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ORIGINAL PAPER

Microbial Source Tracking Analysis Using Viral Indicators in Santa Lucía and Uruguay Rivers, Uruguay

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Abstract

The aim of this study was to determine the origin (human, bovine or porcine) and the concentration of the fecal sources of contamination in waters from Santa Lucía basin and Uruguay River in Uruguay by using host-specific viral markers (adenoviruses and polyomaviruses) as microbial source tracking (MST). Between June 2015 and May 2016, monthly collections of surface water samples were performed in six sites in Santa Lucía basin and four sites in Uruguay River ($n=120$ samples). Viral concentration was carried out using an absorption-elution method. Detection and quantification of human and porcine adenovirus (HAdV and PAdV, respectively) and human and bovine polyomavirus (HPyV and BoPyV, respectively) were performed by quantitative PCR (qPCR). To evaluate the infectivity of circulating HAdV, an integrated cell culture-qPCR (ICC-qPCR) was used. A logistic regression analysis was carried out to estimate the influence of environmental variables on the virus presence in surface waters. Overall, HAdV was the prevalent (18%; 21/120) followed by BoPyV (11%; 13/120) and HPyV (3%; 3/120), whereas PAdV was not detected in this study. The mean concentration ranged from 1.5×10^4 genomic copies/L (gc/L) for HAdV to 1.8×10^2 gc/L for HPyV. Infective HAdVs were observed in two out of ten analyzed samples. A significant effect of environmental temperature ($p=0.001$) and river ($p=0.012$) on the presence of human viruses was found. These results suggest that fecal contamination could affect the water quality of these rivers, showing deficiencies in the procedure of sewage discharge from regional cities, livestock and dairy farms.

Keywords MST · Adenovirus · Polyomavirus · Fecal contamination · Surface waters · Uruguay

Introduction

Environmental waters (mainly rivers, lakes and streams) with fecal contamination used for human consumption, recreational activities and irrigation, constitute a concerning health risk that directly impacts on public health and the countries' economy (Roslev and Bukh 2011). Environmental waters are susceptible to fecal contamination by a wide range of point and diffuse sources. Point sources of fecal contamination are those that come from a clearly and identifiable cause or activity in a defined point or area of a basin, such as effluents from industries and wastewater from human populations and dairy farms. On the other hand, diffuse sources of fecal contamination are those coming from activities that occupy important and extensive areas such as livestock. As a result of the numerous potential sources of contamination, many bodies of water frequently contain traces of fecal material from different sources of various hosts (Roslev and Bukh 2011).

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Rapid and accurate identification of fecal contamination sources is essential for a correct assessment of the risks for human health and for the remediation of this contamination. Biological tools have been developed to establish the microbial source tracking (MST) in aquatic environments mainly through the identification of bacterial and viral indicators associated with the fecal matter of a specific host (Wong et al. 2012).

Enteric viruses constitute a group of infectious agents which are transmitted by the fecal–oral route. These viruses, mainly adenoviruses and polyomaviruses, have been proposed as promising tools in the study of MST. The main advantage of its usage as markers of MST is that these enteric viruses of different host species are identified and differentiated based on the specific differences of each viral gene (host specific). In addition, these viruses are more resistant than bacterial indicators to adverse environment conditions (Chan et al. 2006). Large concentrations of viral particles are disposed daily into sewage networks and treatment plants (when present). Finally, they are dumped into rivers, seas or other water sources (Bosch et al. 2008).

The high stability of these enteric viruses in the environment, the host specificity and the high prevalence worldwide throughout the year, allow the use of highly sensitive, specific and rapid molecular methodologies such as qualitative and quantitative PCR (qPCR) for specific viral identification and quantification (Bofill-Mas et al. 2013). Although qPCR is unable to differentiate between infectious and noninfectious viruses, the integrated cell culture-PCR (ICC-PCR) is a suitable technique to detect infectious viruses with high sensitivity (Cromeans et al. 2008).

Human and Porcine adenoviruses (HAdV, PAdV) belong to the *Adenoviridae* family and possess an icosahedral capsid without envelope. The viral genome is composed of double-stranded linear DNA of approximately 35,000 base pairs (bp) (Berk 2013). HAdV are distributed throughout the world and are responsible for causing diseases related to contaminated water consumption (Jiang 2006). Within the genus *Mastadenovirus*, human adenoviruses are classified into different species or types namely: A, B, C, D, E, F and G and these, in turn, are divided into several serotypes (Berk 2013). The human and bovine polyomaviruses (HPyV, BoPyV, respectively) belong to the family *Polyomaviridae* presenting a small and naked icosahedral capsid with a genome of double-stranded circular DNA of approximately 5000 bp (De Caprio 2013). Similar to HAdV, HPyV is ubiquitously distributed worldwide and more than 80% of humans present antibodies against it (Bofill-Mas et al. 2013).

