Distribution and genetic diversity of the human polyomaviruses JC and BK in surface water and sewage treatment plant during 2009 in Porto Alegre, Southern Brazil

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Abstract

The human polyomaviruses JC and BK (JCPyV and BKPyV) are ubiquitous, species-specific viruses that belong to the family *Polyomaviridae*. These viruses are known to be excreted in human urine, and they are potential indicators of human wastewater contamination. In order to assess the distribution of both JCPyV and BKPyV in urban water samples collected from a sewage treatment plant (STP) and from a canalized water stream of Porto Alegre, Brazil, two nested-PCR assays were optimized and applied to the samples collected. The amplicons obtained were submitted to sequencing, and the sequences were analyzed with sequences of human polyomaviruses previously deposited in GenBank. Twelve out of 30 water samples (40%) were JCPyV positive, whereas six samples (20%) were BKPyV positive. The sequencing results confirmed the presence of JCPyV subtypes 1 and 3, whereas only BKPyV Ia and Ib were found. This study shows for the first time the presence of human polyomaviruses in surface water and in samples collected in a sewage treatment plant in southern Brazil.

Keywords: BKPyV, human polyomaviruses, JCPyV, nPCR, urban wastewater, Arroio Dilúvio.

Distribuição e diversidade genética dos poliomavírus humanos JC e BK em águas superficiais e de estação de tratamento de esgoto durante 2009 em Porto Alegre, sul do Brasil

Resumo

Os poliomavírus humanos JC e BK (JCPyV e BKPyV) são virus ubíquos, espécie-específicos, pertencentes à família *Polyomaviridae*. Estes vírus são conhecidos por serem excretados pela urina humana, sendo considerados potenciais indicadores de contaminação por águas residuais urbanas. Buscando acessar a distribuição de JCPyV e BKPyV em amostras de águas coletadas de uma estação de tratamento de esgoto e de um arroio canalizado de Porto Alegre, Brasil, duas técnicas de nested-PCR foram otimizadas e aplicadas às amostras coletadas. Os amplificados obtidos foram submetidos ao sequenciamento e suas sequências analisadas com base em sequências de poliomavírus humanos previamente depositadas no GenBank. Doze de 30 amostras de água (40%) foram positivas para JCPyV, enquanto 6 amostras (20%) foram positivas para BKPyV. Os resultados do sequenciamento confirmaram a presença dos subtipos 1 e 3 de JCPyV, enquanto apenas os BKPyV Ia e Ib foram encontrados. Este estudo demonstra pela primeira vez a presença de poliomavírus humanos em águas superficiais e em amostras coletadas em uma estação de tratamento de esgoto na região sul do Brasil.

Palavras-chave: BKPyV, poliomavírus humanos, JCPyV, nPCR, efluentes de esgoto, Arroio Dilúvio.

1. Introduction

JC (JCPyV) and BK (BKPyV) are members of the family *Polyomaviridae* (ICTV, 2014). These are nonenveloped viruses containing double-stranded, closed-circular DNA genomes of approximately 5.2 kb. The genomes of these viruses have a common organizational structure and share a nucleotide identity of approximately 72% (Shah, 1995; Flint et al., 2015). Although ubiquitous in the population, human polyomaviruses usually induce diseases in immunocompromised individuals. JCPyV infections have been associated with Acquired Immunodeficiency Syndrome (AIDS) patients and with the development of progressive multifocal leucoencephalopathy (Kazory and Ducloux, 2003), whereas BKPyV may cause polyomavirus-associated nephropathy (PVAN), a major cause of kidney graft failure in kidney transplant patients (Costa et al., 2009). In addition, both species of polyomaviruses have also been related to some types of human cancers (Noch et al., 2012). Asymptomatic infections are common in humans. Serological and virological studies show that more than 80% of adults harbor antibodies to BKPyV or JCPyV (Shah et al., 1973; Bodaghi et al., 2009; Egli et al., 2009; Khanna et al., 2009), and both viruses have been found in the urine of up to 53.4% of asymptomatic, immunocompetent individuals (Chehadeh et al., 2013; Comerlato et al., 2015).

According to the ubiquitous distribution of human polyomaviruses, as well as to their biology and distribution, studies have been conducted to evaluate their potential as attractive indicators of sewage pollution (Bofill-Mas et al., 2000; McQuaig et al., 2006). JCPyV was first suggested as a possible indicator of water contamination by human sewage in 2000 (Bofill-Mas et al., 2000). Subsequently, several groups have reported the presence of JCPyV and also BKPyV in environmental waters (Fumian et al., 2010; Moresco et al., 2012; Ahmed et al., 2015).

