ORIGINAL PAPER

Investigation of Human and Animal Viruses in Water Matrices from a Rural Area in Southeastern Region of Brazil and Their Potential Use as Microbial Source-Tracking Markers

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Abstract

This study assessed the sources of contamination of water matrices in a rural area using detection of a host-specific virus (human adenovirus [HAdV], porcine adenovirus [PAdV] and bovine polyomaviruses [BoPyV]) as potential microbial source-tracking tool, and rotavirus A [RVA], given its epidemiological importance in Brazil. From July 2017 to June 2018, 92 samples were collected from eight points (P1-P8) of surface and raw waters in southeastern region of Brazil. Fifty-five (59.8%) were positive for HAdV, 41 (44.5%) for RVA, 10 (10.9%) for PAdV and four (4.3%) for BoPyV. HAdV and RVA were detected at all sites, and over the entire sampling period, PAdV was detected at a porcine breeding area and at Guarda River site, presenting high concentrations up to 2.6×10^9 genome copies per liter [GC/L], and viral concentrations ranging from 9.6×10^1 to 7.1×10^7, while BoPyV (1.5×10^4 GC/L–9.2×10^5 GC/L) was only detected in samples from the bovine breeding areas. The combination of human and animal virus circulation presents a potential impact in the environment due to raw sewage discharge from regional communities, as well as potential hazard to human and animal health.

Introduction

Human and animal wellbeing and development are dependent on water quality and safety (Korajkic et al., 2019; Wong et al., 2012). Many water-related activities are compromised by the poor treatment of water and wastewater (Rusiñol et al., 2020). Different sources, including the discharge of effluents from human and/or livestock, are responsible for fecal contamination, and the release of pathogens in water resources such as lakes, rivers and oceans (Adelodun et al., 2021). Microbial source tracking (MST) is an important tool that determines the origin of environmental fecal and/or urine contamination using microbial targets. It includes molecular methods to detect, quantify and identify host-specific viruses and bacteria (García-Aljaro et al., 2019; Rusiñol et al., 2014). Many kinds of viruses are excreted in human and animal urine and feces (Bosch et al., 2008). Different species of adenoviruses and polyomaviruses are host-specific and have been investigated using MST as contamination markers, since concentrations remain stable throughout the year and these viruses are more resistant compared to bacterial indicators (Hundesa et al., 2006). In a previous study carried out in five countries, this approach was successful to demonstrate the human and animal fecal contamination of a given area (Rusiñol et al., 2014). Other enteric viruses have been indicated as potential fecal contamination markers, although there may vary in detection according to the geographic region (Ballesté et al., 2021; Barrios et al., 2018; González-Fernández et al., 2021). In developing countries,

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as in Brazil, rotavirus A (RVA) plays an important environmental role, since it is still detected in high concentrations in different environmental matrices, even after the implementation of a vaccination program. RVA are important viral agents of acute gastroenteritis, infecting different animal species, which occasionally can generate genetic reactions by breaching the species barrier (Silva Bandeira et al., 2022; Flores et al., 2021). In rural areas with poor sanitation, it is of great importance to identify the source of contamination to provide data that facilitate mitigating actions thus pointing to possible virus interactions between humans and animals. The aim of this study was to assess the spread of viruses in surface and wastewater generated in a rural area with inadequate basic sanitation and with animal husbandry, which may be affecting the Guarda River, a contributor to the Guandu River basin, of unquestionable strategic importance for the supply of the second largest metropolitan region of the country (Tubbs Filho et al., 2012). For this purpose, we investigated occurrence, concentration, and genotypes of human-specific (HAdV), human-animal (RVA) and animal-specific viruses (porcine-specific adenovirus [PAdV] and bovine-specific polyomavirus [BoPyV]).

Material and Methods

Sampling

The Guandu River basin is formed by the Guandu, Guarda and Guandu-Mirim rivers located in the west side of the Guanabara Bay basin in the State of Rio de Janeiro. This area occupies 3600 km^2 covering 15 municipalities: Seropédica, Itaguaí, Paracambi, Japeri, Queimados, Miguel Pereira, Vassouras, Piraí, Rio Claro, Engenheiro Paulo de Frontin, Nova Iguaçu, Rio de Janeiro, Mendes, Mangaratiba and Barra do Piraí, with approximately 1 million of residents. The Guarda River crosses an urban and an agricultural area and flows in the Sepetiba bay, used for fishing activities and where Itaguaí port is located (Tubbs Filho et al., 2012). The selected sampling sites included areas of potential human and/or animal fecal contamination impact. It is noteworthy that selected animal farming sites lack sewage treatment systems and were chosen according to surrounding areas close to human occupation and/or activities, and included surface water (10 L) and wastewater (40 mL) from areas under human and/or animal fecal contamination (Fig. 1 and Table 1). Sites 1, 2 and 3 are close to a resident area and commercial buildings. Sites 4, 5, 6 and 7 are from effluent disposal inside livestock areas where animals are kept. Finally, site 8 is located close to a resident area at the edge of Guarda River. From July 2017 to June 2018, monthly collections were carried out at eight sites ($n = 92$; for sites P6 and P7 it was not possible to perform the first two collects).

All samples were collected in the first week of the month to maintain a regularity, respecting the exact sites to access the sample according to the GPS locations. Sewage samples were collected from untreated effluent sites that are directly discharged in close water streams or soil. All samples were collected in sterile bottles and immediately transferred to the laboratory for analysis at 4° C.

Genetic Heritage

This article was registered in the "National System for the Management of Genetic Heritage and Associated Traditional Knowledge—SisGen" (registration number A5674E8), in compliance with the Brazilian Law N. 13,123/2015 and its regulations.

