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ORIGINAL PAPER

High Species C Human Adenovirus Genome Copy Numbers in the Treated Water Supply of a Neotropical Area of the Central-West Region of Brazil

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Abstract There is little information about the presence of human adenovirus (HAdV) in drinking water in Neotropical regions. Thus, the present study sought to conduct quantification and molecular characterization of HAdVs detected in treated water samples from an area of the Cerrado ecoregion of Brazil. Between August and November 2012, samples were collected from four treated water reservoirs and their respective sites along the water distribution network of the city of Goiânia, for a total of 80 samples. All samples were concentrated and analyzed by qPCR, and selected samples were sequenced. Overall, 76.6 (10^0–10^9 GC mL^{-1}) and 37.5 % (10^1–10^8 GC mL^{-1}) of samples drawn from reservoirs and their distribution sites, respectively, were positive for virus by qPCR. All samples selected for sequencing were characterized as species C human adenovirus. Such high HAdV counts have in treated water samples. This finding merits special attention, particularly from the sanitation authorities, because the high number of GC mL^{-1} may be an indicative of risk to human health.

Keywords Human adenovirus · Drinking water · Sequencing · QPCR

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Introduction

Traditionally, coliforms and heterotrophic bacteria have been used to test the microbiological quality of water (Lee and Kim 2002). Although bacterial contamination is not correlated with the presence of human enteric viruses (Fong and Lipp 2005), however, it is known that these parameters do not ensure virological quality (Le Guyader et al. 2000; Formiga-Cruz et al. 2003; Hsu et al. 2007).

Thus, human adenoviruses (HAdVs), which are commonly found in human waste, have been strongly advocated as indicators of the virological quality of treated and untreated water (Wyn-Jones et al. 2011; Silva et al. 2011a; Fongaro et al. 2013). HAdVs belong to the family *Adenoviridae*, genus *Mastadenovirus*, which contains 57 different serotypes distributed across seven species (A–G) (ICTV 2013).

With the advent of techniques with improved sensitivity and specificity, such as real-time quantitative PCR (qPCR), and in response to pressures for microbiologically safe water (Albinana-Gimenez et al. 2009), HAdVs have been successfully detected and quantified in treated water across

several world regions (Lambertini et al. 2008; Albinana-Gimenez et al. 2009; Ryu et al. 2010).

In addition to quantification, molecular characterization of circulating serotypes in treated water samples is essential, as it is the foundation for eco-epidemiological studies, risk analyses, and decision making in environmental health. Some divergence is usually observed in the HAdV serotypes detected in different regions. Lee and Kim (2002) found serotypes 40, 41, 1, 5, and 6 in tap water from Korea, whereas van Heerden et al. (2005) detected serotypes 40, 41 and species D in German treated tap water.

Few studies have reported molecular characterization of HAdVs detected in treated water from Neotropical areas. None has been conducted in the Central-West region of Brazil, which is host to the Cerrado biome, a major biodiversity hotspot (Myers et al. 2000). Working in this region, Silva et al. (2010) detected adenoviruses in water samples taken from rivers and lakes in the city of Goiânia but did not conduct any monitoring activities in treated water and did not carry out a quantitative analysis of circulating serotypes.

Within this context, the present study sought to identify, quantify, and perform molecular characterization of HAdV serotypes detected in the treated water supply of the Brazilian Central-West city of Goiânia, state of Goiás.

Materials and Methods

Sample Collection and Preparation

Water samples were collected from four treated water reservoirs that supply the city of Goiânia, Brazil—Senac (R1), Serrinha (R2), Atlântico (R3), and Cristina (R4)—and their respective sites along the drinking water distribution network (P1, P2, P3, and P4) (Fig. 1). These reservoirs are supplied by conventional drinking water treatment plants (treated by coagulation, flocculation, decantation, filtration, chlorination, and pH correction) and redistribute this water to the several districts of the city. The distribution sites chosen for sampling were those at which quality is monitored by the Public Sanitation Company of Goiás (Saneago/GO). A total of 80 samples were collected during the second semester of 2012 (August–November), 10 from each reservoir and distribution site.

Samples were collected into 5-L polyethylene carboys, directly from taps at the outlet of each reservoir or at the street-side water meters of households. All sample collection and concentration procedures were carried out as described by Silva et al. (2010), and concentrates were stored at −80 °C before use. Later, 200-µL aliquots of concentrate were taken for nucleic acid extraction, using the commercially available QIAamp MinElute Virus Spin Kit (QIAGEN) in accordance with the manufacturer's instructions.

