Occurrence of Polyomaviruses in Recreational Freshwaters from Southern Brazil

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ABSTRACT

There is little information about the transmission pathways of polyomaviruses. Since many of them are regularly found in urine and feces, the possibility that the waterborne route could transmit these viruses has not been explored. The aim of this study was to evaluate the presence of human polyomaviruses JCPyV, BKPyV, MCPyV, STLPyV and TSPyV along the Belo River (Caxias do Sul-Rio Grande do Sul-RS, Brazil). Four points were investigated, with 13 monthly samplings (March/2015 - March/2016). A total of 52 samples were concentrated by ultracentrifugation, total genomic material was extracted using commercial extraction kit and human polyomaviruses were investigated through real time polymerase chain reaction. A total of 32/52 (61.5%) positive samples were found. Only JCPyV and BKPyV were identified, and they were present in all sampling points. The average viral load of JCPyV was $7 \times 10^5$ copies/mL and of BKPyV was $5 \times 10^4$ copies/mL after particle concentration process. Finding such polyomaviruses in this environment suggests contamination through human waste and reinforce the notion that fecal-oral route may represent an important transmission mode for these viruses.

Introduction

The Polyomaviridae family consists of 100 viruses described so far, of which 13 can infect humans and 87 infect mainly fish, birds and other mammals [1-3]. Their genomes have relatively high similarity to each other and are homologous to SV40 polyomavirus [4]. Polyomaviruses have DNA genomes with approximately 5k bases that is divided into 3 regions named early, late and non-coding control region [1,2]. The first polyomavirus described was BK (BKPyV), which causes persistent subclinical infection in the kidneys of healthy individuals. In immunocompromised individuals, especially those undergoing renal transplantation, reactivation of BKPyV may cause clinical complications such as BKPyV- associated nephropathy and loss of the graft in the transplanted individual [5]. JC polyomavirus (JCPyV), discovered almost at the same time as BKPyV, is the etiologic agent of an extremely severe disease affecting also immunocompromised individuals, the progressive multifocal leukoencephalopathy (PML) [1,3]. MCPyV (Merkel-cell carcinoma polyomavirus), described in 2008 is the etiologic agent of an aggressive neuroendocrine carcinoma (Merkel Cell Carcinoma - MCC) that targets Merkel cells [6]. TSPyV was identified and sequenced from fragments of injured skin of an immunocompromised individual after cardiac transplantation with clinical evidence of a very rare skin cancer named Tricodisplasia spinulosa [7]. And, one of the most recent human polyomaviruses described is the Saint-Louis polyomavirus (STLPyV). It was detected in human feces by pyrosequencing8, but so far there is no association of STLPyV with diseases.
The mode of transmission of polyomaviruses is still poorly understood, and multiple routes of infection including the contact with urine, feces, saliva and blood are likely [9-11]. Therefore, finding such viruses in water effluents may add a piece to the understanding the polyomaviruses transmission routes. Another concern regarding the presence of these viruses in waters regards to its oncogenic potential [12]. Waterborne diseases represent a serious public health problem in developing and undeveloped countries [13]. Secondary data from health reporting systems highlight viruses as as responsible for more than 7% of the cases of pathologies associated with water infection [14]. The frequency of these pathogens in water matrices and the negative effects on public health indicate the need to dispose of the wastewater before it is discharged into the environment [15]. Recreational water activities are common practice in countries with tropical climate as Brazil and contamination of water bodies can occur by polluting sources as domestic, agricultural and industrial effluents. In fact, recreational use is the main responsible for the onset of illnesses caused by contact with water, leading to disease outbreaks mainly during holidays and periods of warm weather [16,17].

In Brazil, the quality of water for recreational activities must comply with the standards established by CONAMA (Resolution 274 of November 29, 2000). According to this resolution, the fresh water bathing conditions are defined according to the counts of thermotolerant coliforms (E. coli). However, it does not necessarily demonstrate the human origin of fecal material since E. coli is part of human and animal gut microbiota. Although there is no direct relation between coliforms and enteric viruses as indicators of fecal pollution, both parameters can be used as indicators of human waste contamination [18-21]. Some viruses are stable and often found in aquatic environments remaining potentially infectious for long time. Direct and prolonged exposure to enteric virus (i.e. Adenovirus, Rotavirus, Hepatitis A and Enterovirus) is leading cause of gastroenteritis [22,23,17]. Nevertheless, it has been discussed that other viruses, not necessarily related to enteric diseases, would serve as markers for human waste contamination. As an example, the human specific polyomaviruses, which are frequently shed in urine and feces, are very resistant to the environment, are worldwide prevalent and present no seasonality [24]. Some of them can even resist to environmental conditions and to water and sewage treatment since these processes are generally intended to bacterial control [25].

Belo River is one of the tributaries of the Caí River, located in the southwest of the city of Caxias do Sul and contributes to the formation of the Guaíba basin, responsible for the public supply of the capital Porto Alegre. This river has its sources in the urbanized and industrialized areas of Caxias do Sul, thus contributing with domestic and industrial effluents. During its journey, the river is also impacted with effluents from agricultural activities and livestock and close to these activities the waterway is used for leisure [26]. The aim of the present study was to evaluate the presence of human polyomaviruses in samples of the water source collected at distal points along the river flow.

Materials and Methods

The Belo River in Caxias do Sul, RS drains 21% of the city’s urban perimeter and has an area of 75.10 km² and a perimeter of 63.11 km (479,000 inhabitants). Samplings were carried out monthly from March 2015 to March 2016 at four points along the Belo river, resulting in 52 samples (50mL) collected from the surface. The local meteorological estimates were also evaluated. Description of the sampling points and respective GPS coordinates are described in Table 1. Concentration of viral particles was performed by the ultracentrifugation protocol described by Ruskowski [27]. Briefly, a 36mL aliquot was separated from each sample and centrifuged at 41,000Xg at 8°C for three hours. The pellet was re-suspended in 2 mL TE buffer (Tris-EDTA pH 8.0). To allow the material to be used in further studies, total genetic material (RNA and DNA) was extracted using the BioPur® kit from an initial volume of 200μL according to manufacturer instruction. cDNA was synthesized and template containing both DNA + cDNA was used for further experiments. TSPyV molecular detection was performed [28] using TaqMan method. Detection of JCPyV and BKPyV was performed according to Pal et al. [29] also using TaqMan methodology.

Table 1: Name, description and GPS coordinates of collection points along the Belo river.

| Collect Point | Description | GPS Coordinates |
|---------------|-------------|-----------------|
| P1            | Located within the urban area of Caxias do Sul, receives effluent of domestic origin, is upstream of the effluent release from an animal processing industry. It consists of the point of evaluation of the quality of a stream before the contribution of the industrial effluent. | 478024 6770622 |
| P2            | Located in the urban region, it is an established point for monitoring the quality of Belo river after its encounter with the industrial effluent. | 478845 6770010 |
| P3            | Located downstream of the confluence with the monitored stream (P1 and P2), in peri-urban area characterized by native vegetation and agricultural activities. This region receives domestic effluents. | 479051 6769817 |
| P4            | Located after a sewage treatment plant. It comprises the farthest point of the urbanized area upstream of the Belo river basin, on the Caí river. It is characterized by areas of native vegetation and agricultural activities and is used for recreation and aquatic recreation. | 482424 6757598 |
The MCPyV primers were described by Goh et al. [30] and the primers used for STLPyV detection were designed based on reference sequence JX463183.1 using the online primer3 tool [31] (Forward - TACCATTTGCGCCTAA; Reverse - TTTGTTCACTTG- GGGAGAAT) generating a 179bp fragment. In house Real-time PCR (qPCR) protocols were developed for detection of both viruses: Real-time PCR mixes were prepared using 5μl of DNA, 12.5μl of SYBR® Green PCR Master Mix (Applied Biosystems®), 0.5μM of each primer and 5.5μl of MilliQ water for final volume of 20μl. The mixes were cycled 45 times at a Tm of 60°C in an ABI 7300 Real Time (Applied Systems®) and both reactions presented sensitivity of 1E+03 copies/ mL (data not show). All samples were tested in duplicate. For statistical analysis the chi-square test was used with a significant level of 0.05.

Results

A total of 32/52 (61.5%) of samples tested positive for polyomavirus. From these, JCPyV represented 55.8% (29/52), BKPyV was present in 26.9% (14 samples) and 21% of the samples was positive for both viruses. No other polyomaviruses investigated were identified. Regarding to the collect points, 30% of the positive samples were found in P1, 33.3% in P2, 20% in P3 and 23.3% in samples collected in P4 (treated wastewater). The results classified by viruses and by collection points are shown in Table 2. There was no significant difference on the distribution of positive samples according to collection points (p=0.37), nor according to seasonality (p=0.03). Analyzing the viral load (VL) found in positive samples, JCPyV presented an average VL of 7E+05 copies/mL (5E+01 to 1.60E+03 copies/mL), whereas positive samples for BKPyV presented a VL of 5E+04 copies/mL (8E+00 to 1.8E+03 copies/mL) after particle concentration process.