Concentration of Samples, Internal Control Process and Nucleic Acids Extraction

Surface and sewage waters were processed by skimmed milk flocculation (Calgua et al., 2008) and ultracentrifugation (Pina et al., 1998), respectively. Final concentrated (2 mL) were stored at − 80 °C. As an internal process control, each sample was spiked with 100 μL containing 7.5×10^{10} GC of PP7 (ATCC™ 15,692-B4) before the concentration step. Viral genetic materials were extracted from the concentrates using a protocol based on the denaturing properties of guanidine isothiocyanate and binding of nucleic acid to silica particles (Boom et al., 1990). For nucleic acid extraction, 400 μL of each concentrated sample were used and samples were eluted in 120 μL of sterile water.

Detection and Quantification of Host-Specific Markers and RVA

For the detection and quantification of host-specific viral markers, a quantitative real-time PCR/RT-PCR technique using TaqMan® technology was carried out using the ABI PRISM 7500 Real-Time TaqMan System. SuperScript™ III Platinum™ One-Step qRT-PCR Kit (RVA) and TaqMan Universal Master Mix II with UNG (HAdV, PAdV and BoPyV) (both from ThermoFisher Scientific, Foster City, CA, USA) were used for detection and absolute quantification in qPCR reactions. Table 2 presents specific targets, primers, probes, sequences, and amplification conditions. Standard curves using gBlock gene fragments (Integrated DNA Technologies™, Coralville, IA, USA) with sequence fragments of each analyzed virus were used to estimate viral concentrations in the samples. All RNA samples were tested in duplicate as well as undiluted and tenfold diluted to detect the presence of inhibitors. All samples that crossed the threshold line showing a characteristic sigmoid curve with a cycle threshold (Ct) value < 40 for
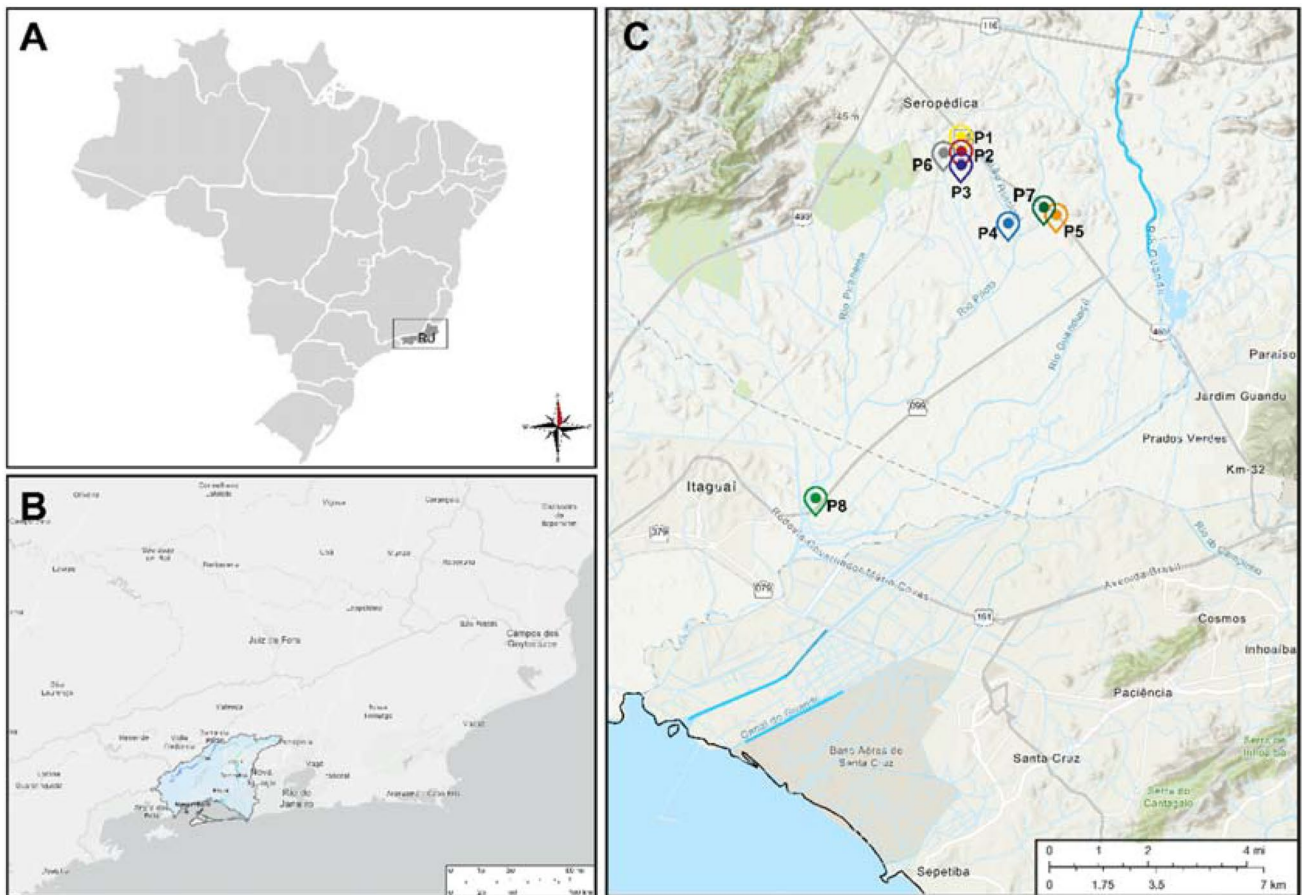
Fig. 1 A Study area in Brazil map. **B** Study area in Rio de Janeiro state. **C** Geographical distribution of sampling points: P1, community area; P2, Artificial Lake 1; P3, Artificial Lake 2; P4, Equine farming area; P5, Porcine farming area; P6, Bovine farming area 1; P7; Bovine farming area 2; P8, Guarda River. Hydrographic data presented in blue lines. Source: ArcGIS Web AppBuilder (Modified from: https://sigaaguas.org.br/sigaweb/apps/guandu/)