To determine the efficiency of the viral recovery by the concentration method, 3.2×10^7 genome copies per milliliter (GC mL^{-1}) of HAdV-2 was spiked into 5 L of MilliQ water and drinking water (previously negative for HAdV by quantitative real-time polymerase chain reaction—qPCR). The samples were concentrated, and the viral recovery was assessed by qPCR.

Viral Genome Quantification by qPCR

Quantitative PCR was performed as reported by Hernroth et al. (2002). DNA samples were diluted 1:10 to avoid inhibition, and all reactions were run in triplicate, using at least five standards (1:10 dilutions of the pBR322 plasmid containing part of the adenovirus hexon gene) and ultrapure water as non-target control (NTC). A StepOnePlus™ Real-Time PCR System (Applied Biosystems) was used for all assays. The result, expressed in GC mL^{-1}, was defined as the mean of the triplicate data obtained.

Polymerase Chain Reaction (PCR)

The primers used in this study were initially described by Allard et al. (2001). PCR was performed only on samples positive by qPCR. Reaction with the hex1 deg/hex2 deg primer set employed 5 µL of extracted DNA in a reaction containing 1× buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 200 µM of each dNTP (dATP, dCTP, dTTP, dGTP), 1.5 mM $MgCl_2$, 0.2 µM of each primer, and 0.25 U of Taq DNA polymerase, in a final volume of 25 µL. Amplification consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension for 1 min at 72 °C. Final extension consisted of 7 min at 72 °C.

For nested PCR, 5 µL of the product of the first PCR run was added to a reaction mix (final volume 25 µL) containing 0.5 µM of each primer (nehex3 deg and nehex4 deg), 1× buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 100 µM of each dNTP, 1.5 mM MgCl2, and 0.25 U Taq DNA polymerase. The amplification reaction was identical to that described above for PCR.

Positive and negative controls (HAdV-5 DNA and ultrapure water respectively) were used in all reactions. The amplified nested PCR products were separated by 1.5 % agarose gel electrophoresis (UltraPure™ Agarose, Invitrogen) for 1 h at 120 V and 70 mA and viewed under UV light. Amplicons that exhibited ~170 bp as compared with a 100 bp DNA ladder (Invitrogen, Brazil) were classified as positive. Nested PCR amplifications exhibiting absence of non-specific bands or smearing and DNA concentrations >50 ng μL^{-1} were selected, purified using the AxyPrep