Several studies reveal the use of adenoviruses and polyomaviruses as viral markers of fecal contamination in environmental samples. These viruses were studied in different geographical areas worldwide assessing the impact of livestock production and human populations in the microbiological quality of rivers and beaches showing the suitability of these MST tools in order to determine the origin of fecal contamination (McQuaig et al. 2012; Rusiñol et al. 2014; Steele et al. 2018).

The aim of this study was to determine the sources of fecal contamination (human, bovine or porcine) in waters from Santa Lucía basin and Uruguay River using host-specific viral markers (adenovirus and polyomavirus) as a MST tool.

Materials and Methods

Sampling of Surface Waters

The Santa Lucía basin, located at the southern region of Uruguay, covers an area of 13,681 km^2 where the Santa Lucía River has an average flow of 2700 m^3/s. This basin is the main source of drinking water supplying 60% of the country's population, mainly located in Montevideo (1,319,000 inhabitants), the capital of Uruguay. Land usage in this basin is predominantly related to agricultural activities with cattle and sheep, dairy, poultry, pigs and also horticulture and fruit production (Barreto et al. 2017).

The watershed of the Uruguay River encompasses a region between Argentina, Brazil and Uruguay, covering a total area of approximately 339,000 km^2. The average flow of this river at Salto city, which is the second most populated city in Uruguay (104,000 inhabitants) is 4622 m^3/s with an international (Argentina and Uruguay) dam located 10 km upstream of this city (CARU 2018).

From June 2015 to May 2016, monthly collections were performed in six points of the Santa Lucía basin and in four points of the Uruguay River ($n = 120$). Each sample consisted in 500 mL of surface water collected in a sterile bottle and immediately transferred to the laboratory at 4 °C (Fig. 1).

Viral Concentration

Concentration of viral particles was performed by viral adsorption and elution to a negatively charged membrane (Katayama et al. 2002; Haramoto et al. 2009). Briefly, $MgCl_2$ was added to the sample to a final concentration of 25 mM and filtered through a type HA negatively charged membrane of 47 mm of diameter and 0.45 µm of pore size. The membrane was rinsed with 200 mL of 0.5 mM H_2SO_4 (pH 3.0) and placed into a petri dish, where the elution of viruses was carried out by the addition of 4 mL of 1 mM NaOH (pH 10.8) and shaking for 5 min. Finally, 40 µL of 50 mM H_2SO_4 and 40 µL of 100× TE Buffer (pH 8.0) were added to neutralize the solution.