Table 1 Description of sampling points

Type of sample	Site point	No. samples	GPS location	Description of location
Surface water	P1	12	22°45´15´´S 43°41´41´´W	Lake
	P2	12	22°45´30´´S 43°41´40´´W	Stream
	P3	12	22°45´43´´S 43°41´40´´W	Lake
Raw wastewater	P4	12	22°46´45´´S 43°40´46´´W	FA Equine
	P5	12	22°46´36´´S 43°39´51´´W	FA Porcine
	P6	10	22°45´31´´S 43°42´00´´W	FA Bovine
	P7	10	22°46´28´´S 43°40´05´´W	FA Bovine
Surface water	P8	12	22°51´36´´S 43°44´26´´W	Guarda River
Total		92		

FA Farming area

at least two of the four wells tested were regarded as positive. Concentration of viruses was expressed as GC per liter (GC/L), considering the volumes of the samples, the concentrate (eluate), the nucleic acid extracts and the RT-qPCR reaction.

Molecular Characterization

The positive samples were subjected to the qualitative PCR for amplification and Sanger sequencing (Table 2).

Table 2 Quantitative and qualitative PCR specific targets, primers, probes, and sequences used in this study

Virus	Primer/probe	Sequence (5′→3′)	Target gene	References
		Quantitative		
HAdV	HAdVF	CWTACATGCACATCKCSGG	Hexon	Hernroth et al. (2002)
	HAdVR	CRCGGGCRAAYTGCACCAG		
	HAdV probe	FAM-CCGGGCTCAGGTACTCCGAGGCGTCCT-BHQ1		
RVA	Foward	ACCATCTWCACRTRACCCTCTATGAG	NSP3	Zeng et al., (2008)
	Reverse	GGTCACATAACGCCCCTATAGC		
	Probe	FAM-AGTTAAAAGCTAACACTGTCAAA-MGB		
PAdV	Q-PAdV-F	AACGGCCGCTACTGCAAG	Hexon	Hundesa et al. (2009)
	Q-PAdV-R	AGCAGCAGGCTCTTGAGG		
	Q-PAdV-P	FAM-CACATCCAGGTGCCGC-BHQ1		
BoPyV	QB-F1-1	CTAGATCCTACCCTCAAGGGAAT	VP1	Hundesa et al. (2010)
	QB-R1-1	TTACTTGGATCTGGACACCAAC		
	QB-P1-2	FAM-GACAAAGATGGTGTGTATCCTGTTGA-BHQ1		
		Qualitative		
HAdV	Hex1deg	GCCSCARTGGKCWTACATGCACATC	Hexon	Allard et al. (2001)
	Hex2deg	CAGCACSCCICGRATGTCAAA		
	Nehex3deg	GCCCGYGCMACIGAIACSTACTTC		
	Nehex4deg	CCYACRGCCAGIGTRWAICGMRCYTTGTA		
RVA	GEN-VP6F	GACGGVGCRACTACATGGT	VP6	Iturriza-Gómara et al. (2002)
	GEN-VP6R	GTCCAATTCATNCCTGGTGG		
PAdV	PALF	GATGTCATGGAYAACGTCAAC	Hexon	Hundesa et al. (2006)
	PARF	CACGGAGGAGTCRAACTGGATG		
	PALN	TACTGCMAGTTYCACATCCAGGT		
	PARN	TACTGCMAGTTYCACATCCAGGT		
BoPyV	BP1F-L	GTGTAGAATAATGATTGAACTAT	Aanoproteina	Hundesa et al. (2006)
	BP2F-R	TGGCCTACCTTTAGTTAAAATCT		
	BP3N-L	TTCTGGACAGTGGGGACTAT		
	BP4N-R	ATTTCAAAGCCCCCTATCATC		

HAdV Human adenovirus, *RVA* human rotavirus A, *PAdV* porcine adenovirus, *BoPyV* bovine poliomavirus
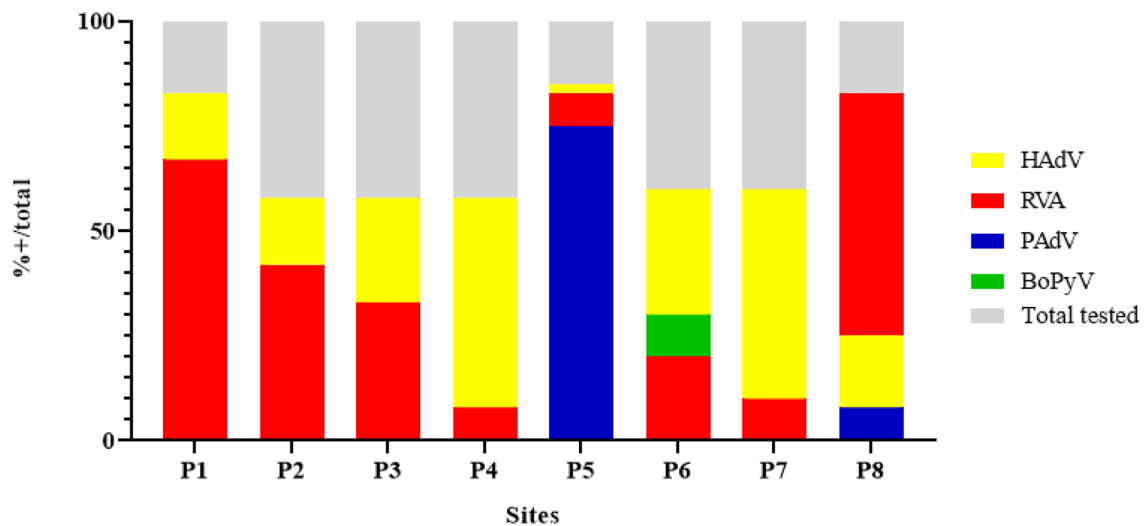
Molecular characterization was performed using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit 1, v. 3.1, and the ABI Prism 3730 Genetic Analyzer (Applied Biosystems™, Foster City, CA, USA) at the FIOCRUZ Institutional Platform for DNA sequencing (PDTIS). Nucleotide sequences were edited and aligned using the Clustal W method in BioEdit Software 7.2.6. Sequences were compared with those available in the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) database using the BLAST (Basic Local Alignment Search Tool). Substitution models used in this study were Tamura 3-parameter (T92) + I (RVA), Kimura 2-parameter—K2 (BoPyV and PAdV) and Jukes-Cantor (JC)—(HAdV). Phylogenetic trees were constructed in MEGA X v. 10.1.7. (Kimura, 1980). The statistical significance at the branch point was calculated by using 2000 pseudo-replicate datasets.