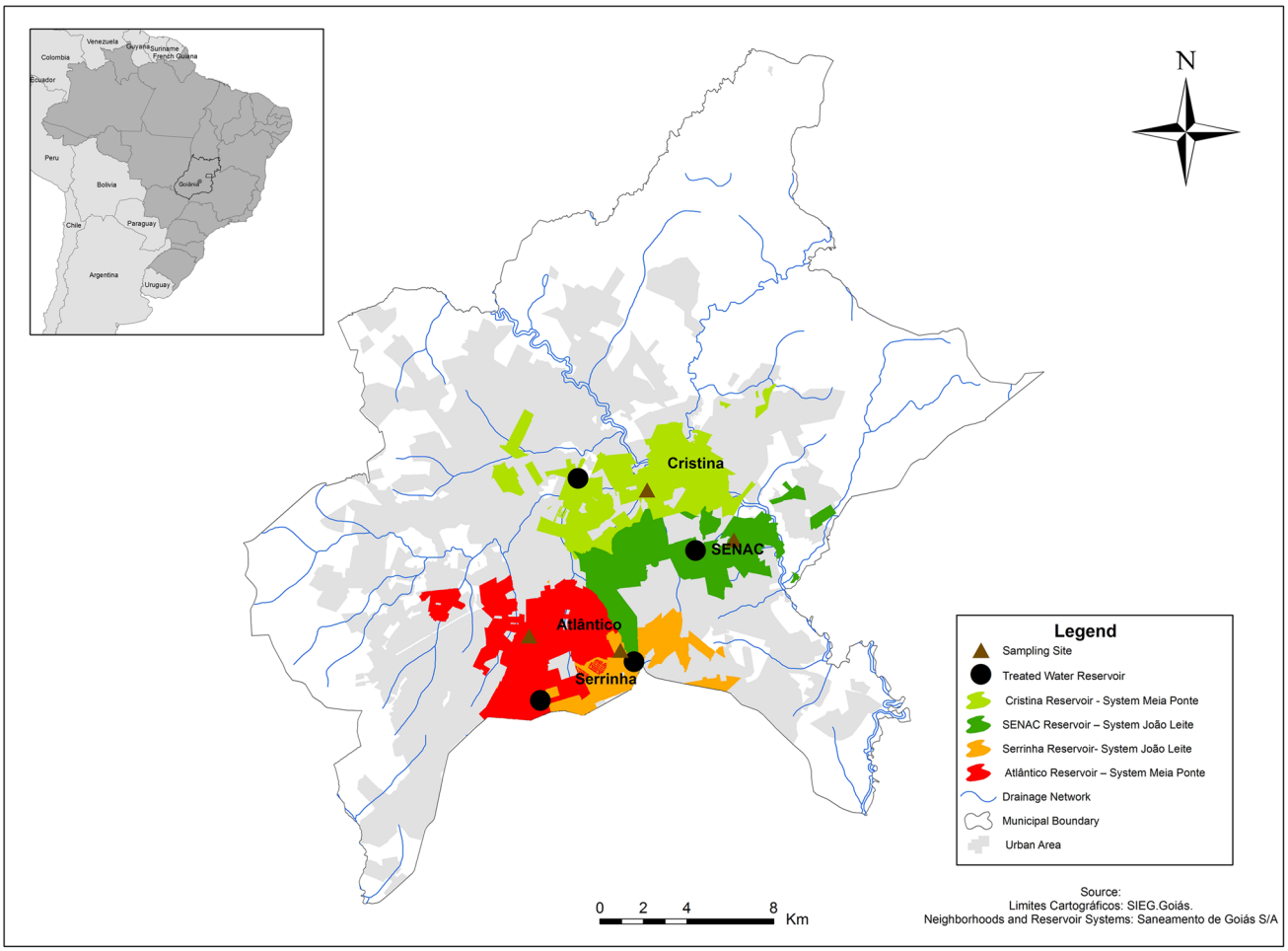

Fig. 1 City of Goiânia, showing the location of the sampled reservoirs and the downstream sampling sites supplied by each reservoir. Coordinates: Senac Reservoir, 16°40′28.82″S 49°14′14.10″W; Serrinha Reservoir, 16°43′26.08″S 49°15′53.69″W; Cristina Reservoir, 16°38′36.03″S 49°17′28.45″W; Atlântico Reservoir, 16°44′29.02″S 49°18′27.49″W; Senac site, 16°40′11.61′’S 49°13′11.05″W; Cristina site, 16°38′53.38″S 49°15′34.83″W; Serrinha site, 16°43′08.13″S 49°16′16.30″W; Atlântico site, 16°42′46.80′’S 49°18′46.77″W. Goiânia is a regional metropolis and the capital of the Brazilian state of Goiás, located in the central region of Brazil, within the Cerrado biome

DNA Gel Extraction kit (AXYGEN), and stored at −20 °C.

Sequencing and Molecular Characterization

Fifteen amplicons were sequenced with both forward and reverse primers using the DYEnamic ET—Dye Terminator Cycle Sequencing Kit for MegaBACE DNA Analysis Systems (GE Healthcare), in accordance with manufacturer instructions. The sequencing reaction program consisted of 30 cycles at 95 °C for 20 s, 55 °C for 15 s, and 60 °C for 1 min. The nested PCR primers were used at a concentration of 0.05 M per run. All sequencing was performed in a DNA 3100 automated analyzer (Applied Biosystems).

A procedure known as base calling was performed to calculate Phred quality scores (Ewing et al. 1998) for each base pair in the DNA sequences obtained. After base calling, the primer fragments present at the ends of each sequence were clipped. Both base calling and clipping were performed in CodonCode Aligner (CodonCode Corporation 2013). High-quality sequences (query sequences) were then compared to subject sequences deposited in the NCBI GenBank database (http://www.ncbi.nlm.gov/BLAST/), using BLASTn. The 15 sequenced samples were aligned and edited in ClustalX2 (Thomposon et al. 1997) and BioEdit (Hall 1999), respectively. After sequence alignment and editing, a genetic distance matrix for these samples was constructed using the JC69 maximum-likelihood model of evolution (Jukes and Cantor 1969). Nucleotide diversity was estimated on the basis of the pi (π) (Nei et al. 1979) and theta (θ) statistics in the MEGA 5.0 software suite (Tamura et al. 2011). GC content and the

rates of transition (Ts) and transversion (Tv) nucleotide substitutions were also estimated in MEGA 5.0 (Tamura et al. 2011).

