of 29 samples were positive for Ad40 and 41
Cho et al. 2000 (*37*)	river water, South Korea	RT-multiplex-nested PCR	10 of 12 samples were positive
Jiang et al. 2001(*55*)	coastal water, Southern California, U.S.	nested PCR	4 of 12 samples were positive
Greening et al. 2002 (*57*)	river water, New Zealand	ICC-PCR and nested PCR	4 of 6 samples were positive by nested PCR but none was positive by ICC-PCR
Van Heerden et al. 2003, 2004, 2005 (*63*, *64*, *86*)	river and dam water used as sources for drinking water, South Africa	ICC-nested PCR, QPCR	13% (of 140 tested) to 44% (of 55 tested) were positive; Most Ads detected were Ad40 and 41; QPCR estimated <1 copy of genome per liter of river water
Jiang and Chu 2004 (*96*)	river water, Southern California, U.S.	nested PCR	approximately 50% of 21 samples were positive
Lee and Jeong 2004 (*39*)	surface water used as source for drinking water, South Korea	tissue culture CPE assay and ICC-nested PCR	positive detection in some samples; ICC-nested PCR showed greater sensitivity
Lee et al. 2004 (*70*)	river water, South Korea	Two cell lines-ICC-multiplex-nested RT-PCR	13 of 40 samples were positive
Lee et al. 2005 (*38*)	surface water, South Korea	one cell line-ICC-multiplex-nested RT-PCR	15.9% of 50 samples were positive by tissue culture; 30% samples were positive by ICC-RT-PCR
Pusch et al. 2005 (*75*)	mining Lake, German	nested PCR	20% of 123 samples were positive
Fong et al. 2005 (*59*)	coastal water, Georgia, U.S.	nested PCR	11 of 30 samples were positive
Haramoto et al. 2005 (*66*)	surface water, Japan	QPCR	45% of 64 samples were positive
Choi and Jiang 2005 (*69*)	river water, Southern California, U.S.	QPCR and tissue culture	16% of 114 samples were positive, up to 10^4 genomes per liter; negative detection on tissue culture

TABLE 5. Occurrence of Adenoviruses in Swimming Pool Waters

reports	sample source	methods of detection	findings
D' Angelo et al. 1979 (*25*)	pool water in a private recreational facility, Georgia, U.S.	tissue culture	isolation of Ad4 associated with outbreak
Martone et al. 1980 (*27*)	pool water in a private recreation center, U.S.	tissue culture	isolation of Ad3 associated with outbreak
Turner et al. 1987 (*26*)	pool water, U.S.	tissue culture	isolation of Ad7a associated with outbreak
Papapetropoulou and Vantarakis 1998 (*29*)	pool water, Greece	nested PCR	positive detection of Ad associated with outbreak
McMillan et al. 1992 (*83*)	swimming pond water for summer camp recreation, North Carolina, U.S.	tissue culture	isolation of Ad3 associated with outbreak
Harley et al. 2001 (28)	pool water for summer camp recreation, Northern Queensland, Australia	tissue culture	isolation of Ad3 associated with outbreak
van Heerden et al. 2005 (84)	pool water, South Africa	nested PCR	10.7% of 28 samples to 21.1% of 38 samples were positive

of Ads detection in surface waters used for drinking water supplies have also been reported in South Korea and South Africa. Interestingly, data from these two regions also suggested that the Ads detected are infectious, in contrast with reports from New Zealand (*57*) and Southern California (*69*). The New Zealand report showed that PCR positive samples are negative by ICC-PCR (*57*); the Southern California study reported high prevalence of Ads by QPCR but negative results on the 293A tissue culture cell line (*69*).

Occurrence in Swimming Pool Water. The reports of Ads in swimming pool water predated the studies of Ads in river and coastal waters (Table 5). These early investigations were motivated by disease outbreaks associated with exposure to the pool water. The first report of Ads in swimming pool waters was in a private recreational facility in Georgia in 1977 (*25*). Adenovirus serotype 4 was isolated from the concentrated pool water. Detection and isolation of Ads were also reported in 1980 and 1987 in both community and private

TABLE 6. Occurrence of Adenoviruses in Finished Drinking Water and Tap Water

reports	sample source	methods of detection	findings
Genthe et al. 1995 (*95*)	treated drinking water, South Africa	gene probe	hybridization positive in some samples
Cho et al. 2000 (*37*)	tap water, South Korea	RT-multiplex-nested PCR	4 of 4 samples were positive; Ad40, 41 and 5 were identified by sequencing
Grabow et al. 2001(*85*)	treated drinking water, South Africa	nested PCR	4% of 413 samples were positive
Van Heerden et al. 2003, 2004, 2005 (*63*, *64*, *86*)	treated drinking water, South Africa	ICC-nested PCR, real-time PCR	4.4% of 205 to 29.8% of 198 samples were positive, specie D dominate
Lee and Kim 2002 (*58*)	tap water, South Korea	ICC-multiplex-nested RT-PCR, followed by sequence identification	39.1% of 23 samples were positive for Ads; negative for coliform bacteria, Ad40, 41 and 5 were identified by sequence analysis
Lee and Jeong 2004 (*39*)	finished drinking water and tap water, South Korea	tissue and ICC-nested-PCR	negative for culture assay, positive for ICC-nested PCR
Lee et al. 2005 (*38*)	tap water, South Korea	one cell line tissue culture and ICC-multiplex-nested PCR	16% of 50 samples were positive by tissue culture; 28% of 50 samples were positive ICC-PCR