Beyond the current studies about JCPyV and BKPyV in the tracking of human pollution, investigations on the genomic variation of different isolates could also be helpful in the assessment of the subtypes' distribution in the population. Based on their nucleotide sequence, BKPyV isolates are classified into four subtypes: I, II, III, and IV (Sharma et al., 2006). Subtype I is prevalent throughout the world, subtype IV shows a geographical distribution biased for East Asia, and subtypes II and III are rarely detected (Zhong et al., 2009). JCPyV shows a higher degree of genomic variability, and it is classified into eight subtypes. Types 1 and 4 have been detected in Europeans, types 3 and 6 in Africans, types 2 and 7 in Asians, and type 8 has been described only in Papua New Guineans, whereas type 5 seems to result from recombination events of other types (Agostini et al., 2001; Yanagihara et al., 2002; Rafique and Jiang, 2008). As each JCPyV subtype is present in specific human ethnicity groups, their phylogenetic analysis has been associated with human migration patterns (Stoner et al., 2000).

Porto Alegre, in Southern Brazil (1.51 million inhabitants; Porto Alegre, 2011), is covered by 12 sewage treatments plants (STPs). The STP called São João/Navegantes receives urban sewage from eleven neighborhoods of the north region of the city (Porto Alegre, 2011). Sewage treatment is performed by activated sludge treatment plants, a methodology that seems to be efficient in removing coliforms and viral agents (Funderburg and Sorber, 1985). On the other hand, the municipality still suffers from the lack of sewage treatment in other parts of the city, and the Arroio Dilúvio, a stream crossing the city from west to south, is the recipient of great amounts of non-treated wastewater. The Brazilian national rules concerning the effluent water treatment accept the presence of up to 200 thermotolerant coliforms in each 100 mL of effluent water. On the other hand, the detection of viruses is suggested but not mandatory (Brasil, 2011, 2005).

Based on the suggestion that JCPyV and BKPyV could be indicators of human pollution, this study aimed to assess the distribution and genetic diversity of these viruses in water samples collected from STP São João/Navegantes and the Arroio Dilúvio stream. The results obtained here support the new strategies and studies aiming at an improved assessment of water quality control, and they contribute to the knowledge about the human polyomaviruses JC and BK distribution in this region.

2. Material and Methods

2.1. Collection of water samples

The water samples were collected from two locations in Porto Alegre: Arroio Dilúvio and STP São João/Navegantes. Arroio Dilúvio is a water stream that crosses the city of Porto Alegre. The canalized portion of Arroio Dilúvio comprises a path of approximately 14 km, flowing into the Guaíba Lake, which is the main source of water for Porto Alegre (Porto Alegre, 2011). Fifteen water samples were collected from five points along the course of Arroio Dilúvio in January, April, and September of 2009. These same samples were also analyzed in the study of Vecchia et al. (2012a) (see Figure 1).

STP São João/Navegantes receives urban sewage from eleven neighborhoods of the north region of the city and assists approximately 150,000 inhabitants (Porto Alegre, 2014). Monthly, two water samples were collected between March and October of 2009. These samples were collected as described in Vecchia et al. (2012b). In total, eight samples of the influent, the pre-treatment sewage and eight of the effluent, post-treatment sewage were collected and analyzed.

The water samples (500 mL) were collected aseptically, taken directly from in sterilized glass bottles. The samples were transported to the laboratory under refrigeration (4 °C) and were kept under this condition until sample concentration.

2.2. Sample concentration

Putative viral particles were concentrated using an adsorption-elution method with negatively charged membranes (HA, Millipore, USA), as described previously

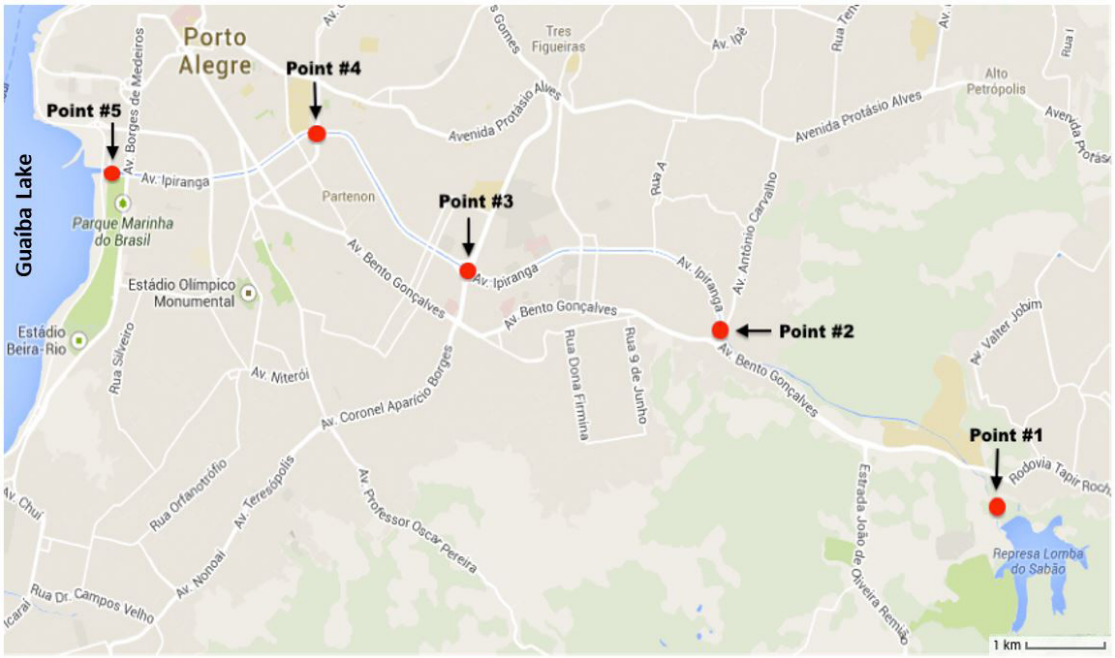

Figure 1. Satellite view of Porto Alegre, showing the collection points along the Arroio Dilúvio from the source (Point 1, East) to the end (Point 5, West) (shown as dots and pointed by black arrows). Figure made with the aid of Google Earth Protm.

by Katayama et al. (2002) with minor modifications (Vecchia et al., 2015). Briefly, 500 mL of each water sample were mixed with 0.3 g $MgCl_2$, and the pH was adjusted to 5.0 with 10% HCl. Subsequently, the resulting mixture was filtered through a type HA negatively charged sterile membrane (0.45 µm pore size; 47 mm diameter). The membrane was rinsed with 87.5 mL of 0.5 mm H_2SO_4 (pH 3.0), followed by elution of viral particles adsorbed to the membrane with 2.5 mL of 1 mM NaOH (pH 10.5). The filtrate was then neutralized with 12.5 µL of 50 mM H_2SO_4 and 12.5 µL of 100 × Tris-EDTA (TE) buffer. The resulting mixture was stored at –80 °C until further processing.

2.3. Viral nucleic acid extraction

Viral nucleic acids were extracted from 400 µL of the concentrated water sample using the RTP® DNA/RNA Virus Mini Kit (Invitek, Berlin, Germany) according to the manufacturer's instructions. The viral DNA obtained was kept at –80 °C until analysis.

2.4. Construction of an internal control molecule

In order to check whether the DNA extracted from water samples would present any inhibitory effect on the PCR, an internal control (IC) molecule was constructed to be amplified by the primers BJ2 (PF 5'- AACATTTTCYCCTCCTG -3') and BJ1 (PR 5'- TATTGCMCCAGGAGGT -3'), primers used in the first PCR to amplify BKPyV and JCPyV DNA. These primers were the same used by Bofill-Mas et al. (2000). The IC consisted of a 540 base pair (bp) long fragment, arising from an unspecific amplification in a low annealing temperature and cloned in pCR 2.1 plasmid (Invitrogen). The IC was sequenced and showed 100% identity with a segment of the human genome, which corresponds to part of the intraflagellar transport complex B protein present in ciliated human cells. The detection of the IC product in the absence of a viral product implied a negative result or at least a result below the detection limit of the method. On the other hand, the lack of amplification of both the IC and viral products implied an amplification failure and a false negative result. The discrimination of the IC and viral products was achieved following the electrophoresis analyses when the differences in the fragments' sizes were observed.

2.5. PCR assay to detect JCPyV/BKPyV simultaneously

The first PCR was carried out using the external primers BJ2 and BJ1, which anneal to a region localized between the VP1 and VP2/VP3 genes of both viruses. The resulting amplicons are 797 bp and 773 bp long for BKPyV and JCPyV, respectively. Modifications were introduced in the method described by Bofill-Mas et al. (2000). Each reaction was performed in 25 µL reaction mixtures containing 3 mM of $MgCl_2$ (Invitrogen), 0.4 µM of each primer (IDT), 1.5 U Taq DNA polymerase (Invitrogen), 10% PCR buffer (Invitrogen), and 0.6 mM dNTPs (ABgene) per reaction. In each reaction, 65 molecules of the IC were added. Reactions were performed in an Eppendorf Mastercycler under the following cycle conditions: 5 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 42 °C, and 1 min and 15 seconds at 72 °C. A final elongation period of 5 min at 72 °C was applied. Positive

controls of both viruses were used in every set of ten reactions. For BKPyV, a known positive sample was the positive control, whereas the positive control of JCPyV was a plasmid containing the whole JCPyV genome strain Mad-1 cloned in pBR322, a kind gift from Dr. Rosina Gironés from University of Barcelona, Department of Microbiology and Andrew M. Lewis from the Office of Vaccine Research and Review, CBER/FDA, MD, USA. BK: KC534839.

2.6. Nested PCR to discriminate between JCPyV and BKPyV

To differentiate JCPyV and BKPyV, two nested PCRs were developed using the product of the first PCR as a template. The primers used here were designed based on the work previously described by Bofill-Mas et al. (2000). The JCPyV-specific DNA primers were as follows: JLP16 (PF 5'- TAAAGCCTCCCCCCCAACAGAAA -3') and JLP15 (PR 5'- ACAGTGTGGCCAGAATTCCACTACC -3'). These are expected to give rise to a product that is 215 bp long. To detect BKPyV, the primer pair BK6 (PF 5'- CCAGGGGCAGCTCCCAAAAAG -3') and BK4 (PR 5'- AGTAGATTTCCACAGGTTAGGTCCTC -3') was used to give rise to a 296 bp long amplicon. Both amplicons correspond to part of the major capsid protein VP1 genome. The nPCRs were performed in 25 µL reaction mixtures containing 1 mM of $MgCl_2$ (Invitrogen), 0.2 µM of each primer (IDT), 1.5 U Taq DNA polymerase (Invitrogen), 10% PCR buffer (Invitrogen), and 0.6 mM dNTPs (ABgene). The cycling conditions were 5 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C, and a final extension time of 5 min at 72 °C.

2.7. Sequencing and phylogenetic analysis

To confirm the identity of the amplicons, nPCR products were submitted to sequencing in an automatic sequencer (ABI-PRISM 3100 Genetic Analyzer). The BigDye™ Terminator Chemistry was used (Applied Biosystems, Foster City, CA). A sequence analysis was performed with the BLAST software, and the sequences were aligned with the BioEdit Sequence Alignment Editor program. The BKPyV and JCPyV sequences used in the alignments were obtained from the Genbank database and from a previous report in which the presence of these viruses were demonstrated in urine samples of humans (McQuaig et al., 2006). Phylogenetic analysis was carried out using MEGA 5.0 for both trees. The neighbor-joining method and the maximum likelihood method were used to build the phylogenetic trees. Statistical significance of clades was measured by 500 bootstrap replicates.

3. Results

3.1. Sensitivity of the first PCR assay

The sensitivity of the first PCR reaction was determined using tenfold serial dilutions of known quantities of IC molecules. Amplicons were easily observed on an ethidium bromide-stained agarose gel when the PCR was performed with the addition of between 65 and 6 IC molecules (data not shown). This implies that the lower detection limit of the PCR was of about 6 IC molecules.

3.2. Prevalence of BKPyV and JCPyV

Water samples were taken at five sampling sites along the water stream Arroio Dilúvio and from STP São João/Navegantes in Porto Alegre. From the fifteen samples collected along the Arroio Diluvio, only fourteen were analyzed, one of them was lost during the process. One of the Arroio Dilúvio' samples was positive only for BKPyV (7.1%), while 4 samples were positive only for JCPyV (28.6%) and four samples (28.6%) were positive for both viruses (Table 1). The analysis of virus DNA obtained from the water samples collected from STP São João/Navegantes showed that five out of 16 samples were positive for virus DNA. A higher frequency of positive samples was found in the influent samples (Table 2). Among these samples, JCPyV was more frequently detected (3/8 or 37.5% of the samples), while only 1 sample was BKPyV positive (12.5%). In the effluent, only one sample, collected in July, was positive for JCPyV (Table 2).

3.3. Characterization of JCPyV and BKPyV DNA

Five amplicons of each polyomavirus detected were submitted to sequencing. The sequences were analyzed and compared with sequences available in GenBank using the BLAST program. The JCPyV strains detected here clustered mainly in two different groups, 1 and 3 (see Figure 2). The BKPyV positive samples were all characterized as subtypes Ia and Ib (see Figure 3).

Table 1. Detection of BKPyV and JCPyV DNA in water samples collected from the Arroio Diluvio, Porto Alegre, Brazil.

Harvesting points	Period of collection					
	January		April		September	
	BKPyV	JCPyV	BKPyV	JCPyV	BKPyV	JCPyV
P 1	+ [a]	+	-	+	+	+
P 2	+ [a]	-	-	-	-	-
P 3	-	-	-	+	-	-
P 4	-	+ [a]	+ [a]	+	+ [a]	+ [a]
P 5	-	-	-	+	N/T	N/T

[a]Sequenced samples; + (Positive); – (Negative); N/T (Not tested).

Table 2. Detection of BKPyV and JCPyV DNA in water samples collected from STP São João/Navegantes, Porto Alegre, Brazil.

		Influent		Effluent	
		BKPyV	JCPyV	BKPyV	JCPyV
Period of collection	March	-	+	-	-
	April	-	-	-	-
	May	-	-	-	-
	June	-	-	-	-
	July	+ [a]	+ [a]	-	+ [a]
	August	-	+ [a]	-	-
	September	-	-	-	-
	October	-	-	-	-

[a]Sequenced samples; + (Positive); – (Negative).

4. Discussion

The present study shows the detection of BKPyV and JCPyV in environmental water samples collected in Porto Alegre, Southern Brazil. The nPCR assay used here, based on the study developed by Bofill-Mas et al. (2000), allowed the detection of BKPyV and JCPyV positive water samples with a high sensitivity. In addition, as the target of the nPCR is the VP1 gene, which encodes the major capsid protein and is responsible for the antigenic variability among these viruses, its amplification and sequence analysis allowed the phylogenetic characterization of these samples (Jin et al., 1993).

To be sure that polyomavirus DNA could be amplified in the presence of inhibitors, which are commonly contaminating