The model of evolution for phylogenetic reconstruction was selected on the basis of the Akaike (1974) and Bayesian information criteria, both determined in jModelTest2 (Darriba et al. 2012). The phylogenetic tree was constructed using a parsimony-based approach in PAUP 4.0 (Swofford 2003) and using Bayesian methods in the Mr.Bayes software environment (Huelsenbeck and Ronquist 2001). The model of evolution was included in the analysis with 10,000 bootstraps to estimate node consistency for the phylogenetic tree. For phylogenetic reconstruction using the Bayesian approach, four independent runs were carried out with 12,500-step burn-in followed by 10 million iterations of Markov chain Monte Carlo (MCMC). The phylogenetic topology was visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree).

Results

The virus recovery efficiency of the adsorption–elution method of Silva et al. (2010) was evaluated. No significant differences were observed between MilliQ water and drinking water. Quantitative PCR gave adenovirus average recovery rate of 6.5 %. Overall, 52.5 % of samples (42/80) were positive for HAdV by qPCR: 18.75 % collected at distribution sites (15/80) and 33.75 % collected at reservoirs (27/80) (data not shown). On separate analysis, 67.5 % of reservoir samples (27/40) were positive, versus 37.5 % (15/40) of those collected at distribution sites along the supply network (Table 1). Copy numbers ranged from 10^0 to 10^9 GC mL^{-1} (mean 1.01×10^9) in reservoir samples, versus 10^1–10^8 GC mL^{-1} (mean, 3.42×10^7) in street-side samples. The results of qPCR quantification of each sample are shown in Table 1. In all reservoir samples except those from R2, GC mL^{-1} values were markedly elevated. Conversely, the distribution site corresponding to R2 (P2) had the highest viral genome copy number as compared with the other sampled distribution sites (Table 1).

Regarding sequencing, the mean fragment size was 165 bp. The results of BLASTn analysis showed a mean sequencing coverage of 87.26 % of amplicons in relation to samples deposited in the NCBI database (subject sequences). On average, 93.4 % of sequenced nucleotides (query sequences) had recognizable identity to database sequences, for a mean e value of 4.10^{-56}. The accession numbers for the BLASTn analysis of each sequence and the percentage of GC content are shown in Table 1.

All sequenced samples exhibited a high degree of genetic similarity (0.921), with a mean genetic distance of approximately 0.07 and a mean standard deviation of 0.024. Table 2 shows the genetic distance matrix for the 15 samples, as estimated by the JC69 model.

The high degree of DNA sequence identity between the sampled adenoviruses and the sequences deposited in the NCBI database suggested that all 15 samples were species C human adenovirus (HAdV-C). Of these, sample R4 was characterized as HAdV-5 and all others as HAdV-2 or HAdV-C. Overall, six samples were characterized as HAdV-2, three as the 14-11 strain, and the remainder as strains RKI-0762/02 and Gab153. Of the HAdV-C samples, three corresponded to strain Pitts_00109/1992/2[P2H2F2], three to strain VT5544/2003/2[P2H2F2], and two to strain ARG/A8649/2005/2[P2H2F2]. The mean genetic distance among the 15 samples was approximately 0.079, which is indicative of a high degree of similarity among the samples collected at different sampling sites (Table 2).

Nucleotide diversity analysis was carried out on 165 sites (the number of base pairs in the largest sample). Of these sites, only 56 (33.93 %) were considered polymorphic, whereas approximately 66 % of sites were conserved, corroborating the high degree of genetic similarity across samples. A total of 26 sites (15.75 %) were identified as phylogenetically informative. Overall, the samples had intermediate nucleotide diversity (π), with $\pi = 0.073$ and $\theta = 0.082$. The number of haplotypes was the same as the number of samples, i.e., 15.