Statistical Analysis

Pearson's chi-squared test was used to determine whether there was a statistically significant difference between frequencies. For analyses, a p-value < 0.05 was considered statistically significant.

Results

In this study, we used the MST tool to analyze 92 surface and sewage samples from an urban/rural metropolitan area with poor sewage system treatment presenting risk of human and animal pathogens contamination. Figure 2 shows the prevalence of each analyzed virus detected by qPCR. HAdV and RVA were more frequently detected than animal host-specific ones. Overall, HAdV was the most prevalent viral agent, detected in 59.8% of analyzed samples, followed by

Fig. 2 Prevalence of human adenovirus (HAdV), rotavirus A (RVA), porcine adenovirus (PAdV) and bovine polyomavirus (BoPyV) according to sampling sites (above) and samplings months (bottom). P1, community area; P2, Artificial Lake 1; P3, Artificial Lake 2; P4, Equine farming area; P5, Porcine farming area; P6, Bovine farming area 1; P7; Bovine farming area 2; P8, Guarda River

RVA (44.5%), PAdV (10.9%) and BoPyV (4.3%). HAdV and RVA were detected throughout the period of study, except for July 2017, when RVA was not detected. Comparing livestock viruses' detection, PAdV was more frequently detected than BoPyV, which was detected in detected in January, March, and April 2018. It is worth mentioning that undiluted and 1:10 diluted nucleic acid in the PCR/RT-PCR reactions to rule out possible false-negative results was used.

Concerning viral concentrations, the higher value was observed for RVA (2.6×10^9 genome copies [GC/L]) in site P5 from samples collected in December 2017. Meanwhile, PAdV was detected with the lowest viral concentration (9.6×10^1 GC/L) in site P8 in November 2017. HAdV viral concentration (GC/L) ranged from 1.2×10^2 to 1.2×10^8 GC/L, RVA ranged from 3.4×10^2 to 2.6×10^9 GC/L, PAdV from 9×10^1 to 7.1×10^7 GC/L and BoPyV concentrations ranged from 1.5×10^4 to 9.2×10^5 GC/L (Fig. 3). HAdV and RVA were detected at higher concentrations compared to livestock viruses (PAdV and BoPyV). In addition, HAdV and RVA were detected at all sampling sites, while PAdV was detected only in sites P5 and P8, and BoPyV in P6 and P7. Considering seasonality distribution, we observed a higher detection of HAdV and RVA during spring and summer months, respectively. Nevertheless, only the HAdV detection frequency for the spring months showed statistical

significance. PAdV was detected during all seasons, while BoPyV was only detected during summer and fall (Table 3).

Phylogenetic analyzes of HAdV and RVA detected in environmental samples, showed that they belong to the same genotypes associated with cases of acute gastroenteritis in different regions of Brazil at the time of the study. RVA strains were characterized as RVA I2 and I1 and HAdV as F40 and F41 (Fig. 4a, b). Analyzes of the PAdV and BoPyV strains showed that belong to genotypes PAdV-3 and PAdV-5 and BoPyV-1, respectively (Fig. 5a, b). A summary of sequenced samples according to accession number, collection year, collection site, virus species and genotype or subtype is presented in Table 4.

Discussion

In our study, HAdV, PAdV and BoPyV were used as viral indicators to investigate the source of fecal contamination in water samples via MST. Additionally, RVA was included in our study in attempt to investigate its impact and a potential genetic heterogeneity, despite not being considered as a host-specific viral indicator. Several studies showed the detection of RVA in Brazil, even after the implementation of the Rotarix® vaccine (GlaxoSmithKline Biologicals, Rixensart,
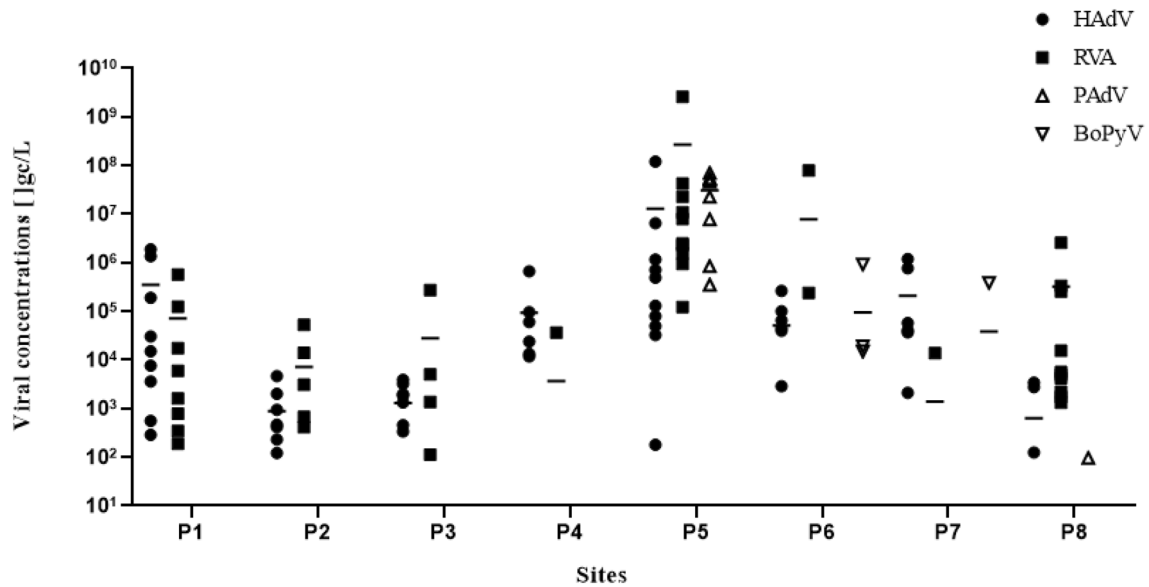
Fig. 3 Concentration of human and animal viruses according to sampling sites. GC/L, genome copies per liter; HAdV, human adenovirus; RVA, human rotavirus A; PAdV, porcine adenovirus and BoPyV, bovine poliomavirus. P1, community area; P2, Artificial Lake 1; P3, Artificial Lake 2; P4, Equine farming area; P5, Porcine farming area; P6, Bovine farming area 1; P7; Bovine farming area 2; P8, Guarda River