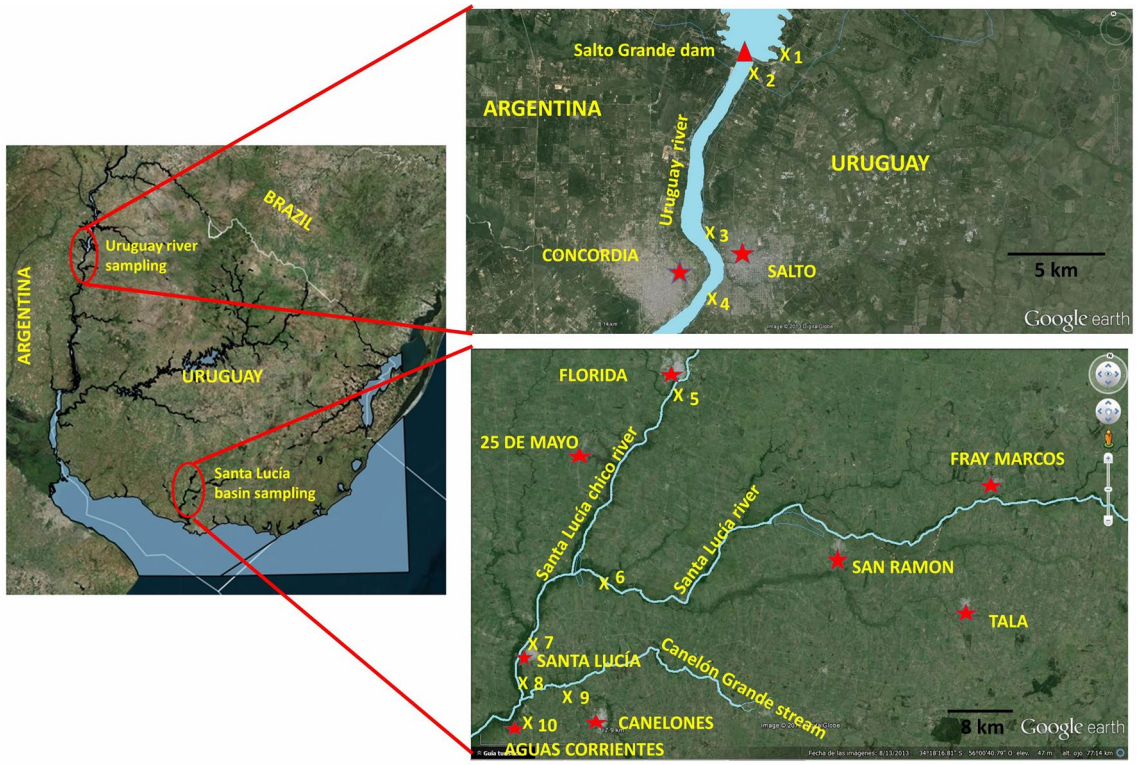

Fig. 1 Maps showing the sites of surface water collection. In Uruguay River: upstream dam (1), downstream dam (2), drinking water intake for Salto city (3), and downstream Salto city (4). In Santa Lucía basin (SL): downstream Florida city (5), Paso Pache (6), upstream SL city (7), downstream SL city (8), Canelón Grande stream (9), and drinking water intake for Montevideo city (10). The red triangle represents the dam, and the red stars represent the cities located in the studied area

Nucleic Acids Extraction and Quantitative PCR (qPCR) Detection

Nucleic acids extraction was performed with the *QIAmp Cador Pathogen mini kit* (QIAGEN®, Hilden, Germany) according to the manufacturer's instructions.

For the detection and quantification of host-specific viral indicators of fecal contamination, a Real-Time PCR technique with *TaqMan*® technology was carried out. The *SensiMix™ II Probe kit* (BIOLINE®, England) and the *Rotor Gene Q* thermocycler (Qiagen®, Germany) were used following the manufacturer's instructions. Briefly, for the detection and quantification of HAdV, HPyV, BoPyV and PAdV, the PCR assays were performed as follows: a mix containing 5.0 µL of RNAse/DNAse free water (Amresco®, USA), 12.5 µL of 2X SensiMix™ II Probe kit (Bioline Reagents Ltd, England), 1.0 µL of forward and reverse primers (10 µM) and 0.5 µL of probe (10 µM) were added to 5.0 µL of the template. Cycling conditions for HAdV, BoPyV and PAdV were as follows: denaturation at 95 °C for 5 min followed by 45 cycles of denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 50 s. The HPyV cycling conditions were the same as previously mentioned with the exception that the annealing extension step was at 60 °C for 60 s. The sensitivity of HAdV, HPyV, BoPyV and PAdV qPCR assays was 3, 4, 5 and 50 genomic copies (gc)/reaction, respectively. Primers' details were included as supplementary material.

Viral concentrations are shown as the average of two replicates. Those samples that presented negative results in these qPCR assays were analyzed again in a 1:10 dilution from the extracted nucleic acid in order to overcome the presence of enzymatic inhibitors. Plasmids containing HAdV, PAdV, HPyV and BoPyV genes for qPCR assays were kindly provided by Dr. Marize Miagostovich from the Laboratory of Comparative and Environmental Virology, FIOCRUZ, Brazil, and Dr. Viviana Mbayed from the Chair of Virology, Universidad de Buenos Aires, Argentina.

Determination of HAdV Infectivity by ICC-qPCR

ICC-qPCR was performed as previously described by Rigotto et al. (2005). To assess HAdV infectivity, ten samples with the highest viral concentration (determined by qPCR) were inoculated in A549 cells (lung carcinoma cell line). Viral concentrates (196 µL) were treated with 2 µL of Penicillin/Streptomycin (100×) (Capricorn Scientific, Ebsdorfergrund, Germany) and 2 µL of amphotericin (100 mg/mL)

(Capricorn Scientific, Ebsdorfergrund, Germany). These samples were 1:2 diluted, inoculated in duplicate in 24-well plates containing A549 cells and incubated during 1 h at 37 °C, with shaking every 10 min. Then, the supernatant was removed and the monolayer from each well was washed three times with PBS 1× followed by the addition of 1 ml of DMEM/Ham's F-12 medium with L-glutamine (Capricorn Scientific, Ebsdorfergrund, Germany) supplemented (for final concentration) with amphotericin (2.5 mg/L) (Capricorn Scientific, Ebsdorfergrund, Germany), 1× tryptose phosphate broth (Gibco, New York, USA), 1× nonessential amino acids (Capricorn, Ebsdorfergrund, Germany) and 1× penicillin–streptomycin (Capricorn, Ebsdorfergrund, Germany) with 2% of inactivated fetal bovine serum (SFB) (GE HealthCare, UK). Cells were incubated for 7 days at 37 °C and 5% CO_2. After this incubation, plates were frozen and thawed 3 times and the nucleic acid extraction and qPCR assays were performed in order to detect HAdV infective particles.

Sequencing and Phylogenetic Analysis

ICC-qPCR positive samples were amplified by nested PCR as described by Allard et al. (2001), corresponding to the hexon gene of the genome for further purification and sequencing analysis. Amplicons were purified using *PureLink™ Quick Gel Extraction* and *PCR Purification Combo kit* (Invitrogen, Carlsbad, California, USA) and sequenced (with forward and reverse primers) by Macrogen (South Korea) in an *ABI3730XL Genetic Analyzer*® (Applied Biosystems, California, USA). Sequences were assembled and edited with *SeqMan*® Software (DNAstar Lasergen®) and aligned with reference sequences obtained from the NCBI Database using *MUSCLE*® program (Edgard 2004). The phylogenetic tree was constructed with MEGA® 7 (Kumar et al. 2016) software using the *Neighbor-Joining* method and *Kimura 2 parameters* model with gamma distribution. As a measure of robustness of each node, the bootstrap method with 1000 pseudo replicates was used (Kumar et al. 2016). HAdV sequences obtained in this study were submitted to GenBank under the following accession numbers: MK473843 and MK473844.

Statistical Analysis

Statistical analyses were performed using the *STATA® v. 14.1* software (College Station, TX: StataCorp LP, USA). Confidence intervals for proportions were obtained to estimate the frequency of detection of HAdV, HPyV and BoPyV by river, and test of proportions was used to test differences. Frequency of viral detection by selected environmental variables (environmental temperature < 20 °C or ≥ 20 °C; river level as regular or flood; season) was calculated for descriptive purposes. Summary statistics were estimated for the concentration (g.c./L) of HAdV, HPyV and BoPyV by river, and mean-comparison analyses (*T* tests) were carried out. Subsequently, a logistic regression analysis was performed to estimate the influence of the environmental temperature (as categorical variable) and to evaluate if there was a significant effect of the river (Santa Lucía basin or Uruguay River) on the virus presence in surface waters. This analysis was performed separately for viral indicators of human fecal contamination (HAdV and/or HPyV) and the viral indicator of bovine fecal contamination (BoPyV), including rainfall as a control variable. Odds ratios (ORs) and 95% confidence intervals were estimated as association measures. The calculation of the Akaike Information Criterion (AIC) was performed to determine the best models suited to our data.

Results

In this study, a total of 120 samples of surface water collected in the Santa Lucía basin and Uruguay River in Uruguay were analyzed to determine the origin of fecal contamination using HAdV, HPyV, BoPyV and PAdV as MST tools.

Table 1 shows the frequency of the positive samples detected by qPCR for each analyzed virus. Overall, HAdV was detected in 18% of the analyzed samples which was higher than the frequency of BoPyV (11%) ($p = 0.069$). It is worth mentioning that for HAdV, 8% of negative samples were positives when the nucleic acid was diluted to 1:10 and for BoPyV 5% were positives after this dilution. HPyV was only detected in 3% (1% of the negative samples were positives after the dilution) which was significantly lower than the frequencies observed for HAdV ($p = 0.0001$) and BoPyV

Table 1 Frequency of detection of HAdV, HPyV and BoPyV according to the river analyzed

River	HAdV		HPyV		BoPyV	
	Frequency (%)	95% CI	Frequency (%)	95% CI	Frequency (%)	95% CI
Uruguay ($n = 48$)	6	[1; 17]	0	–	8	[2; 20]
Sta Lucía ($n = 72$)	25	[15; 37]	4	[9; 12]	13	[6; 22]
Total ($n = 120$)	18	[11; 25]	3	[0.5; 7]	11	[6; 18]

CI confidence interval

Table 2 Quantification of HAdV, HPyV and BoPyV (expressed as g.c./L) according to the analyzed river

	HAdV			HPyV			BoPyV		
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max
Uruguay	15,904 (±21,277)	113	40,100	–			6896 (±8895)	360	19,600
Sta. Lucía	15,295 (±14,216)	630	46,900	180 (0)	180	180	14,803 (±30,831)	319	93,400
Total	15,382 (±14,734)	113	46,900	180 (0)	180	180	12,370 (±25,844)	319	93,400

SD standard deviation, *Min* minimum, *Max* maximum

Table 3 Positive samples for each virus according to the environmental variables

Environmental variables	HAdV, *n* (%)	HPyV, *n* (%)	BoPyV, *n* (%)
Temperature (°C)			
<20 (*n*=60)	17 (28.3)	2 (3.3)	9 (15.0)
≥20 (*n*=60)	4 (6.7)	1 (1.7)	4 (6.7)
River level			
Regular (*n*=90)	19 (21.1)	3 (3.3)	9 (10.0)
Flood (*n*=30)	2 (6.7)	0 (0)	4 (13.3)
Season			
Winter (*n*=30)	11 (36.7)	1 (3.3)	7 (23.3)
Spring (*n*=30)	9 (30.0)	2 (6.7)	2 (6.7)
Summer (*n*=30)	0 (0)	0 (0)	1 (3.3)
Autumn (*n*=30)	1 (3.3)	0 (0)	3 (10.0)

($p=0.0048$). On the other hand, PAdV was not detected in any of the analyzed samples. In Santa Lucía basin, HAdV was more frequently detected than BoPyV ($p=0.0273$) and HPyV ($p=0.002$). Moreover, BoPyV presented a higher frequency of detection when compared with HPyV ($p=0.0352$). Considering the samples collected from the Uruguay River, both HAdV and BoPyV were detected with similar frequencies and no detection of HPyV was observed.

HAdV was detected with a mean concentration of 1.5×10^4 gc/L, similar to that determined for BoPyV (1.2×10^4 gc/L). On the other hand, HPyV presented a mean concentration of 1.8×10^2 gc/L. No significant difference was observed for the concentration of HAdV ($p=0.949$) or BoPyV ($p=0.631$) when comparing both analyzed rivers (Table 2).

Considering the environmental variables such as temperature, river level and season, we observed that human viruses (HAdV and HPyV) were more frequently detected in days with low temperature (< 20 °C), in regular river level and mainly in winter and spring seasons. BoPyV was also detected more frequently in low temperature, although they were more present in flood and in winter and autumn seasons (Table 3).

According to the logistic regression analysis performed, there were seven and six times more chances to detect human viruses in colder weather (< 20 °C vs. ≥ 20 °C) (OR 7.4; p = 0.001) and in the Santa Lucía basin (Santa Lucía basin vs. Uruguay River) (OR 5.6; $p=0.012$), respectively (Table 4).

In Santa Lucía basin, HAdV was detected in all the collection points and mainly from June to October of 2015 corresponding to winter and spring with concentrations ranging from 6.3×10^2 to 4.7×10^4 gc/L. BoPyV was sporadically detected in 5 points from June to October 2015 and March to May 2016 (the coldest period of the year) and this virus was not detected in the warmest period of the year (from November 2015 to February 2016). Regarding HPyV, it was only detected three times (June, September and October of 2015) downstream of Florida city, at the drinking water intake for Montevideo city and Paso Pache with 1.8×10^2 gc/L (Fig. 2a). In Uruguay River, HAdV was detected in 3 different points of the 4 sampled with concentrations between 1.1×10^2 gc/L and 4.0×10^4 gc/L. BoPyV was found in 4 occasions, at 3 different collection points, twice at the water

Table 4 Logistic regression analysis for human viruses (HAdV and/or HPyV) and BoPyV according to environmental temperature and river

	Human viruses (model I[a], AIC = 97.12)			BoPyV (model II[a], AIC = 87.51)		
	OR	[95% CI]	*p* value	OR	[95% CI]	*p* value
Temperature (°C)						
≥20	Ref.	–	–	–	–	–
<20	7.4	[2.22; 24.46]	0.001	2.5	[0.72; 8.65]	0.148
Uruguay River	Ref.	–	–	–	–	–
Santa Lucía basin	5.6	[1.47; 21.40]	0.012	1.6	[0.44; 5.47]	0.493

OR odds ratio, *CI* confidence interval, *AIC* Akaike Information Criterion; *Ref.* reference

[a]Adjusted for rainfall

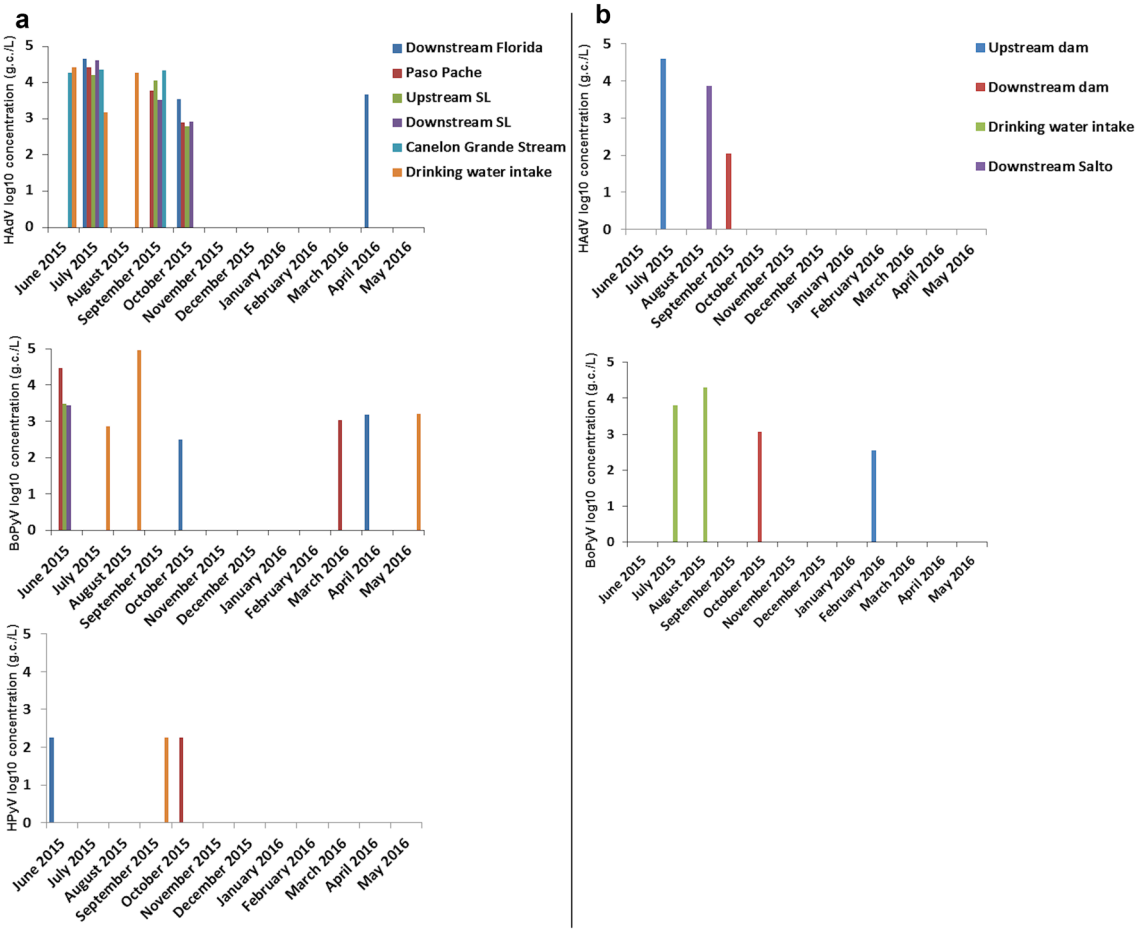

Fig. 2 Distribution of viral concentrations according to the date and collection site in **a** Santa Lucía basin and **b** Uruguay River (Color figure online)

intake for Salto city with concentrations between 3.6×10^2 gc/L and 2.0×10^4 gc/L (Fig. 2b).

To test the infectivity of the HAdV detected in the water samples, ten HAdV positive samples (previously tested by qPCR) were tested by ICC-qPCR. Two of them were positive and genotyped through sequencing and phylogenetic analysis as HAdV species B type 3 (Fig. 3).

Discussion

This study showed the presence of virus indicators of fecal contamination from human (HAdV and HPyV) and bovine (BoPyV) origin, mainly due to dairy, human and agricultural activities in two important rivers from Uruguay. Although human, bovine and porcine fecal contamination was assessed, PAdV was not found in both rivers suggesting that no fecal contamination of swine origin was present in the period of this study. Some factors, including water volume of the samples, qPCR analytical sensitivity and the presence of putative inhibitors may influence the detection rates of viral genomes in water and should be considered in the present study (Girones et al. 2010). Also, the absence of PAdV and the presence of BoPyV in both rivers could be explained due to the higher bovine (11,993,000 cows) production compared to swine (187,000 pigs) production in Uruguay (MGAP and DIEA 2018). An intensive bovine production related to dairy activities is registered in the Santa Lucía basin considered as the main dairy basin in Uruguay (MGAP and DIEA 2018).

The increase in positive samples after 1:10 nucleic acid dilution, found in our study, suggested the existence of inhibitory elements in the samples that, when diluted, no longer inhibited the enzymatic reactions like reverse transcription and DNA polymerization. There are some disadvantages using PCR virus detection from environmental samples, including the presence of inhibitors' compounds in concentrated samples and the inability to differentiate between infectious and noninfectious virus particles. PCR-inhibitory compounds are naturally present in the environment (i.e.,

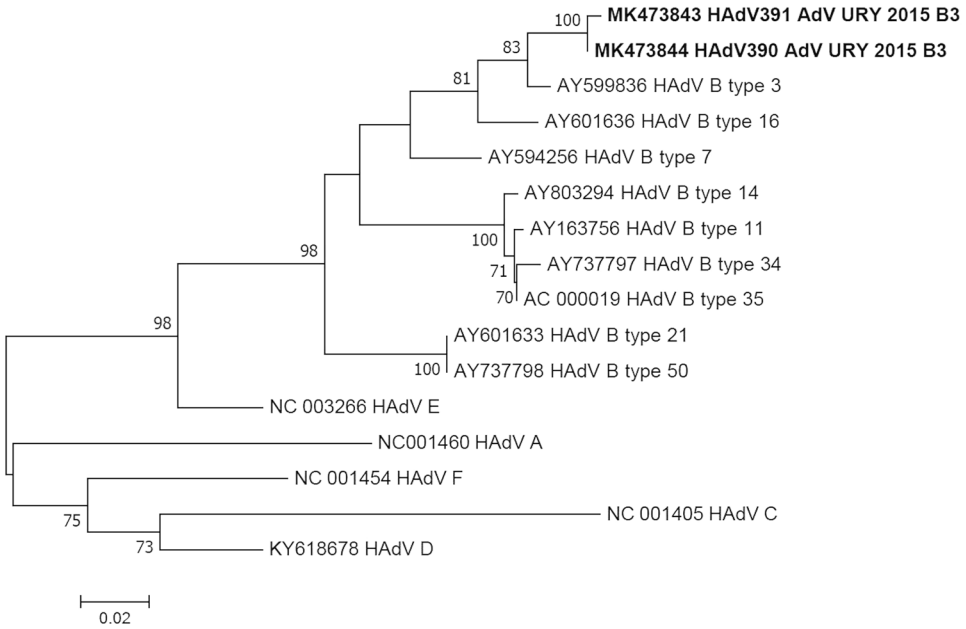

Fig. 3 Phylogenetic analysis of the infective HAdV sequences obtained from samples A390 and A391 (in bold) corresponding to a 171 bp fragment of the hexon gene. Accession numbers HAdV reference strains are shown along with species and type. Bootstrap values over 70% are shown. The scale bar at the bottom represents substitutions per nucleotide position

humic and fulvic acids, proteins, metals and salts) preventing enzymes reactions from amplifying a target sequence, leading to a false-negative result (Reynolds 2004).

HAdV presented a mean concentration of 1.5×10^4 gc/L, similar to that determined for BoPyV (1.2×10^4 gc/L) in both rivers. Rusiñol et al. (2014) observed in the Umea River in Sweden a similar concentration about 5.2×10^4 gc/L for BoPyV, and for HAdV and HPyV the concentrations ranged between 10^2 and 10^3 gc/L. Moreover, in our work, HPyV presented a mean concentration of 1×10^2 gc/L, also similar to the concentration detected in rivers from North Wales (Farkas et al. 2018). Differences in concentration values for the same virus among different geographical areas could be attributed to the viral concentration and/or detection methods used in each study or due to the local epidemiology of these viruses and its analysis was beyond the aim of this work.

Environmental factors such as UV radiation, temperature, rainy or dry periods, are highly relevant to the global dissemination of viral pathogens (Carratalá et al. 2013). In our study, viral indicators of fecal contamination of human origin in both basins were found mostly when the river presented a normal water level although they were also detected during periods of flooding. These results are similar to a recent work reported in Brazil, which showed no significant differences on HAdV concentration between dry and flood seasons (Vieira et al. 2016).

Human fecal contamination is considered more significant than animal fecal pollution in terms of human health risk. However, in some cases, fecal contaminants of animal origin can also have a significant impact on microbial water quality, thus, on risk for human health (WHO 2018). Moreover, some studies indicated that the risk for human health associated with fecal residues of different animals varies according to the species. Therefore, the risk associated with exposure to water affected by cattle feces is greater than risks associated with exposure to water contaminated with gull, pig or chicken feces (Soller et al. 2010). Both, in the Santa Lucía basin and in the Uruguay River, virus indicators of human fecal contamination were found more frequently than those of animal origin, which shows that the cities located on the riverside have a major direct effect on the microbial quality of those waters. Although a lower frequency of BoPyV was detected in this study, it should not be underestimated their impact in the human health.

Overall, the positive samples were registered in periods when the temperature was lower than 20 °C. These findings suggested a greater stability of these viruses in the environment, mainly in winter and therefore a greater circulation and a continuous threat to the exposed population. In addition, animal viruses were also observed predominantly in cold months. However, in the Uruguay River they were detected in February. During that particular month, there was a flooding suggesting that this river reached areas contaminated with cattle feces. Temperature is also an important factor for virus stability in the environment and heat treatments have shown to inactivate viral pathogens in water and food (Bertrand et al. 2012; Carratalá et al. 2013). Cold weather is related to a high frequency of gastroenteritis cases caused by rotavirus and adenovirus (Celik et al. 2015). In our work, it could be observed that the chances of finding human viruses were greater in colder months, similar to that noticed by

Corsi et al. (2014) report, observing that the occurrence of viruses from human origin was more prevalent in cold weather months.

Comparing the cities located at the riversides of both rivers, we observed two different scenarios. HAdV detected upstream of Salto city in Uruguay River likely originated from cities about 40 km upstream of these collection sites, which showed that despite being subjected to environmental stressors, these HAdV persisted in these waters. These results were in agreement with previous reports where it was observed 83%, 66% and 58% undamaged HAdV particles (defined as those in which the genetic material is protected by the viral capsid) at Peri Lagoon, spring source water and public supply system water, respectively, in the southern region of Brazil (Fongaro et al. 2013). The other scenario showed that Santa Lucía basin had several cities on its riverside; therefore, this area is more densely populated comparing with the study area in Uruguay River, which supports our results where HAdV was more frequently detected in Santa Lucía basin than in Uruguay River. These results highlighted the fecal pollution generated by those cities in the waters of these rivers and the risk of gastrointestinal diseases of the local population which are constantly exposed.

Two out of ten HAdV positive samples tested by qPCR were considered infective since they were positive after their inoculation in A549 cells and qPCR assays (ICC-qPCR). These samples corresponded to the Santa Lucía basin collected in the sites Paso Pache and upstream of Santa Lucía city, both samples were collected in winter (July 2015). After the phylogenetic analysis, we observed that these samples belonged to HAdV specie B type 3, which is related to different illnesses like conjunctivitis, acute respiratory disease and hemorrhagic cystitis (Berk 2013). On the other hand, several studies showed that HAdV-2 is one of the most common serotypes excreted by humans, suggesting that this serotype is intermittently excreted in feces of most individuals, even in asymptomatic cases (Mena and Gerba 2009; Wyn-Jones et al. 2011).

In conclusion, to our knowledge, this is the first work using MST tools in order to assess the origin and burden of fecal contamination in surface waters in Uruguay. An important fecal contamination was revealed mainly from human and bovine origin in both basins evidencing that this approach was appropriated in order to access the origin of the fecal contamination in surface waters. The results obtained in this study suggested deficiencies in the elimination of enteric viruses in wastewater treatment mainly from cities and dairy farms located in both basins. This originates a potential health risk for the population using these surface waters for recreational activities, crop irrigation and as a drinking water source.

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