The model of evolution selected for phylogenetic tree construction, as determined by a jModelTest analysis, showed different results for the Akaike and Bayesian information criteria. However, the Jukes and Cantor (1969) model fit to a gamma distribution (JC+G) showed the best agreement and was thus used to construct the phylogenetic tree using Bayesian methodology. In other words, the topology that demonstrated a phylogenetic relationship among the 15 adenovirus samples was estimated by means of parsimony and Bayesian criteria, assuming the JC+G model. The phylogenetic tree demonstrated two groups, i.e., part of the amplicons could be clearly structured into two subgroups (Fig. 2), which did not correspond to specific collection sites. Group 1 comprised five samples, three collected at distribution sites (P2, P3, and P4) and two collected at reservoirs (R1 and R2), whereas group 2 comprised four samples, all collected at distribution sites (P1, P2, and P4). The samples were so similar from a genetic standpoint that phylogenetic analysis was unable to identify distinct genetic structuring among serotypes.

Discussion

The primary water sources of the city of Goiânia are the Meia Ponte River and the João Leite Creek, which supply roughly 48.0 and 52.0 % of the population, respectively. A

Table 1 Percentage of samples positive for human adenovirus (Pos %), mean genome copy number per milliliter (GC mL^{-1}), standard deviation (SD), GenBank accession number, and percentage of GC content (% GC) of human adenovirus detected in treated water samples collected between August and November 2012 in the city of Goiânia, Goiás, Brazil ($n = 80$)

Site	Pos %	GC mL^{-1}	SD	GenBank	% GC
Reservoirs					
R1	40.0	6.45×10^6	8.98×10^6	1—JX173077.1	1—61.69
				2—KJ425142.1	2—60.00
R2	40.0	5.81×10^0	8.21×10^0	JX173077.1	60.61
R3	90.0	3.00×10^9	2.18×10^9	EU867453.1	61.01
R4	100.0	1.04×10^9	2.00×10^9	AB330086.1	57.32
Mean	67.5	1.01×10^9	1.05×10^9	–	60.12
Distribution sites					
P1	30.0	9.70×10^4	5.04×10^5	1—JX173084.1	1—59.01
				2—KC585031.1	2—60.38
				3—KF268310.1	3—60.76
P2	20.0	1.36×10^8	2.71×10^8	1—JX173084.1	1—60.49
				2—KC585031.1	2—61.39
P3	30.0	7.14×10^5	1.01×10^6	1—EU867453.1	1—61.59
				2—KC585031.1	2—61.39
P4	70.0	8.80×10^3	1.14×10^4	1—JX173084.1	1—62.35
				2—KF268310.1	2—60.00
				3—KF268310.1	3—60.38
Mean	37.5	3.42×10^7	6.81×10^7	–	60.77

R1, R2, R3, and R4: Senac, Serrinha, Atlântico, and Cristina reservoirs respectively. P1, P2, P3, and P4: sites supplied by water from the Senac, Serrinha, Atlântico, and Cristina reservoirs respectively

Table 2 Genetic distance matrix for the 15 samples, estimated by the JC69 model

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]
[1]	0.000	0.033	0.02	0.022	0.02	0.029	0.029	0.027	0.02	0.02	0.019	0.019	0.021	0.017	0.011
[2]	0.129	0.000	0.031	0.038	0.036	0.044	0.041	0.043	0.039	0.039	0.037	0.038	0.041	0.038	0.035
[3]	0.054	0.120	0.000	0.02	0.019	0.027	0.025	0.026	0.023	0.023	0.022	0.025	0.027	0.025	0.02
[4]	0.062	0.165	0.054	0.000	0.007	0.017	0.017	0.015	0.02	0.02	0.017	0.02	0.028	0.026	0.022
[5]	0.054	0.156	0.046	0.007	0.000	0.018	0.019	0.017	0.019	0.019	0.015	0.019	0.027	0.025	0.02
[6]	0.103	0.213	0.095	0.038	0.046	0.000	0.015	0.017	0.027	0.027	0.024	0.027	0.032	0.033	0.029
[7]	0.103	0.184	0.078	0.038	0.046	0.030	0.000	0.017	0.027	0.027	0.025	0.028	0.034	0.033	0.029
[8]	0.095	0.203	0.086	0.030	0.038	0.038	0.038	0.000	0.023	0.023	0.023	0.026	0.033	0.032	0.028
[9]	0.054	0.174	0.070	0.054	0.046	0.095	0.095	0.070	0.000	0.000	0.01	0.017	0.027	0.024	0.019
[10]	0.054	0.174	0.070	0.054	0.046	0.095	0.095	0.070	0.000	0.000	0.01	0.017	0.027	0.024	0.019
[11]	0.046	0.165	0.062	0.038	0.030	0.078	0.078	0.070	0.015	0.015	0.000	0.013	0.025	0.022	0.017
[12]	0.046	0.165	0.078	0.054	0.046	0.095	0.095	0.086	0.038	0.038	0.023	0.000	0.027	0.023	0.019
[13]	0.054	0.193	0.095	0.095	0.086	0.120	0.138	0.129	0.086	0.086	0.078	0.086	0.000	0.019	0.017
[14]	0.038	0.174	0.078	0.086	0.078	0.129	0.129	0.120	0.070	0.070	0.062	0.070	0.046	0.000	0.013
[15]	0.015	0.147	0.054	0.062	0.054	0.103	0.103	0.095	0.046	0.046	0.038	0.046	0.038	0.023	0.000

Distance matrix (main diagonal) and standard deviation of the estimate (upper diagonal)

[1] C1R3AT_EU867453.1, [2] C1R4CR_AB330086.1, [3] C1P1SE_JX173084.1, [4] C1R1SE_JX173077.1, [5] C1R2SR_JX173077.1, [6] C1P4CR_JX173084.1, [7] C1P3AT_EU867453.1, [8] C1P2SR_JX173084.1, [9] C2P4CR1_KF268310.1, [10] C2P1SE_KF268310.1, [11] C2P4CR2_KF268310.1, [12] C2P2SR_KC585031.1, [13] C2R1SE_KJ425142.1, [14] C2P3AT_KC585031.1, [15] C2P1SE_KC585031.1

seasonal study by Silva et al. (2010) found that 47.60 % of samples drawn from the João Leite Creek and 55.50 % of those collected from the Meia Ponte River were positive for adenoviruses. These findings are consistent with ours: R1 and R2, which are supplied by the João Leite system, yielded eight positive samples, whereas R3 and R4, which

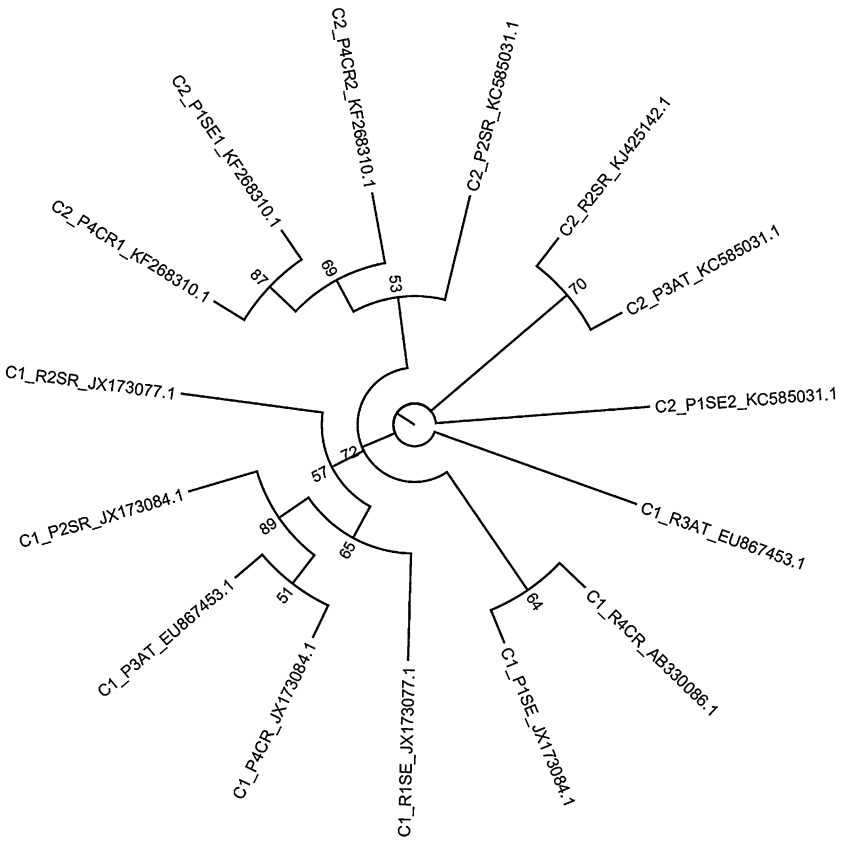

Fig. 2 Phylogenetic tree estimated by analysis of the hexon region of the 15 samples of human adenovirus detected in water samples from treated water reservoirs and downstream water distribution sites in the city of Goiânia, Goiás, Brazil. The topology represented by the tree was estimated using the parsimony method, and revealed two major groups: Group 1 (samples P1, P2, P4) and Group 2 (samples R1, R2, P2, P3, P4)

are supplied by the Meia Ponte system, yielded 19 positive samples. These results can also be extrapolated to the supply network distribution sites: 10 positive samples were collected from P3 + P4, versus five positive samples from P1 + P2. Hence, one may infer that adenoviral contamination is greater in the waters of the Meia Ponte system. It is known that untreated sewerage is discharged into the Meia Ponte River upstream from sites where water is collected for treatment (Silva et al. 2010), which may account for this increased contamination.

We carried out the detection of positive HAdVs in environmental samples only by qPCR because in our laboratory routine and even to other researchers (Jiang and Chu 2004), this method is more sensible than PCR and provides robust diagnostic in shorter time and has the advantage of performing viral quantification.

The low recovery rate of HAdV in this study may be due to a loss of viruses during the elution step or to incomplete elution of viruses from the membrane. Fong et al. (2010) evaluated the virus recovery efficiency by adsorption–elution method of Haramoto et al. (2005) and reported a mean recoveries from the MilliQ water and river water of 2.08 (range 0.17–6.98 %) and 0.98 % (range 0.32–1.47 %), respectively. So, recover viral from environmental is always very low; the result found here is satisfactory because the method is fast and inexpensive.

Moreover, the detection of viral pathogens in water is strongly influenced by the concentration method (Silva et al. Silva et al. 2011b). The sampling volume used to concentrate adenoviruses in treated water usually ranges from 10 to 1500 L (Lambertini et al. 2008; Albinana-Gimenez et al. 2009; Dong et al. 2010). The volume used in the present study (5 L) was half of that used by Silva et al. (2010) to detect adenoviruses in river and lake waters from the city of Goiânia with the exact same methodology. We expected that positivity rates in treated waters from the same city would be lower; however, we observed the opposite.

The high positivity and contamination rates of our samples were confirmed by GC mL^{-1} values (Table 1). In previous studies of HAdVs quantification in water, values have ranged from 10^0 to 10^3 GC mL^{-1} in treated water (Albinana-Gimenez et al. 2009; Lambertini et al. 2008, Fongaro et al. 2013) and from 10^0 to 10^8 GC mL^{-1} in river and lake samples (Xagoraraki et al. 2007; Albinana-Gimenez et al. 2009; Calgua et al. 2011; Fongaro et al. 2012). Adenoviruses are stable in the environment and are the most prevalent virus in sewerage systems (Metcalf et al. 1995; Parasidis et al. 2013), with mean counts of 10^2–10^5 CG mL^{-1} (He and Jiang 2005; Muscillo et al. 2008). Therefore, the measurements obtained in the present study, both at reservoirs and at downstream sites along the

distribution network, far exceed those reported elsewhere in untreated environmental water samples (Table 1). Nevertheless, it bears stressing that measurements were widely variable, particularly in samples from R4, R3, and P2, where the overall mean was elevated due to the presence of samples with very high GC mL^{-1} counts.

The mean GC mL^{-1} values measured in reservoir samples were higher than those measured at their respective distribution sites (with the exception of R2). Furthermore, positivity rates were higher in reservoirs than at downstream distribution sites along the supply network (Table 1). This may be explained by dilution, adsorption, and degradation of viral particles in the water as it makes its way to households, assuming that the distribution system is integrated, continuous, and free of contamination.

The qPCR is frequently employed for viral detection in environmental samples but do not predict viral infectivity (Wyn-Jones et al. 2011). Cell culture methods can be an alternative to infer viral viability and be a tool for future risk assessments studies, but it is laborious and can be difficult to detect viral agents present in very low concentrations (Rodríguez et al. 2009). So, the application of molecular techniques, despite unable to assess the infectivity of pathogens, are considered a gold standard in environmental virology as rapid diagnostic tool.

Silva et al. (2011a) proposed the HAdVs detection in the environment only by molecular methods because these viruses are prevalent and stable in environment (Lee and Kim 2002; Wong et al. 2009). Furthermore, this is the aquatic pathogen more resistant to inactivation by ultra violet light (UV) (Liden et al. 2007). According to Charles et al. (2009) PCR offered good correlation with the infectivity for adenovirus detected in groundwater and can be used as a tool for risk assessment in public health.

Recently, a study contemplating the research group from the Virobathe project proposed the use of a mathematical model that predicts the existence of adenovirus in treated water using only qPCR (Wyer et al. 2012). The model uses data of monitoring of *Escherichia coli* and *Enterococcus intestinales* and can be an efficient and quick alternative for the detection of HAdVs in environment.

So, despite the limitations of qPCR for assessment of infectivity, several studies conducted in different areas worldwide have reported detection of infectious HAdVs in treated water (Rigotto et al. 2010; Dong et al. 2010; Calgua et al. 2011). This suggests that qPCR can be used to evaluate virus removal from treated waters (Albinana-Gimenez et al. 2009), and calls into question the current water treatment model, which was unable to eliminate the HAdVs present in untreated water before purification. Koopmans et al. (2002) note that current water treatment models are indeed ineffective in removing and eliminating viruses (Koopmans et al. 2002; Bosch et al. 2008) and unable to ensure that water is free of viral contamination (Bosch et al. 2008). In Brazil and other Neotropical countries, this situation is compounded by the absence of specific laws that require viral detection and monitoring in water for human consumption. In industrialized nations at the forefront of environmental virology, these pathogens are implicated as the leading cause of gastroenteritis (Lopman et al. 2003).

Regarding molecular characterization, species C HAdVs are known as respiratory pathogens (Wold and Horwitz 2007). Serotypes 2 and 5 cause acute pharyngitis, particularly in children and neonates (Mena and Gerba 2008).

Phylogenetic trees estimated by two distinct methods (parsimony and Bayesian inference) yielded the same topology. Bootstrap values were $\geq$50 %, which is indicative of good confidence/support for nodes; this denotes the presence of monophyletic groups in the phylogenetic tree (Fig. 2). These two groups—Group 1 (samples P1, P2, and P4) and Group 2 (samples R2, P2, P3, and P4)—encompassed 60 % of the collected samples and were phylogenetically well delimited. Analysis of the phylogenetic tree strongly suggests that all P3 and R4 samples belong to serotypes 2 and 5, respectively.

The higher occurrence of HAdV-C in Neotropical waters is corroborated by the work of Moresco et al. (2012) and Fongaro et al. (2013), who found an increased prevalence of HAdV-2 in environmental and treated water samples collected in the South of Brazil. Conversely, a greater occurrence of the gastroenteric serotypes HAdV-40 and 41 has been reported in environmental waters in North America and Europe (Chapron et al. 2000; Wyn-Jones et al. 2011).

Both respiratory and gastroenteric serotypes can be isolated from environmental waters (Mena and Gerba 2008). However, data on the prevalence of these pathogens—particularly in treated water and in Neotropical regions—are still scarce. Additional studies on this topic are required to improve our understanding of the dynamics of HAdVs in different water sources worldwide.

In the city of Goiânia, de Freitas et al. (2010) reported clinical detection of HAdVs in diarrheal stool samples from children; HAdV-5 and, due to the nature of the sample, the gastroenteric serotypes (HAdV40/41) were those most frequently detected. These clinical findings and the results of the present study show that treated water can still pose a viral risk, particularly to the pediatric population. This assertion should be evaluated in future studies, assessing viral infectivity and including seasonal data analysis.

Furthermore, a more in-depth assessment of the sampled water distribution sites is warranted, as the GC mL^{-1} values detected were extremely high. Sampling occurred during the rainy season, a time of increased percolation of

organic matter from human waste, which may have influenced the high copy numbers observed. In addition, supply lines along the water distribution network are subject to infiltration/inflow and exogenous contamination, which were not assessed in this study.

The high rate of HAdV replication in individuals and the mutability of HAdVs should also be taken into account, as the frequent passage of these viruses through hosts may produce viral particles with increased pathogenicity. This heightens the need for development of efficient methods for the monitoring, removal, and inactivation of viruses in treated water, particularly as the most susceptible segments of the population (such as older adults and immunosuppressed and immunodeficient individuals) grow.

The data presented herein may contribute greatly to a better understanding of the eco-epidemiology of adenoviral infections in the Cerrado areas of the central region of Brazil, and even of the epidemiology of other waterborne pathogens. We hope it will also be useful for risk analysis and decision making in public health.

Conclusion

HAdV-C is present in the treated water supply of the city of Goiânia, Brazil. The current treatment model is ineffective in removing viruses from drinking water, which suggests that HAdV could be implemented as a marker for assessment of virus removal after water treatment.

Conflict of interest The authors declare that they have no conflict of interest.

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