Table 3 Detection of human and animal viruses according to season

Season (n)	HAdV	RVA	PAdV	BoPyV
Winter (20)	11 (55.0%)	5 (25.0%)	1 (5.0%)	0
Spring (24)	21 (87.5%*)	12 (50.0%)	3 (12.5%)	0
Summer (24)	13 (54.2%)	14 (58.3%)	3 (12.5%)	3 (12.5%)
Fall (24)	10 (41.6%)	10 (41.6%)	3 (12.5%)	1 (4.2%)
Total (92)	55 (59.8%)	41 (44.6%)	10 (10.9%)	4 (4.3%)

HAdV Human adenovirus, *RVA* human rotavirus A, *PAdV* porcine adenovirus, *BoPyV* bovine poliomavirus

*p < 0.05

Belgium) in the Brazilian National Immunization Program in 2006 (Fumian et al., 2013, 2018, 2011; Miagostovich et al., 2020).

Our results showed the presence of fecal viral indicators from human (HAdV), porcine (PAdV), bovine (BoPyV) origin at all sites selected for sampling, related to human or livestock' activities. In our study, it is likely that RVA are from human origin since in molecular analysis, sequences clustered with human strains. Overall, HAdV and RVA were frequently detected in all sites during the period of the study, while PAdV and BoPyV were less frequently detected. Previous studies investigating the presence of HAdV in water samples demonstrated its suitable use as a viral indicator for human fecal contamination (Gularte et al., 2019; Hundesa et al., 2006; Viancelli et al., 2012). The fact that HAdV was detected at high viral concentrations in all sites (up to 10^8

GC/L), denotes a frequent and steady excretion of this virus in the sites screened. In Brazil, other studies showed frequent HAdV detection with similar viral concentrations in several water matrices, such as rivers, beaches and lagoons (Girardi et al., 2019; Gulorte et al., 2019; Graça et al., 2021). In Uruguay, a study also showed HAdV as the most prevalent virus, followed by BoPyV in waters from a basin and a river, with viral concentrations varying from 10^2 to 10^4 GC/L. The authors also showed that HAdV particles from analyzed samples were infective (Bortagaray et al., 2019). In our study, RVA was detected less frequently than HAdV but presented higher viral concentrations (up to 10^9 GC/L). RVA was also detected in animal farming sites, suggesting the hypothesis of cross contamination of sewage collection systems. The cross-contamination may happen during seasons when run-off water or storm water could combine sewer systems (García-Aljaro et al., 2019).