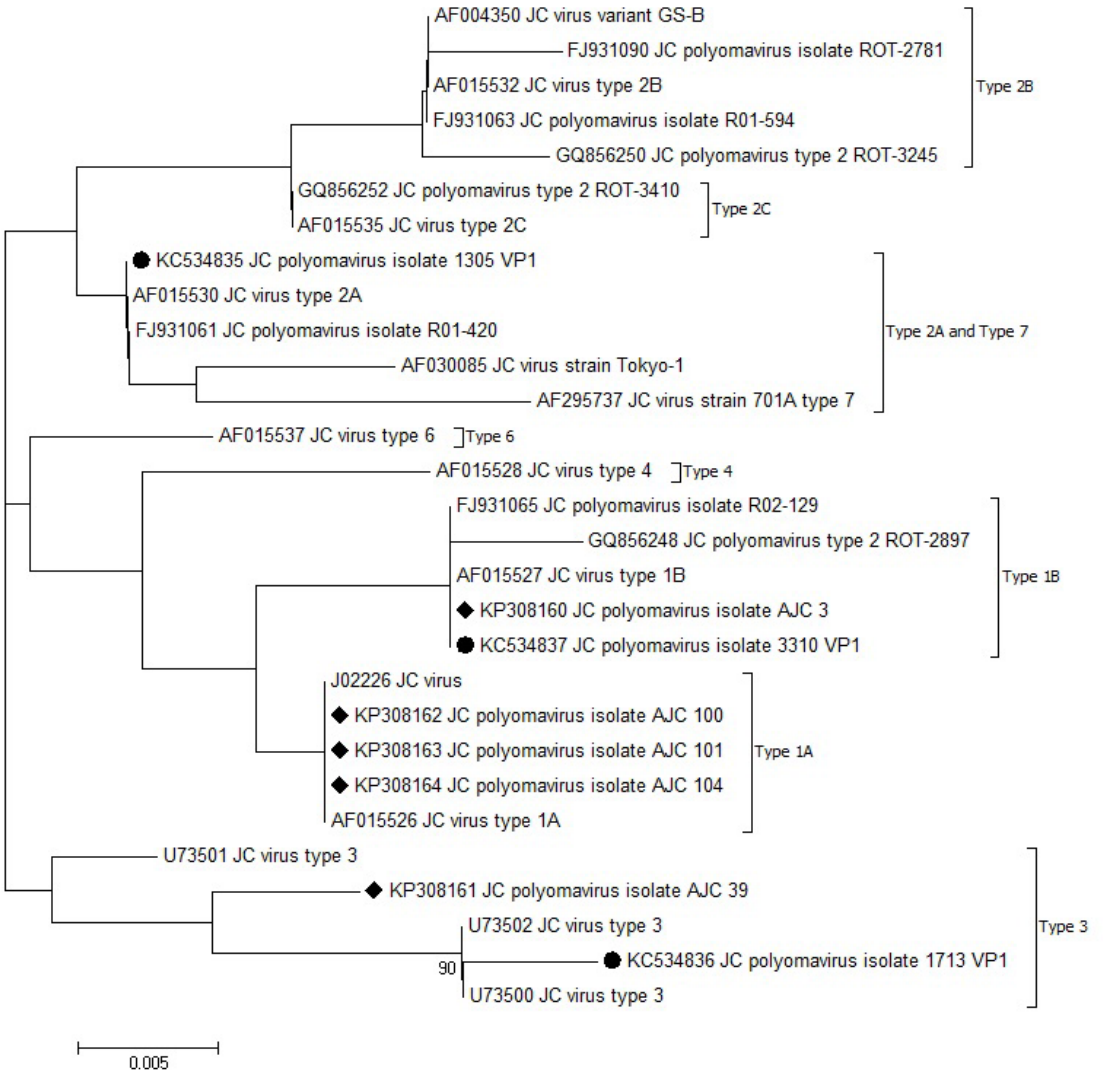

Figure 2. Phylogenetic analysis of the nucleotide sequences of a 215bp fragment of the VP1 region of JCPyV. To elaborate this tree 29 nucleotide sequences were aligned. Five water samples were analyzed and the JCPyV genotypes found were compared with JCPyV sequences obtained from urine samples and other sequences available in GenBank. The black rhombuses represent the analyzed water samples, whereas the black circles refer to the urine samples.

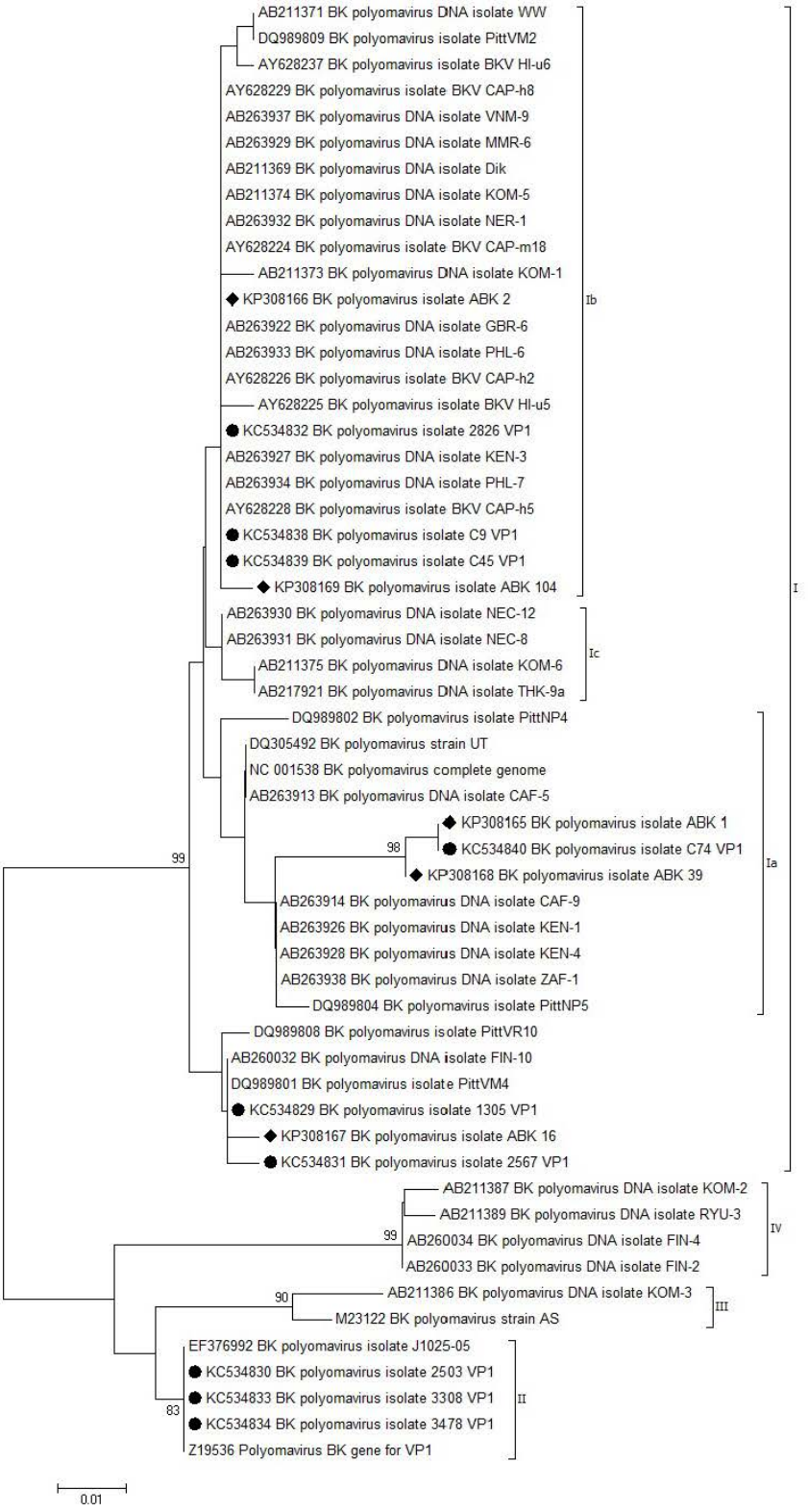

Figure 3. Phylogenetic analysis of the nucleotide sequences of a 296bp fragment of the VP1 region of BKPyV. To elaborate this tree 56 nucleotides sequences were aligned. Five water samples were analyzed and the BKPyV sequences found were compared with sequences of urine samples and other sequences available in GenBank. The black rhombuses represent the analyzed water samples, whereas the black circles refer to urine samples.

environmental samples, an IC molecule was constructed that could be amplified with the same primers as the ones used for the amplification of polyomavirus DNA. The IC construct used here was shorter than the polyomavirus product and could then be easily differentiated from the viral product on gel. The construct was added to each PCR reaction. The absence of both a viral product and an IC product implied an amplification failure. On the other hand, the presence of the IC product in the absence of a viral product implied a negative result, at least a result below the detection limit of the first PCR method. The IC construct was used here particularly to detect false negative results, as described in previous studies (Campos et al., 2009; Oliveira et al., 2011).

Of the samples collected from Arroio Dilúvio, virus DNA was found at a higher frequency among the samples collected from harvesting points 1 and 4. Point 1 is located at a low environmental impact area (Menegat, 2006) and, at first, the suggestion of sewage contamination at this point was not expected. However, the use of this site for touristic activities and the presence of low cost dwellings without sewage connection may explain these results. In addition, in a previous study, enteric viruses DNA were also found in this location (Vecchia et al., 2012a). On the other hand, point 4 is the closest to Guaíba Lake, it is located at a high environmental impact area (Menegat, 2006), and the presence of virus DNA in these samples was expected. This same harvesting point was also found to be contaminated by enterovirus and torque teno virus (TTV) in a previous study (Vecchia et al., 2012a).

Polyomavirus DNA, especially from JCPyV, was found more frequently in the second collection period (April) in all harvesting points. These results could be related to climatic factors such as precipitation and temperature. The precipitation level in April 2009 was reduced when compared with other periods of the year (169 and 293 mmHg in January and September, respectively, against 31 mmHg in April) (INMET, 2009). The reduced precipitation could be related to the high frequency of virus nucleic acid found here, however, the same correlation was not observed when Vecchia et al. (2012a) described the presence of nucleic acids of different enteric viruses in the same samples. Environmental parameters such as water temperature, air humidity and insolation may indeed be associated to viral detection (Wong et al., 2009). However, here, anthropogenic factors may be the main determinant of viral detection. The comparison between these previous results and the results shown here indicate that JCPyV may be, among other viruses, a good choice to be used in virological analysis of waters.

Most of the samples collected from the STP São João/Navegantes were free of polyomavirus DNA, except from the influent and effluent collected in July and the influents collected in March and August. Most of the positive samples consisted of influents and contained JCPyV DNA. In a previous study, the same samples were analyzed in search of enteric viruses, and the highest detection rate observed was for adenoviral genomes (37.5%) (Vecchia et al., 2012a). In that study, samples of effluents were more frequently positive than samples of influents. Our results are limited to the analysis of monthly collected samples during only one year; in addition, it is important to highlight that the presence of virus DNA does not implicate in the presence of viable virus particles. However, the presence of virus DNA in the effluent samples suggests that the sewage treatment used by the STP São João/Navegantes does not eliminate virus particles in total, and viable viruses might be present in these samples.

In Brazil, Moresco et al. (2012) analyzed 132 seawater samples collected from 11 beaches of Florianopolis Island for the presence of different enteric viruses, and the most prevalent virus detected was the human adenovirus (55%), whereas JCPyV DNA was found in only 3% of all samples. However, in a study developed by Fongaro et al. (2012), also in Florianopolis, JCPyV DNA was found in 21% of the water samples collected from a lagoon. This data is similar to our results. Nevertheless, in search of virus DNA in sewage samples collected in Rio de Janeiro (Brazil), JCPyV was found in 96% and 43% of raw and treated sewage samples, respectively (Fumian et al., 2010). Such results suggest a reduction of viral loads in Rio de Janeiro and agree with the results presented here. These differences in percentages of detection may be due to several factors, including different concentration methods, sensitivity differences in the molecular assays performed, and differences in the composition of the environmental sample evaluated (Girones et al., 2010).

The JCPyV and BKPyV were ubiquitous in untreated sewage and septage samples collected in Florida and California, USA. This study, conducted by McQuaig et al. (2009), employed a qPCR able to amplify both viruses, differentiating BKPyV of JCPyV only by sequencing. They found a similar frequency between the viruses in the environmental samples. However, in Japan, only 5.6% of a total of 18 river water samples were positive to JCPyV DNA detection, while no sample was positive for BKPyV (Haramoto et al., 2010). The present study also corroborates the results found by other authors who have reported a wide distribution of human polyomavirus DNA in environmental water samples. These studies have been carried out in Cairo, Egypt; Patras, Greece; Barcelona, Spain; Nancy, France; Pretoria, South Africa; Umea, Sweden; Tokyo, Japan; California and Washington, USA; and Germany (Bofill-Mas et al., 2000; Albinana-Gimenez et al., 2006; Bofill-Mas et al., 2006; Rafique and Jiang, 2008; Hamza et al., 2009; Haramoto et al., 2010).

4.1. JCPyV and BKPyV characterization

JCPyV has been studied as a marker of human populations due its role as a human pathogen since the emergence of modern humans 100,000-200,000 years ago. Therefore, the characterization of this virus in a specific geographic area allows the knowledge on the population diversity in the local of study (Stoner et al., 2000). In addition, the JCPyV strains found in sewage and other urban water bodies seems to represent the viruses that are excreted by

the local population. In this study we found JCPyV 1 and 3, which are associated with populations of European and African origins, respectively - corroborating with the structure of the south of the Brazilian population (Parra et al., 2003). Previous studies performed in other regions of Brazil have also demonstrated the presence of these JCPyV subtypes population (Fumian et al., 2010; Fink et al., 2010).

The characterization of BKPyV has been mainly focused on the involvement of specific BKPyV subtypes with the development of diseases in humans, and there is limited data on the characterization of BKPyV obtained from environmental samples (Albinana-Gimenez et al., 2006; Montagner et al., 2010; Pires et al., 2011; Machado et al., 2011; Zalona et al., 2011). Here, only BKPyV Ia and Ib were found in the water samples. A study that suggested the co-migration hypothesis of BKPyV, detected primarily in subgroup Ib in the American and European populations (Zhong et al., 2009), similar to our results. In Brazil, Zalona et al. (2011) published the only report on the molecular characterization of BKPyV - positive samples. These authors detected BKPyV in renal transplant patients of Rio de Janeiro and found the subtypes I, II, and III.

The same subtypes of JCPyV (1 and 3) and the subgroup of BKPyV (I) characterized in the water samples were found in a previous study with urine samples collected in the same geographic region (Comerlato et al., 2015), reinforcing that the polyomaviruses detected in urban waters belong to the same subtypes that have been excreted by the local population. This is the first report on the presence and genetic diversity of BKPyV and JCPyV circulating in this region of the South of Brazil.

5. Conclusion

The present study demonstrated the circulation of JCPyV and BKPyV in environmental waters of the city of Porto Alegre, in the south of Brazil. According to our results, the higher frequency of JCPyV in water samples seems to indicate that JCPyV is a better microbial source tracking than BKPyV. The subtypes and subgroups found here could be related with the population diversity of the geographic area of this study. The widespread geographic distribution of these human polyomaviruses encourages the use of molecular assays for the worldwide detection of JCPyV and BKPyV, making them potential viral markers for human waste contamination within the environment.

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