Table 2: Polyomavirus positivity according to the collect points.

| Collect Point | Virus | P1 | P2 | P3 | P4 |
|---------------|-------|----|----|----|----|
| JCPyV         | 14.2% | 28.6% | 28.6% | 28.6% |
| BKPyV         | 31%   | 34.5% | 10.3% | 24.25% |

The average annual temperature of the municipality is 16.3°C. The warmest months are January and February, both averaging 20.6°C, while June is the coldest, averaging 12.1°C (BDMEP - Historical Series - Daily Data - Minimum Temperature (°C) - Caxias do Sul - National Institute of Meteorology); the average of the annual rainfall index was 120 mm (BDMEP - Historical Series - Daily Data - Precipitation (mm) - Caxias do Sul. National Institute of Meteorology). No seasonality was seen in the present data since the detection of the JCPyV and BKPyV DNA occurred in all months. The rainfall index and temperature variation during the seasons did not impact on the positivity found suggesting no variability between warmer (rainy) and colder months. For example, the highest rainfall index (199mm) was recorded in September and the lowest rainfall index (118mm) was recorded in May. Both months presented 3 positive samples each.

Discussion

Samples collected in water resources with anthropogenic influence can harbor enormous microbiological diversity. In this work the presence of polyomaviruses JCPyV, BKPyV, MCPyV, STLPyV and TSPyV was evaluated and although polyomaviruses have already been found in human fluids1-5 including urine and feces, only JCPyV and BKPyV were found. BKPyV and JCPyV are globally distributed. Seroepidemiological data show that between 60 and 80% of adults in the US and Europe carry these viruses, suggesting a common exposure route [32-35]. Despite the plenty of studies on distribution and excretion, the transmission routes of these viruses remain unknown. We detected JCPyV and BKPyV in all sampling points, ranging from 10 to 31% of positivity according to the local. These findings agree to the high prevalence of polyomavirus shedding in Brazil, ranging between 62% and 80% in immunocompetent and immunocompromised individuals respectively [36-38]. Besides the urine shed, it was already demonstrated that polyomaviruses are also found in feces of children and adults, with BKPyV being the most frequent in young children [10,39].

Therefore, intense human activity due to the agricultural, urban and livestock runoffs at or near these collection points would explain such level of contamination since in addition to recreation, the river is used as a resource for survival and work [40]. Besides the present study, several groups have shown high prevalence of HPVs in sewage and other water environments worldwide [41-43]. Polyomaviruses have also been described as being very resistance over time and is also resistant to treatments at low pH [41,44]. This would explain why polyomaviruses are resistant to most sewage treatments protocols, in particular to chlorine treatment as JCPyV and BKPyV were also found in P4, after a sewage plant. Rivers located in the Mediterranean area in Barcelona (Spain) and Rio de Janeiro (Brazil), which receives domestic sewage from urbanized areas also were subject of analysis, revealing 100% positivity for JCPyV [45]. The presence of the JCPyV in water from the metropolitan area of Berlin was also demonstrated and agrees to that JCPyV is the most prevalent polyomavirus in natural environments due to contamination with human wastes [46]. In agreement to our results, it was recently described the presence of JCPyV and BKPyV in water samples from a nearby region in Porto Alegre [47].

We did not observe MCPyV, TSPyV, and STLPyV at any sampling point. MCPyV virus was found in 50.3% of samples from urban sewage collected at wastewater treatment plants in several regions in Italy [43] and in 50% in Barcelona (Spain) and Rio de Janeiro (Brazil) [45], suggesting that it may also be transmitted by fecal oral route. Although there is no report regarding to the MCPyV shedding in Brazil, approximately 20% of excretion was observed in the immunocompetent population (Urbano et al, unpublished data), which is much lower than the observed to BKPyV and JCPyV. Nevertheless, no positivity to MCPyV at all in our samples was an unexpected finding. It is possible that these viruses were present...
in the samples but at such low level that our methods were unable to detected them. To our knowledge, there is no data regarding to the rate of STLPyV in urine or TSPyV urine and fecal excretion in healthy individuals. Since these viruses also have high prevalence in general population [48-50], one may expect to find them at least in a proportion of the samples if these viruses would also use the fecal-orlal route. In fact, it has been proposed that the respiratory tract may be the main route for TSPyV infection [51] and not the fecal-orlal as it is suggested for JCPyV, BKPyV and MCPyV.

Freshwater is considered appropriate for use when it is in accordance with the bacterial levels accepted by CONAMA [52]. However, there is no treatment for viral removal in WWTP suggested by CONAMA and the presence of viruses can represent a public health problem [52-54]. As an example, RVA, NoV and HAdV were detected in the surface waters of the Rodrigo de Freitas Lagoon, which is considerate suitable for recreation according to the microbiological level’s evaluation [53]. Some authors have suggested that the quality of river water (in terms of viruses presence) may be closely linked to the health status of the local population [54-56]. However, studies that also estimated viral load could not establish any relationship between viral load and probability of transmission [53,56]. In sum, as already seen in other regions, polyomaviruses are common in water matrices and we found them also in Belo River. Positive samples were detected during different months, regardless of season, temperature, rainfall, and direct or indirect human influence. Although no experiments were performed to evaluate the infectivity of viruses found in the Belo River; this data support the environmental spread and point to the need to further investigate the waterborne transmission of polyomaviruses.

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