swimming pools (*26*, *27*) and from a swimming pond that was used for summer-camp recreation in North Carolina (*83*). In most cases, the disease outbreaks were attributed to the low chlorine residual in water. More recently, Van Heerden et al. (*84*) showed that Ads were prevalent in both indoor and outdoor pools in South Africa, where there was no report of disease outbreak. The risk of infection from the pool water was estimated to be between 2 and 4 per 1000 swimmers (*84*).

Occurrence in Drinking Water. The occurrence of Ads in finished drinking water and tap water has been reported in South Korea and South Africa (Table 6). Cho et al. (*37*) first reported that all four tap water samples collected in South Korea tested positive for Ads by RT-multiplex-nested PCR. Sequencing analysis confirmed the presence of Ad40, 41, and 5. Using nested-PCR method, Grabow et al. (*85*) reported the detection of Ads in 4% of 413 finished drinking water tested in South Africa. This study was followed by three additional reports from the same group of researchers showing Ads in 4% of 204 to 30% of 198 finished drinking water and tap water tested in South Africa (*63*, *64*, *86*). Surprisingly, this detection frequency is higher than reports of Ads in river and coastal waters from many other regions. Similarly, in South Korea, surprisingly high frequency of Ads detection was reported in finished drinking water and tap waters (*38*, *39*, *58*). Lee et al. (*58*) showed that infectious Ads were found in 39% of 23 tap water samples that were negative for coliform bacteria. However, there are also discrepancies among different reports from South Korea. For example, Lee et al. (*38*) reported 28% of 50 tap water samples were positive by ICC-multiplex-nested PCR and 16% positive by tissue culture assay. Conversely, another group of researchers from South Korea reported negative detection of Ads by tissue culture, but positive detection by ICC-nested-PCR (*58*). These discrepancies may be due to the differences in tissue culture cell lines used or the cell line condition. Nevertheless, such high frequency of infectious Ads detection in drinking water from these two regions is alarming. However, there has not been any documented waterborne Ads outbreak in South Africa or South Korea.

Risk based analyses using a rate of Ads occurrence of 1/100−1/1000 L in drinking water indicates an illness rate between 8.3/1000 and 8.3/10 000 in drinking water (*87*). This estimated illness rate is below our current definition of waterborne outbreak. However, the estimated risk for Ads infection due to drinking water is significantly higher in South Africa, falls between 10/100 and 17/100. This level of risk is within the definition of outbreak and should have been observed and reported if a good illness surveillance system existed. Alternatively, the infection and immunity of Ads might not fit the traditional model of waterborne outbreaks. The persistent low level of exposure to Ads in water may result in the endemic cases of Ads infection and establishment of immunity against the virus, which prevent the large scale outbreak of disease.

Final Remarks

In the past 20 years, we have witnessed the rapid development of pathogen detection technologies in water. Examination of current methods for virus detection as required by USEPA ICR suggests that they are not optimal at detecting human Ads in the aquatic samples. A method that combines the newest molecular biological technique with improved infectivity assay is necessary to accurately assay the occurrence of Ads in the water. Streamline of experimental protocols, standardize assay methods, and development of testing kits are also necessary before these technologies are practical for routine water quality testing and public health protection. Furthermore, these new technologies are currently expensive, and therefore, unlikely to be practical in underdeveloped regions of the world, where water quality problems are most urgent. Research that leads to a better understanding of the physical and biological property of Ads and their survival and transport characteristics in the environment should be a priority in order to develop strategies to eliminate this and other viral pathogens from drinking and recreation waters in both developing and developed nations.

It is also interesting to note that non-enteric Ads outbreaks are mostly reported from more developed countries including U.S., Greece, and Australia. However, little work has been done in these countries to test for Ads in swimming pool water other than those associated with disease outbreaks. Furthermore, the past epidemiological data point the public health significance of both enteric and non-enteric Ads. So far, there has not been an indicator for non-enteric related illness in recreational water. Adenovirus, with its prevalent association with human sewage, human waste specificity, environmental stability, and resistance to water treatment processes, perhaps can serve as a conservative indicator for assessing viral quality and efficacy of water treatment processes. It can also be used as one of the indicators to distinguish human waste contamination from fecal waste of non-human origin. Moreover, an epidemiological investigation in drinking and recreational waters may be necessary to include viral indicators such as Ads in addition to traditional fecal indicator bacteria in order to understand the link between human health risk and the occurrence of human viral pathogens.

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