Between livestock viruses, PAdV was detected more frequently compared to BoPyV in P5, a porcine farming area. Nevertheless, it was also detected in one sample from Guarda River (P8) during spring, 2017. The low detection of PAdV could be attributed to the water type and the volume of samples since they were collected from an area of running water. This area is located close to a residential area in Itaguaí municipality. Despite the notable pollution in the area, residents have contact with the water to swim, to use boats for fishing activities and transport. Although human fecal contamination offers more risk to human health, this data shows the need for a better infrastructure for basic
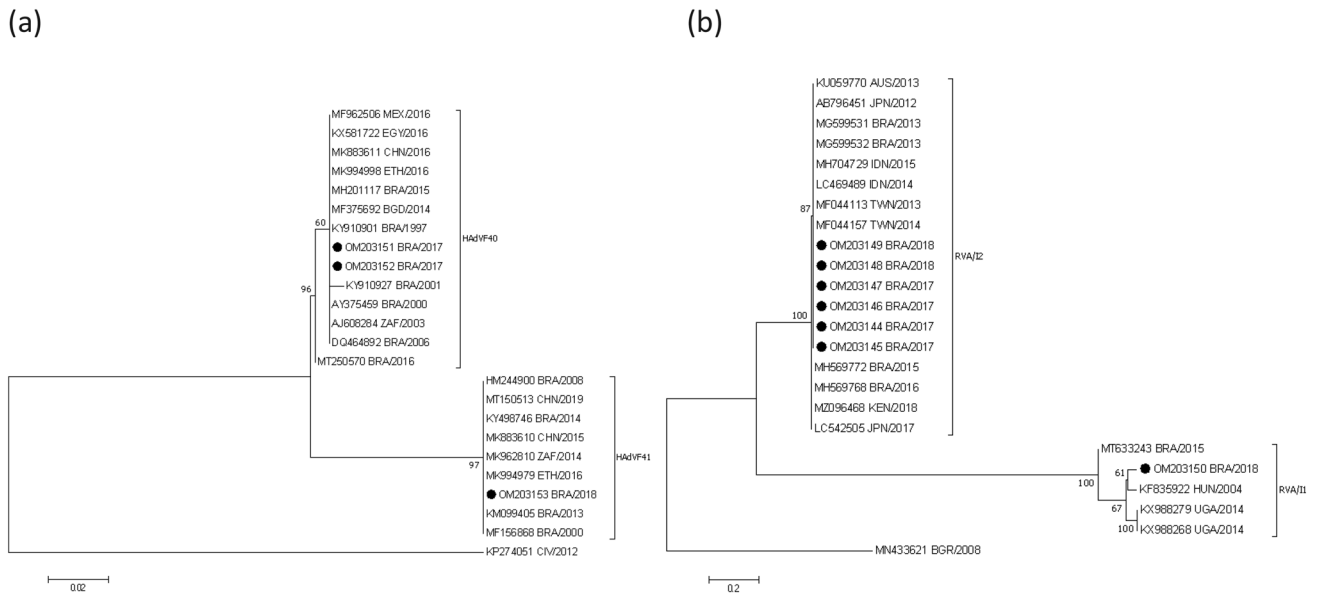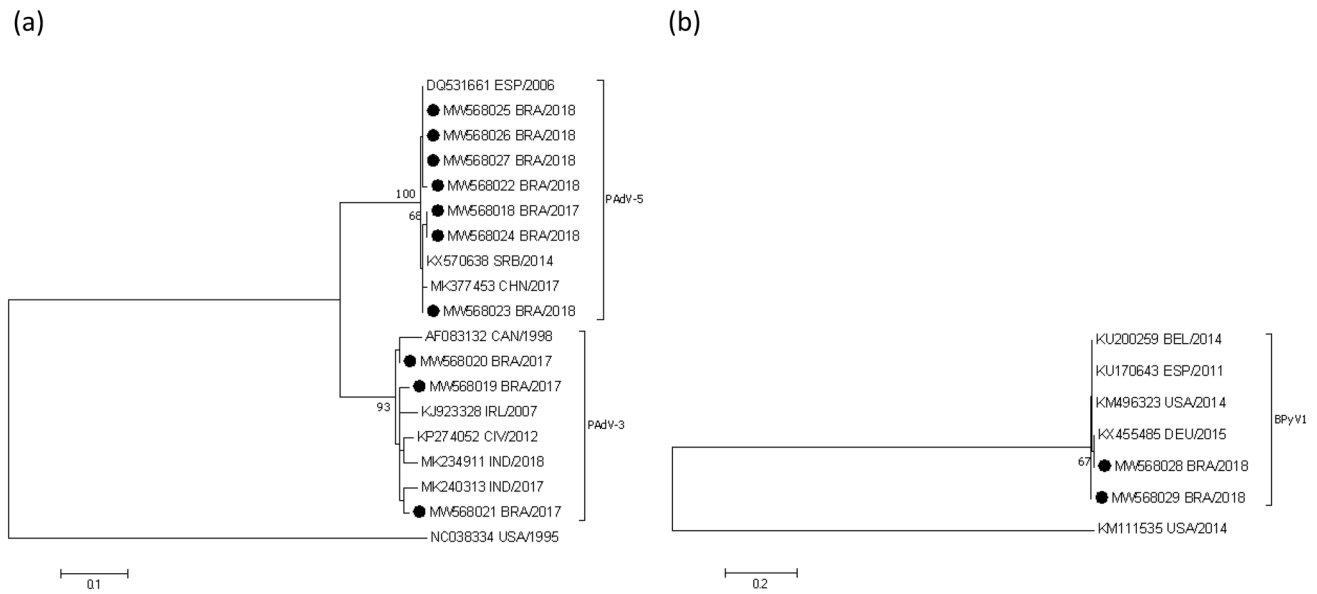
(a)

(b)

Fig. 4 a Phylogenetic analysis of human adenovirus (HAdV) sequences (black dots) corresponding to a 171 bp fragment of the hexon gene. HAdV reference strains are shown along with species and types. **b** Phylogenetic analysis of rotavirus A (RVA) sequences (black dots) corresponding to a 379 bp fragment of the VP6 gene. RVA reference strains are shown along with species and types. Bootstrap values over 70% are shown. The scale bar at the bottom represents substitutions per nucleotide position

(a)

(b)

Fig. 5 a Phylogenetic analysis of porcine adenovirus (PAdV) sequences (black dots) corresponding to a 308 bp fragment of the hexon gene. PAdV reference strains are shown along with species and types. **b** Phylogenetic analysis of bovine polyomavirus (BoPyV) sequences (black dots) corresponding to a 217 fragment of the gp2 gene. BoPyV reference strains are shown along with species and types. Bootstrap values over 70% are shown. The scale bar at the bottom represents substitutions per nucleotide position

sanitation. BoPyV was not detected frequently, and this could be due to local low dispersion from contamination sources (effluent pipes disposal) associated to viral concentration and/or geographical factors. Nevertheless, BoPyV was detected at high viral concentrations in sewage samples

from both sites of bovine farming sites and dairy production, which could represent a risk of contamination of milk and/or other products of animal origin.

Another study evaluated the prevalence and potential infectivity and showed that PAdV can be resistant to

Table 4 Summary of molecular characterization of viruses according to sampling site, year and GenBank number

Site	Year	Virus	Genbank accession number
P1	2018	RVA I2	OM203148
P2		RVA I2	OM203147
P3	2017	HAdV F40	OM203152
		RVA I2	OM203144
	2018	HAdV F41	OM203153
P4	2017	RVA I2	OM203146
P5		PAdV-5	MW568018
		PAdV-3	MW568020
		PAdV-3	MW568021
		HAdV F40	OM203151
	2018	PAdV-5	MW568022
		PAdV-5	MW568023
		PAdV-5	MW568024
		RVA-I1	OM203150
		PAdV-5	MW568025
		PAdV-5	MW568026
		PAdV-5	MW568027
P6	2018	BoPyV-1	MW568028
P7		BoPyV-1	MW568029
	2017	RVA-I2	OM203145
P8	2017	PAdV-3	MW568019

HAdV Human adenovirus, *RVA* human rotavirus A, *PAdV* porcine adenovirus, *BoPyV* bovine poliomavirus

wastewater treatment (Viancelli et al., 2012). In our investigation, molecular methods were used for viral detection and quantification. The detection and quantification of viruses by qPCR/RT-qPCR can be compromised by the presence of inhibitors, nor does it address infectivity (Girones et al., 2010). These points may represent a limitation to our study.

The virus concentrations in our study tended to be higher than in similar studies. In Brazil, a study showed viral concentrations ranging from 8×10^2 to 2×10^5 GC/L for HAdV in water samples from a coastal ecosystem area in Rio de Janeiro, Brazil (Graça et al., 2021). Another surveillance study performed in Negro River, in the Brazilian Amazonic region, HAdV mean viral concentrations were similar to our study (10^6 GC/L) and RVA mean viral concentrations were lower (10^4–10^5 GC/L) (Girones et al., 2010). Bortagaray et al. (2019) found mean viral concentration of 10^4 GC/L of both HAdV and BoPyV in water samples from a river in Uruguay. In a study using water samples from a river in Sweden, HAdV and BoPyV mean viral concentrations were of 5.2×10^4 GC/L and 1.0×10^2 GC/L, respectively. Differences in mean viral concentration values among different geographical areas may be related to the local epidemiology

of these viruses and to the viral concentration and/or detection methods used in different studies.

In our study, HAdV and RVA were detected mostly during spring and summer. This may be attributed to the pluviometric factors. A study developed in the area showed that December, January and March presented rainfall levels over 120 mm (Oliveira Júnior et al., 2014). As showed in another study, environmental factors, such as temperature and rainy or dry periods, could determine the global distribution of viral pathogens (Carratalà et al., 2013).

Molecular analysis of gene VP6 characterized RVA strains of this study as I2 and one strain as I1 (recovered from the porcine farming area in the summer of the year 2018). It is known that genotype I1 is associated with strains that belong to *Wa-like* genogroup (most detected in humans and pigs), and which internal genes are associated to genotype 1. A study from 2016, also conducted in a rural area of Rio de Janeiro, detected RVA genotype I1 in fecal samples from pigs, demonstrating its broad circulation among pigs in the state (Flores et al., 2021). As for genotype I2, it is known that it is associated with *DS-1-like* RVA genogroup, related to variants whose internal genes are associated with genotype 2, e.g., R2, C2, M2, I2, A2, N2, T2, E2, and H2, for VP1-3, VP6, NSP1-5, respectively. Studies conducted in Brazil showed in the last decade, an increase in detection of RVA from equine G3P[8] *DS-1-like* profile, as well as in other genotypic combinations compatible with the DS-1-like genogroup, as G1P[8] and G12P[4/8] strains with internal genes associated to genotype 2 (Guerra et al., 2016; Luchs et al., 2019; Silva-Sales et al., 2020). HAdV sequences were classified as F40 and F41, enteric types most associated to acute gastroenteritis (AG) cases in young children (Rafie et al., 2021; Tahmasebi et al., 2020). The presence of enteric viruses may reflect the epidemiological scenario of AG in the metropolitan area of Rio de Janeiro, since this area suffers from inadequate water distribution, sanitation, and investments in hygiene programs (Bacha et al., 2021; Faria et al., 2021).

Phylogenetic analysis of PAdV showed the circulation of two genotypes: PAdV-3 and PAdV-5. The sample detected in Guandu River (P8) was closely related to a sample detected in porcine farming site (P5), which reinforces the evidence for a potential contamination source. Both genotypes were already detected in animal and environmental samples from different countries, usually in rural regions with livestock activities (Haack et al., 2015; Hundesa et al., 2006). Our study corroborates a study developed in Taiwan that showed these two genotypes circulating in the outflow of livestock farm wastewater channel (Nagarajan et al., 2021). PAdVs have been associated to gastrointestinal disease, diarrhea, and multifactorial respiratory disease in swine (Kumthip et al., 2019).

BoPyV was characterized as BoPyV-1, closely related to BoPyV detected in cattle beef muscle and ground beef samples from Germany and USA (Gräfe et al., 2017; Peretti et al., 2015). The areas where BoPyV was detected are involved with educational purposes and dairy productions in a university campus. Since these viruses offer risk of infection and disease, further risk assessment studies are necessary. BoPyV-1 was first described as a contaminant in fetal bovine serum used in laboratory procedures (Wang et al., 2005). Despite the lack of clinical evidence of polyomavirus infection in animals, it has been detected on aborted cattle (Van Borm et al., 2016).

Conclusion

In conclusion, our study shows that the water matrices studied are affected by anthropogenic and livestock contamination, with evidence for cross-contamination. It also highlights the need to improve sewage treatment inside the university campus, since students and staff are at risk of exposure during classes or recreational activities. This scenario creates a potential contamination risk for groundwater, waters for recreational activities and crop irrigation with impact to public and animal health.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Nucleotide sequences are available in GenBank database under the deposited numbers cited within the manuscript.

Declarations

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

References

Adelodun, B., Ajibade, F. O., Ighalo, J. O., Odey, G., Ibrahim, R. G., Kareem, K. Y., Bakare, H. O., Tiamiyu, A. O., Ajibade, T. F., Abdulkadir, T. S., Adeniran, K. A., & Choi, K. S. (2021). Assessment of socioeconomic inequality based on virus-contaminated water usage in developing countries: A review. *Environmental Research., 192*, 110309. https://doi.org/10.1016/j.envres.2020.110309

Allard, A., Albinsson, B., & Wadell, G. (2001). Rapid typing of human adenoviruses by a general PCR combined with restriction endonuclease analysis. *Journal of Clinical Microbiology, 39*, 498–505. https://doi.org/10.1128/JCM.39.2.498-505.2001

Bacha, L., Ventura, R., Barrios, M., Seabra, J., Tschoeke, D., Garcia, G., Masi, B., Macedo, L., de Godoy, J. M. O., Cosenza, C., de Rezende, C. E., Lima, V., Ottoni, A. B., Thompson, C., & Thompson, F. (2021). Risk of collapse in water quality in the Guandu River (Rio de Janeiro, Brazil). *Microbial Ecology*. https://doi.org/10.1007/s00248-021-01839-z

Ballesté, E., Blanch, A. R., Mendez, J., Sala-Comorera, L., Maunula, L., Monteiro, S., Farnleitner, A. H., Tiehm, A., Jofre, J., & García-Aljaro, C. (2021). Bacteriophages are good estimators of human viruses present in water. *Frontiers in Microbiology, 12*, 619495. https://doi.org/10.3389/fmicb.2021.619495

Barrios, M. E., Blanco Fernández, M. D., Cammarata, R. V., Torres, C., & Mbayed, V. A. (2018). Viral tools for detection of fecal contamination and microbial source tracking in wastewater from food industries and domestic sewage. *Journal of Virological Methods, 262*, 79–88. https://doi.org/10.1016/j.jviromet.2018.10.002

Boom, R., Sol, C. J., Salimans, M. M., Jansen, C. L., Wertheim-van Dillen, P. M., & van der Noordaa, J. (1990). Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology, 28*, 495–503. https://doi.org/10.1128/JCM.28.3.495-503.1990

Bortagaray, V., Lizasoain, A., Piccini, C., Gillman, L., Berois, M., Pou, S., del Pilar, D. M., Tort, F. L., Colina, R., & Victoria, M. (2019). Microbial source tracking analysis using viral indicators in Santa Lucía And Uruguay Rivers. *Food and Environmental Virology, 11*, 259–267. https://doi.org/10.1007/s12560-019-09384-2

Bosch, A., Guix, S., Sano, D., & Pintó, R. M. (2008). New tools for the study and direct surveillance of viral pathogens in water. *Current Opinion in Biotechnology, 19*, 295–301. https://doi.org/10.1016/j.copbio.2008.04.006

Calgua, B., Mengewein, A., Grunert, A., Bofill-Mas, S., Clemente-Casares, P., Hundesa, A., Wyn-Jones, A. P., López-Pila, J. M., & Girones, R. (2008). Development and application of a one-step low cost procedure to concentrate viruses from seawater samples. *Journal of Virological Methods, 153*, 79–83. https://doi.org/10.1016/j.jviromet.2008.08.003

Carratalà, A., Rusiñol, M., Rodriguez-Manzano, J., Guerrero-Latorre, L., Sommer, R., & Girones, R. (2013). Environmental effectors on the inactivation of human adenoviruses in water. *Food and Environmental Virology, 5*, 203–214. https://doi.org/10.1007/s12560-013-9123-3

da Graça Pedrosa de Macena, L., Feitosa, R. C., Vieira, C. B., Araújo, I. T., Taniuchi, M., & Miagostovich, M. P. (2021). Microbiological assessment of an urban lagoon system in the coastal zone of Rio de Janeiro Brazil. *Environmental Science and Pollution Research, 28*, 1170–1180. https://doi.org/10.1007/s11356-020-10479-8

da Silva Bandeira, B., dos Santos Souto, L. C., de Souza, L. C., Linhares, A. C., & Mascarenhas, J. D. A. P. (2022). Detection of a novel species A, DS-1-like, G4P[6] rotavirus strain from a Brazilian child with gastroenteritis. *Journal of Medical Virology, 94*, 610–615. https://doi.org/10.1002/jmv.27283

Faria, C. P., Almendra, R., Dias, G. S., Santana, P., do Céu, S. M., & de Freitas, M. B. (2021). Evaluation of the drinking water quality surveillance system in the metropolitan region of Rio de Janeiro. *Journal of Water and Health, 19*, 306–321. https://doi.org/10.2166/wh.2021.217

Flores, P. S., Costa, F. B., Amorim, A. R., Mendes, G. S., Rojas, M., & Santos, N. (2021). Rotavirus A, C, and H in Brazilian pigs: Potential for zoonotic transmission of RVA. *Journal of Veterinary Diagnostic Investigation, 33*, 129–135. https://doi.org/10.1177/1040638720967673

Fumian, T. M., Gagliardi Leite, J. P., Rose, T. L., Prado, T., & Miagostovich, M. P. (2011). One year environmental surveillance of rotavirus specie A (RVA) genotypes in circulation after the introduction of the Rotarix® vaccine in Rio de Janeiro Brazil. *Water Research, 45*, 5755–5763. https://doi.org/10.1016/j.watres.2011.08.039

Fumian, T. M., Vieira, C. B., Leite, J. P. G., & Miagostovich, M. P. (2013). Assessment of burden of virus agents in an urban sewage treatment plant in Rio de Janeiro Brazil. *Journal of Water and Health, 11*, 110–119. https://doi.org/10.2166/wh.2012.123

Fumian, T. M., Victoria, M., Vieira, C. B., Fioretti, J. M., Rocha, M. S., Prado, T., Guimarães, F. R., da Gama, N. P., de Oliveira, J. M., Mendes, A. C. O., Gaspar, A. M. C., Santos, J. D. O., Chame, M., Leite, J. P. G., & Miagostovich, M. P. (2018). Enteric viruses' dissemination in a private reserve of natural heritage. *Letters in Applied Microbiology, 66*, 313–320. https://doi.org/10.1111/lam.12848

García-Aljaro, C., Blanch, A. R., Campos, C., