Assessment of diversity of adenovirus DNA polymerase gene in recreational waters facilitated by ultracentrifugal concentration

Viviane Girardi, Meriane Demoliner, Caroline Rigotto, Vania Elisabete Schneider, Suelen Paesi and Fernando Rosado Spilki

ABSTRACT

Adenoviruses (AdV) are related to respiratory and gastrointestinal diseases in animals and human beings. Their wide genetic diversity in water bodies and their resistance to environmental conditions allow the use of AdV as a reliable marker for detection of fecal contamination. In this work, the diversity of AdV along Belo Stream – in the city of Caxias do Sul, Rio Grande do Sul, Brazil – was evaluated. Samples were compared in both concentrated and unconcentrated forms. The identification of different AdV species was performed by amplifying a partial sequence of the DNA polymerase gene. AdV was detected in 24 out of 55 concentrated samples (43.6%) and the following species were identified: human adenovirus (HAdV) species C (4/55; 7.2%), D (6/55; 10.9%), E (2/55; 3.6%), and F (9/55; 16.3%). AdV related to other mammalian hosts, such as bovine adenovirus (1/55, 1.8%) and murine adenovirus (2/55, 3.6%), have also been identified; 23.6% (13/55) of the unconcentrated samples were positive, and identified as HAdV species C (6/55, 10.9%), D (1/55, 1.8%), and F (6/55, 10.9%). Results obtained evidenced the presence and the great diversity of AdV, mainly of human origin, circulating in Belo Stream. As expected, the concentration step performed helped to detect AdV in more samples.

Key words | adenovirus diversity, DNA polymerase gene, environmental sanitation, nested-PCR, primary contact waters, water resources

Viviane Girardi (corresponding author)
Meriane Demoliner
Caroline Rigotto
Fernando Rosado Spilki
Laboratório de Microbiologia Molecular,
Universidade Feevale,
ERS 239 n° 2755, Novo Hamburgo, RS 93352-000,
Brasil
E-mail: *0178103@feevale.br*

Vania Elisabete Schneider
Instituto de Saneamento Ambiental (ISAM),
Universidade de Caxias do Sul,
Rua Francisco Getúlio Vargas, n° 1130, Caxias do Sul, RS 95070-560,
Brasil

Suelen Paesi
Laboratório de Diagnóstico Molecular, Instituto de Biotecnologia,
Universidade de Caxias do Sul,
Rua Francisco Getúlio Vargas, n° 1130, Caxias do Sul, RS 95070-560,
Brasil

INTRODUCTION

Waterborne diseases, such as gastroenteritis, represent a serious public health problem in developing countries. Several studies in literature confirm that there is a relation between the lack of basic sanitation and the dissemination of viral waterborne diseases, such as the ones caused by adenoviruses (AdV). The poorer the sanitation systems, the greater the impact of these illnesses. In addition, the lack or ineffectiveness of sewage treatment systems aggravates and leads to the deterioration of aquatic ecosystems (Heller *et al.* 2003; Prado & Miagostovich 2014; Spilki 2015).

The use of water for recreation purposes is a common practice in tropical climate countries. The contamination of these water bodies can be generated by several polluting sources, such as domestic, agricultural, and industrial effluents, the first being the main factor responsible for diseases caused by contact with water. During the summer, waterborne disease outbreaks increase due to recreational activities and contact with water contaminated by domestic sewage. Therefore, the quality of the recreational waters should be analyzed more thoroughly, due

doi: 10.2166/wh.2017.144

to the risk offered to human health by direct and prolonged exposure to pathogenic organisms, such as AdV (Bosch *et al.* 2008; Sinclair *et al.* 2009; Lodder *et al.* 2015).

Human adenoviruses (HAdV) are non-enveloped viruses with icosahedral capsid 60–100 nm in diameter and DNA double-stranded genome, and they belong to the family *Adenoviridae*. This family is composed of five genera; among them is the genus *Mastadenovirus*, which includes 57 serotypes of HAdV that are subdivided into seven species that go from A to G. In addition to HAdV, bat, bovine (BAdVs), canine, equine, murine (MAdVs), sheep, swine, simian and viruses from other hosts are included in this genus. HAdVs may have different tropisms, causing a wide variety of diseases that include acute febrile pharyngitis, respiratory infections, acute conjunctivitis, cystitis, gastroenteritis, and systemic infections in immunocompromised patients. HAdV-A, B, C, and E affect the respiratory system, while species D, F and G are more prone to cause gastrointestinal infections (Ghebremedhin 2014; Santos & Soares 2015).

Species classification and AdV serotypes found in aquatic environments help to identify different sources of contamination, as well as to have a better knowledge of their occurrence in water, thus improving the assessment of infectious risk for humans exposed to specific types of HAdV (Kidd *et al.* 1990; García 2006; Robinson *et al.* 2013). AdVs are stable in the aquatic environment and may remain potentially infectious for a long time in water, including diverse environmental conditions and water and sewage treatment processes that are normally intended for bacterial control. Moreover, they can adsorb solid particles of the aquatic environment, promoting greater stability. Due to all these characteristics, AdVs may be used as viral markers for detection of fecal contamination in water (García 2006; Yates *et al.* 2006; Ogorzaly *et al.* 2015).

Belo Stream is one of the tributaries of the Caí River Basin, located in the mountainous region of the state of Rio Grande do Sul (RS), in southern Brazil. Its springs are located in the city of Caxias do Sul, in both urbanized and industrialized areas, which add to domestic and industrial effluents. In the countryside, the impacts are the result of agricultural activities and animal husbandry when the area near to its mouth is used for recreation. Until now, no investigation has been carried out on the presence of enteric viruses in this region.

In this context, the aim of the present work was to compare results obtained from concentrated and unconcentrated samples for the presence and diversity of the mammalian polymerase DNA gene of AdV along a river known to be polluted by human and animal waste.

MATERIAL AND METHODS

Study area and sampling

Sampling was performed from March 2015 to April 2016, monthly, at four points along Belo Stream in Caxias do Sul (RS, Brazil) as shown in Figure 1, in accordance with the Brazilian Association of Technical Standards (ABNT) 9897 (Planning of sampling of liquid effluents and receiving bodies) and ABNT 9898 (Preservation and sampling techniques of liquid effluents and receiving bodies) (ABNT 1987a, 1987b). Belo Stream drains 21% of the city urban perimeter; it has an area of 75.10 km^2 and a perimeter of 63.11 km.

A total of 55 samples were collected from four different points: 13 samples from P1, and 14 from P2, P3, and P4. A volume of 500 mL of surface water was collected in sterile flasks. These samples were stored at 4 °C until the concentration process took place. A short description of the points is given in Table 1.

Viral concentration

A total of 55 samples containing 500 mL were collected. From these 500 mL, 36 mL were concentrated by ultracentrifugation method, following the protocol: An aliquot was centrifuged at the rate of 41,000 × *g* at 8 °C for 3 h. Thereafter, the precipitate was resuspended in 2 mL of Tris-EDTA buffer (pH 8.0), and vigorously homogenized in vortex for 1 min. The resuspended samples were aliquoted and stored in microtubes at −80 °C until the DNA extraction process. Unconcentrated samples (55) were also evaluated and aliquoted into microtubes and stored at −80 °C for further DNA extraction.

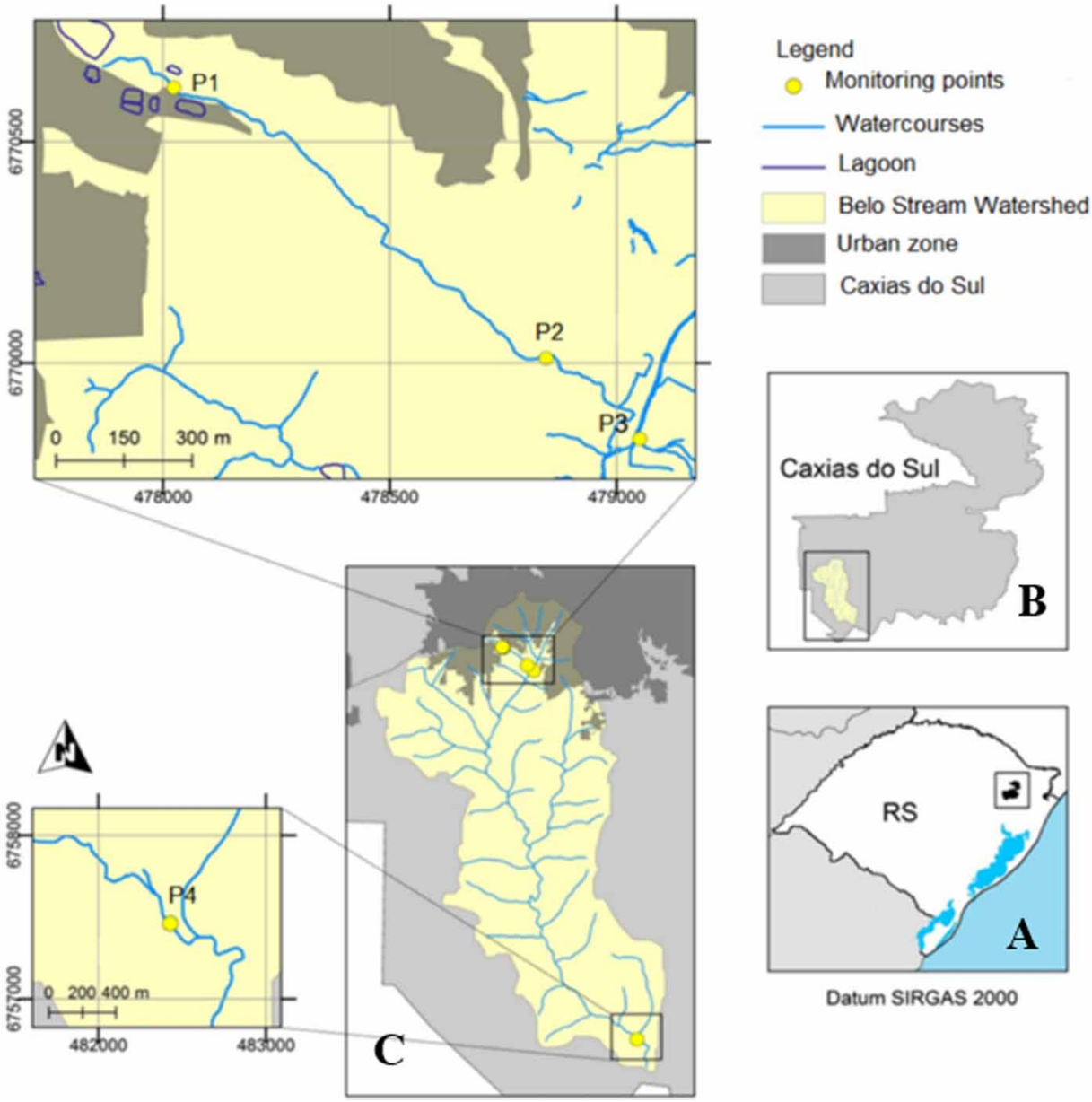

Figure 1 | (a) Map of Rio Grande do Sul – Brazil, in which the location of the city of Caxias do Sul is highlighted. (b) Map of Caxias do Sul, in which the outline and location of Belo Stream are highlighted. (c) Distribution of the sampling points along Belo Stream (elaborated by Geise Macedo dos Santos).

Extraction of genetic material

Genetic material from the concentrated and unconcentrated samples was extracted with a BioPur® Kit from an initial volume of 200 μL according to the instructions described by the manufacturer. The final elution was performed in microtubes free of DNAse/RNAse, in which they were stored and maintained at −80 °C until further processing.

Viral detection

To evaluate the presence of different AdV species, a partial sequence of the DNA polymerase (pol) gene was amplified by nested polymerase chain reaction (nested-PCR). Measurements of the reaction were carried out for a final volume of 50 μL, as follows: 1 μL of Pol-F primer (5′-CAGCCKCKGTTRTGYAGGGT-3′), 1 μL of the primer

Table 1 | Name, description and coordinates of collection points along Belo Stream

Point	Description	Coordinates	
		X	Y
P1	It is located in the urban area of the city of Caxias do Sul. It receives effluent from domestic origins. It is upstream of the effluent release of an animal processing industry. It is a sampling point located in a stream that is a tributary of Belo Stream	478,024	6,770,622
P2	It is located in a periurbanized region. It is upstream of the confluence of the stream with Belo Stream and downstream of P1	478,845	6,770,010
P3	It is located downstream of the confluence with the monitored stream (P1 and P2). It receives domestic effluents. Native vegetation and agricultural activities characterize this setting	479,051	6,769,817
P4	It is upstream from the confluence with the Caí River. P4 is characterized by areas of native vegetation and agricultural activities, and it is used for leisure and aquatic recreation because there is a camping area	482,424	6,757,598

Pol-R (5′-GCHACCATYAGCTCCAACTC-3′), both primers at 20 pmoles, 18 μL of DNAse/RNAse free water, 25 μL of GoTaq® Green Master Mix (Promega, USA) and 5 μL of nucleic acid extracted from each sample. After initial incubation at 94 °C for 5 min, 30 cycles of amplification were performed. These consisted of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. Amplification was performed in a Bio-Rad® thermal cycler. The second PCR reaction was performed using the same reagents and quantities from the first one, as well as the same amplification cycles carried out with the products of the first PCR; however, both primers were replaced with Pol-nF (5′-GGGCTCRTTRGTCCAGCA-3′) and Pol-nR (5′-TAYGACATCTGYGGCATGTA-3′) (Li *et al.* 2010).

PCR products were analyzed on 2% agarose gel, 0.5 mg of ethidium bromide/mL was added and the running time was 60 min at 70 V. Molecular sizes of the products were compared with a 100 bp DNA standard (Ludwig brand). The bands stained with ethidium bromide were visualized in UV light; subsequently images were photographed with an Easy Doc 200 UV transilluminator equipment.

Samples testing positive for the DNA polymerase gene by nested-PCR were submitted to DNA sequencing for species identification. Sequencing was carried out by Ludwig Biotec (Sequencing Service) using an automated sequencer (ABI-PRISM 3500 Genetic Analyzer Applied Biosystems). Phylogenetic analysis was performed by comparing the genomic sequences obtained by direct DNA sequencing with other nucleotide fragments available from GenBank, in accordance with the Neighbor-Joining methodology (Saitou & Nei 1987). A phylogenetic tree was elaborated from the calculation of evolutionary distances, using the Kimura-parameter 2 method (Kimura 1980) and operating with Molecular Evolutionary Genetics Analysis software version 5 (MEGA5) (Tamura *et al.* 2011).

RESULTS

Evaluation of the presence of the AdV DNA polymerase gene

The AdV DNA polymerase gene was detected in 43.6% (24/55) of the concentrated samples. On the other hand, 25.4% (14/55) of the unconcentrated samples were positive for AdV. Thus, more positive samples were obtained when submitted to the concentration step in contrast to the unconcentrated ones.

The greatest number of positive samples for both concentrated and unconcentrated samples was from the P3 site (concentrated samples 57.1% (8/14); unconcentrated: 50.0% (7/14)) as seen in Tables 2 and 3. The sites with the lowest positivity were P2 (21.4% (3/14)) and P4 (7.1% (1/14)) (Tables 2 and 3) for concentrated and unconcentrated samples, respectively.

Among the concentrated samples (Figure 2), AdV was found in at least one collection point every month.

Table 2 | Number of positive samples detected by nested-PCR along the collection points of Belo Stream (concentrated samples)

Concentrated samples

AdV species	P1	P2	P3	P4	Total (per species)
HAdV-C	0	2	0	2	7.3% (4/55)
HAdV-D	4	0	2	0	10.9% (6/55)
HAdV-E	0	0	1	1	3.6% (2/55)
HAdV-F	1	1	4	3	16.4% (9/55)
BAdV	1	0	0	0	1.8% (1/55)
MAdV	1	0	1	0	3.6% (2/55)
Total (per collection point)	7/13 (53.8%)	3/14 (21.4%)	8/14 (57.1%)	6/14 (42.8%)	

Table 3 | Number of positive samples detected by nested-PCR along the collection points of Belo Stream (unconcentrated samples)

Unconcentrated samples

AdV species	P1	P2	P3	P4	Total (per species)
HAdV-C	1	3	1	1	10.9% (6/55)
HAdV-D	0	0	1	0	1.8% (1/55)
HAdV-F	2	0	5	0	12.7% (7/55)
Total (per collection point)	3/13 (23.0%)	3/14 (21.4%)	7/14 (50.0%)	1/14 (7.1%)	

Furthermore, October and November of 2015 and February of 2016 were the months which presented the highest number of positive results for AdV (three samples tested positive in each of these months). Based on unconcentrated samples (Figure 3), July, October, and November of 2015 and March of 2016 presented greater positivity, as two positive samples were detected in each of these months.

Diversity of the AdV DNA polymerase gene

Based on the results obtained by nucletotide sequencing and phylogenetic analysis, it was found that the most prevalent species of AdV was F in both unconcentrated and concentrated samples, being found at a rate of 16.4 and 12.7%, respectively. On the other hand, the BAdV species was the one with less predominance in concentrated samples,

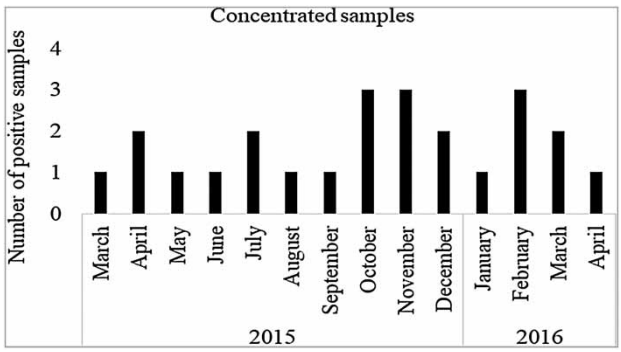

Figure 2 | Total number of positive samples per month of collection along the four sampling points of Belo Stream (concentrated samples).

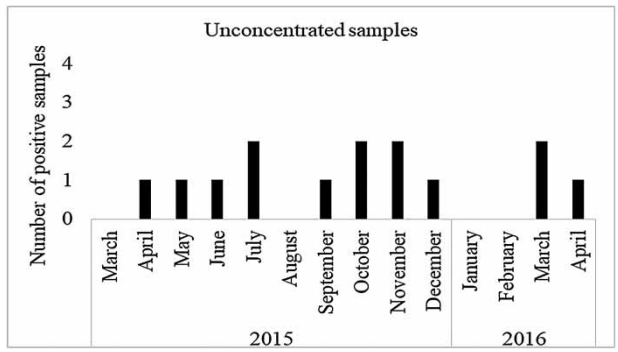

Figure 3 | Total number of positive samples per month of collection along the four sampling points of Belo Stream (unconcentrated samples).

being present in only one sample (1.8%) from P1. In the unconcentrated samples group D was the one less found, being in only 1.8% of the samples. It is important to point out that the HAdV-E, BAdV and MadV species were only identified among the water samples that were submitted to the concentration method (Tables 2 and 3, and Figure 4). Therefore, it may be claimed that the concentration method allows superior results to be obtained, since a great diversity of AdV species was identified, including from other hosts in addition to the human ones.

DISCUSSION

Water resources suffering anthropogenic influence can harbor immense microbiological diversity, as they can be contaminated by different effluent sources. AdV is often found in aquatic ecosystems, since current sewage treatment methods are not fully effective in the removal of viral particles. In the present study, the presence of AdV was detected in 43.6% of the concentrated samples. Taking into consideration this study and the sites investigated, the occurrence of AdV was higher than in other studies. Aw & Gin (2011) evaluated AdV in surface waters in Singapore and reported positivity in 38% of the samples. Kundu *et al.* (2013) found the presence of AdV in 11% of recreational water samples from the USA. However, Maurer *et al.* (2015) found superior results compared with this study. They described the presence of the AdV genome in 77.8% of samples collected in recreational waters in southern Brazil.

Usually, when comparing concentrated with unconcentrated samples, the number of samples found positive is higher after the concentration stage, which allows the finding of a greater diversity of AdV species. This is an important fact to consider when comparing both protocols, since it is not always possible to find the same diversity if samples are not concentrated. Therefore, the concentration method is fundamental to surface water samples, because it allows an increase in the frequency of positive samples. Furthermore, unconcentrated samples contain a number of substances that inhibit or interfere with the viral detection and quantification, due to the receipt of different effluents, such as domestic, industrial, and agricultural (Fumian *et al.* 2010; Silva *et al.* 2011).

HAdV found in recreational waters has been considered to cause gastroenteritis outbreaks and other diseases of the respiratory system due to one of its main characteristics: the resistance to variations in environmental conditions (Sinclair *et al.* 2009). Considering concentrated samples, the total number of positive samples found at P4 (place used for leisure and aquatic recreation) was six (6/14 (42.8%)) (Table 1). It is worth highlighting that among these six positive samples, three belong to group F, a major virus that causes gastroenteritis, and is also considered one of the major etiological agents responsible for infantile gastroenteric infections. Thus, this constitutes a health risk for the population that bathe in these waters, since viral analysis in recreational waters in Brazil is not mandatory (Filho *et al.* 2007; Kundu *et al.* 2013; La Rosa *et al.* 2015). Caxias do Sul (RS, Brazil) is one of the cities that face gastroenteritis problems. Paesi & Magrini (2015) conducted a study on the number of cases of acute diarrheal disease in this municipality from 2004 to 2013. The authors reported that during this period 61,246 cases were recorded, the highest numbers being in low-income neighborhoods. Furthermore, the authors also reported that the area with the second highest register was Desvio Rizzo. In this same area is located P1, which, as previously mentioned (Table 2), was the point with the second highest number of positive samples (7/13 (53.8%)).

In contrast to what is found in the literature, HAdV belonging to the F group was detected more frequently in this study. In the literature, water sample analyses have shown that AdV belonging to group C are in a greater quantity (Bibby & Peccia 2013; Barrios *et al.* 2016; Staggemeier *et al.* 2017). On the other hand, there are studies that have found similar results. Kuo *et al.* (2015), and Wieczorek *et al.* (2015), when analyzing the presence of AdV genome in sewage samples from Taiwan and Poland, respectively, described a higher number of AdV from the F group. Other species of HAdV have also been identified, such as those of groups D and E, viruses that affect the gastrointestinal and respiratory tract, respectively. It is described in literature that HAdV-A, C, and F are more frequently found in water bodies when compared with groups B, D, and E (Kuo *et al.* 2015; Ogorzaly *et al.* 2015; Wieczorek *et al.* 2015). Serotypes 40 and 41 belonging to the F group have been considered one of the most prevalent viruses in

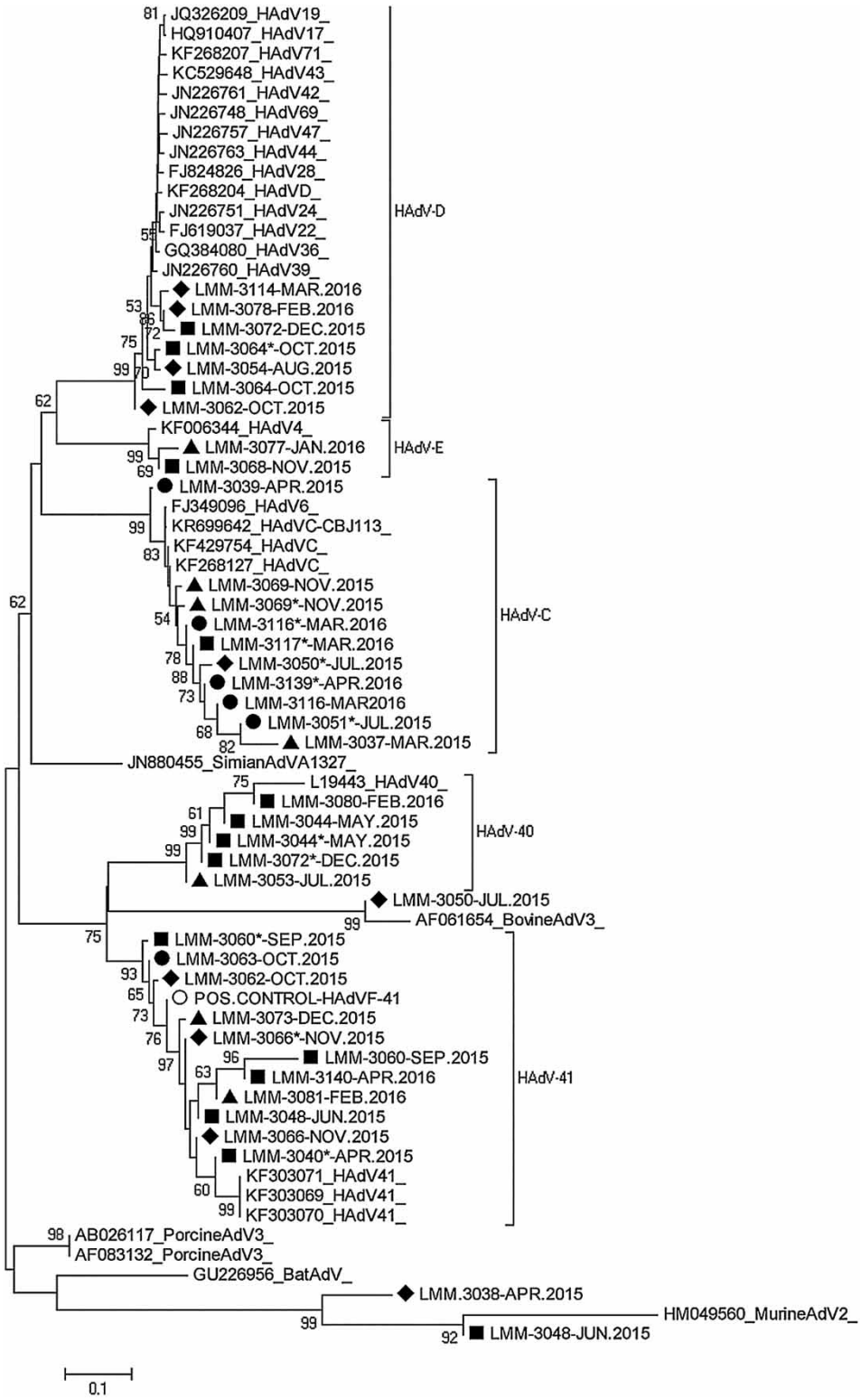

Figure 4 | Phylogenetic analysis showing the identification of six different AdV species (HAdV-C, HAdV-D, HAdV-E, HAdV-F, BAdV and MAdV) from unconcentrated and concentrated samples along four points of Belo Stream. * Unconcentrated samples; ◆ Point 1; ● Point 2; ■ Point 3; ▲Point 4.

acute gastroenteritis in children, and may also cause mortality in immunocompromised individuals. These viruses are released for long periods in feces, urine and respiratory secretions of infected persons. Thus, the high number of positivity found in this species, when compared with others, suggests a high incidence of HAdV-F infections in the population of the region of the present study (Jiang 2006; Filho *et al.* 2007; La Rosa *et al.* 2015).

AdV from other mammalian hosts were also detected, such as MAdV and BAdV. Recently, three types of MAdVs were described, and in this study, type 2 was identified. MAdVs have potential for vector screening in genes and cancer therapy, and may complement vector studies derived from HAdV. Furthermore, MAdVs provide insight into the pathogenesis process of the host, which includes acute and persistent infections (Nguyen *et al.* 1999; Weinberg *et al.* 2005; Klempa *et al.* 2009; Robinson *et al.* 2009; Hemmi *et al.* 2011). In spite of the low detection of BAdV (1.8% (1/55)) in the present study, it is worth noting that this virus can be used as a marker of fecal animal contamination in groundwater and surface waters in rural environment. Due to its structural characteristics, this type of the virus is more stable in the environment and may cause disease in animals. Bovine excrements are often applied to the soil as untreated fertilizer. Because of this, both groundwater and surface water can be contaminated through the dragging of fecal material to water due to the runoff. As a result of this, environmental degradation and the transmission of diseases caused by these pathogens are concerning, since the animals have access to contaminated rivers and streams (Spilki *et al.* 2009; Wong & Xagoraraki 2010).

In the face of the presented results, it is worth mentioning that the great diversity of AdV identified by the nested-PCR technique followed by sequencing represents a result that has not been reported in the literature. Most of the studies on diversity use techniques that are restricted to PCR or quantitative PCR (qPCR) using specific primers for a given species, thus requiring more detailed work (Filho *et al.* 2007; La Rosa *et al.* 2015; Wieczorek *et al.* 2015; Adefisoye *et al.* 2016; Barrios *et al.* 2016).

In general, AdV detection occurred during all the months of the study, and it can be stated that they did not present seasonally, a fact also observed by other studies (Wieczorek *et al.* 2015; Adefisoye *et al.* 2016). Among the months that presented the highest number of positive samples, the month of October 2015 stands out the most. According to INMET (National Meteorological Institute), the months with the highest rainfall in 2015 were September and October. Therefore, a possible reason for the increase in the number of positive samples in October is the increase in precipitation index during this period. Rainfall events can introduce a large quantity of microbial contaminants, which include human enteric viruses, through flow of contaminants and flow of fecal material to the sampling sites (Hata *et al.* 2014; Rodrigues *et al.* 2015).

In this study, the identification of AdV was performed by nested-PCR, which does not necessarily mean the presence of infectious viral particles, since PCR techniques detect both infective and non-infective genomes. However, literature confirms the risk of individuals getting sick when they bathe in water contaminated with AdV (Ogorzaly *et al.* 2010). In order to identify the true presence of infectious particles in water, PCR-integrated cell culture (ICC-PCR) studies should be performed (Rigotto *et al.* 2010).

CONCLUSIONS

The results obtained in the present study are rare in literature regarding diversity, since it is not always possible to find the same amount of species by using the nested-PCR technique. AdV belonging to the F group, an important virus that causes gastroenteritis, was present in most samples (in both unconcentrated and concentrated samples). The concentration step is necessary, for it allows the identification of a greater diversity and quantity of AdV in analyzed samples when compared with unconcentrated ones. However, results obtained from unconcentrated samples should be considered, since three different species of HAdV were found. Because they were detected in all analyzed months, the AdV detected did not present seasonally. The analysis of AdV in these waters reveals mainly human fecal contamination along Belo Stream, which demonstrates the inefficiency or the absence of adequate sewage treatment processes to remove AdV. Considering that part of this stream is used for leisure and recreation, such contamination can put the exposed population at risk.

ACKNOWLEDGEMENTS

The authors are grateful for the financial and structural support from University Feevale, the Brazilian Coordination for the Improvement of Higher Level Personnel (CAPES), the Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS), the Brazilian National Council for Scientific Development (CNPq), and the Institute of Environmental Sanitation/ University of Caxias do Sul.

REFERENCES

ABNT – Associação Brasileira de Normas Técnicas 1987a *Planejamento de amostragem de efluentes líquidos e corpos receptores – NBR 9897*. ABNT, Rio de Janeiro.

ABNT – Associação Brasileira de Normas Técnicas 1987b *Preservação e técnicas de amostragem de efluentes líquidos e corpos receptores – NBR 9898*. ABNT, Rio de Janeiro.

Adefisoye, M. A., Nwodo, U. U., Green, E. & Okoh, A. L. 2016 Quantitative PCR detection and characterisation of human adenovirus, rotavirus and hepatitis A virus in discharged effluents of two wastewater treatment facilities in the Eastern Cape, South Africa. *Food Environ. Virol.* **8** (4), 262–274.

Aw, T. G. & Gin, K. H. 2011 Prevalence and genetic diversity of waterborne pathogenic viruses in surface waters of tropical urban catchments. *J. Appl. Microbiol.* **110** (4), 903–914.

Barrios, J. C., Russomando, G. & Espínola, E. E. 2016 Diversidad de adenovirus detectados en niños menores de 5 años hospitalizados por infección respiratoria aguda baja en Paraguay, 2010–2013. *Pediatría (Asunción)* **43** (2), 115–122.

Bibby, K. & Peccia, J. 2013 Prevalence of respiratory adenovirus species B and C in sewage sludge. *Environ. Sci. Process Impac.* **15** (2), 336–338.

Bosch, A., Guix, S., Sano, D. & Pinto, R. M. 2008 New tools for the study and direct surveillance of viral pathogens in water. *Curr. Opin. Biotechnol.* **19** (3), 295–301.

Filho, E. P., Faria, N. R. C., Fialho, A. M., Assis, R. S., Almeida, M. M. S., Rocha, M., Galvão, M., Santos, F. B., Barreto, M. L. & Leite, J. P. G. 2007 Adenoviruses associated with acute gastroenteritis in hospitalized and community children up to 5 years old in Rio de Janeiro and Salvador, Brazil. *J. Med. Microbiol.* **56** (3), 313–319.

Fumian, T. M., Leite, J. P. G., Castello, A. A., Gaggero, A., Caillou, M. S. L. & Miagostovich, M. P. 2010 Detection of rotavirus A in sewage samples using multiplex qPCR and an evaluation of the ultracentrifugation and adsorption-elution methods for virus concentration. *J. Virol. Methods* **170**, 4246.

García, M. E. 2006 Virus en águas de consumo. *Higiene y Sanidad Ambiental* **6**, 173–189.

Ghebremedhin, B. 2014 Human adenovirus: viral pathogen with increasing importance. *Eur. J. Microbiol. Immunol.* **4** (1), 26–33.

Hata, A., Katayama, H., Kojima, K., Sano, S., Kasuga, I., Kitajima, M. & Furumai, H. 2014 Effects of rainfall events on the occurrence and detection efficiency of viruses in river water impacted by combined sewer overflows. *Sci. Total Environ.* **468–469**, 757–763.

Heller, L., Colosimo, E. A. & Antunes, C. M. D. F. 2003 Environmental sanitation conditions and health impact: a case-control study. *Rev. Soc. Bras. Med. Trop.* **36** (1), 41–50.

Hemmi, S., Vidovszky, M. Z., Ruminska, J., Ramelli, S., Decurtins, W., Greber, U. F. & Harrach, B. 2011 Genomic and phylogenetic analyses of murine adenovirus 2. *Virus Res.* **160** (1), 128–135.

Jiang, S. C. 2006 Human adenoviruses in water: occurrence and health implications: a critical review. *Environ. Sci. Technol.* **40** (23), 7132–7140.

Kidd, A. H., Erasmus, M. J. & Tiemessen, C. T. 1990 Fiber sequence heterogeneity in subgroup F adenoviruses. *Virology* **179** (1), 139–150.

Kimura, M. 1980 A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16** (2), 111–120.

Klempa, B., Krüger, D. H., Auste, B., Stanko, M., Krawczyk, A., Nickel, K. F., Überla, K. & Stang, A. 2009 A novel cardiotropic murine adenovirus representing a distinct species of mastadenoviruses. *J. Virol.* **83** (11), 5749–5759.

Kundu, A., Mcbride, G. & Wuertz, S. 2013 Adenovirus-associated health risks for recreational activities in a multi-use coastal watershed based on site-specific quantitative microbial risk assessment. *Water Res.* **47** (16), 6309–6325.

Kuo, H. W., Chen, L. Z. & Shih, M. H. 2015 High prevalence of type 41 and high sequence diversity of partial hexon gene of human adenoviruses in municipal raw sewage and activated sludge. *J Appl. Microbiol.* **119** (4), 1181–1195.

La Rosa, G., Della Libera, S., Petricca, S., Iaconelli, M., Donia, D., Saccucci, P., Cenko, F., Xhelilaj, G. & Divizia, M. 2015 Genetic diversity of human adenovirus in children with acute gastroenteritis, Albania, 2013–2015. *Biomed Res. Int.* **15**, 1–8.

Li, Y., Ge, X., Zhang, H., Zhou, P., Zhu, Y., Zhang, Y., Yuan, J., Wang, L. F. & Shi, Z. 2010 Host range, prevalence, and genetic diversity of adenoviruses in bats. *J. Virol.* **84** (8), 3889–3897.

Lodder, W. J., Schijven, J. F., Rutjes, S. A., de Roda Husman, A. M. & Teunis, P. F. M. 2015 Entero-and parechovirus distributions in surface water and probabilities of exposure to these viruses during water recreation. *Water Res.* **75**, 25–32.

Maurer, C. P., Simonetti, A. B., Staggemeier, R., Rigotto, C., Heinzelmann, L. S. & Spilki, F. R. 2015 Adenovirus, enterovirus and thermotolerant coliforms in recreational waters from Lake Guaíba beaches, Porto Alegre. *J. Water Health* **13** (4), 1123–1129.

Nguyen, T. T., Nery, J. P., Joseph, S., Rocha, C. E., Carney, G. E., Spindler, K. R. & Villarreal, L. P. 1999 Mouse adenovirus (MAV-1) expression in primary human endothelial cells and generation of a full-length infectious plasmid. *Gene Ther.* **6** (7), 1291–1297.

Ogorzaly, L., Bertrand, I., Paris, M., Maul, A. & Gantzer, C. 2010 Occurrence, survival, and persistence of human adenoviruses and F-specific RNA phages in raw groundwater. *Appl. Environ. Microbiol.* **76** (24), 8019–8025.

Ogorzaly, L., Walczak, C., Galloux, M., Etienne, S., Gassiloud, B. & Cauchie, H. M. 2015 Human adenovirus diversity in water samples using a next-generation amplicon sequencing approach. *Food Environ. Virol.* **7** (2), 112–121.

Paesi, S. & Magrini, F. E. 2015 Incidência de doença diarreica aguda em Caxias do Sul, Rio Grande do Sul, Brasil, em uma série histórica de 10 anos. *Sci. Med.* **25** (2), 1–8.

Prado, T. & Miagostovich, M. P. 2014 Environmental virology and sanitation in Brazil: a narrative review. *Cad. Saúde Pública* **30** (7), 1367–1378.

Rigotto, C., Victoria, M., Moresco, V., Kolesnikovas, C. K., Corrêa, A. A., Souza, D. S. M., Miagostovich, M. P., Simões, C. M. O. & Barardi, C. R. M. 2010 Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. *J. Appl. Microbiol.* **109** (6), 1979–1987.

Robinson, M., Li, B., Ge, Y., Ko, D., Yendluri, S., Harding, T., VanRoey, M., Spindler, K. R. & Jooss, K. 2009 Novel immunocompetent murine tumor model for evaluation of conditionally replication-competent (oncolytic) murine adenoviral vectors. *J. Virol.* **83** (8), 3450–3462.

Robinson, C. M., Singh, G., Lee, J. Y., Dehghan, S., Rajaiya, J., Liu, E. B., Yousuf, M. A., Betensky, R. A., Jones, M. S., Dyer, D. W., Seto, D. & Chodosh, J. 2013 Molecular evolution of human adenoviruses. *Sci. Rep.* **3** (1812), 1–7.

Rodrigues, M. T., Henzel, A., Staggemeier, R., De Quevedo, D. M., Rigotto, C., Heinzelmann, L. & Spilki, F. R. 2015 Human adenovirus spread, rainfalls, and the occurrence of gastroenteritis cases in Brazilian basin. *Environ. Monit. Assess.* **187** (11), 1–12.

Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4** (4), 406–425.

Santos, N. S. O. & Soares, C. C. 2015 Viroses entéricas. In: *Virologia Humana* (N. S. O. Santos, M. T. V. Romanos & M. D. Wigg Coord.) Guanabara Koogan, Rio de Janeiro, pp. 209–215.

Silva, H. D., Santos, S. F., Lima, A. P., Silveira-Lacerda, E. P., Anunciação, C. E. & Garcíazapata, M. T. 2011 Correlation analysis of the seasonality of adenovirus gene detection and water quality parameters based on yearly monitoring. *Water Qual. Expo. Health.* **3** (2), 101–107.

Sinclair, R. G., Jones, E. L. & Gerba, C. P. 2009 Viruses in recreational water-borne disease outbreaks: a review. *J. Appl. Microbiol.* **107** (6), 1769–1780.

Spilki, F. R. 2015 Crise hídrica, saúde e parâmetros de qualidade microbiológica da água no Brasil. *Revista USP* **106**, 71–78.

Spilki, F. R., Oliveira, L. K., Dalla Vecchia, A., Comerlato, J., Freza, R. & Silva, J. V. S. 2009 *Detecção e desinfecção de vírus em dejetos de ruminantes.* Revista Conhecimento Online, Novo Hamburgo, v. 1. Available from: http://aplicweb.feevale.br/site/files/documentos/pdf/32677.pdf.

Staggemeier, R., Heck, T. M., Demoliner, M., Ritzel, R. G., Röhnelt, N. M., Girardi, V., Venker, C. A. & Spilki, F. R. 2017 Enteric viruses and adenovirus diversity in waters from 2016 Olympic venues. *Sci. Total Environ.* **586**, 304–312.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011 MEGA5: molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28** (10), 2731–2739.

Weinberg, J. B., Stempfle, G. S., Wilkinson, J. E., Younger, J. G. & Spindler, K. R. 2005 Acute respiratory infection with mouse adenovirus type 1. *Virology* **340** (2), 245–254.

Wieczorek, M., Krzystoszek, A. & Witek, A. 2015 Species-specific identification of human adenoviruses in sewage. *Polish J. Microbiol.* **64** (1), 23–28.

Wong, K. & Xagoraraki, I. 2010 Quantitative PCR assays to survey the bovine adenovirus levels in environmental samples. *J. Appl. Microbiol.* **109** (2), 605–612.

Yates, M. V., Malley, J., Rochelle, P. & Hoffman, R. 2006 Effect of adenovirus resistance on UV disinfection requirements: a report on the state of adenovirus science. *J. Am. Water Works Assoc.* **98** (6), 93–106.

First received 27 June 2017; accepted in revised form 5 October 2017. Available online 3 November 2017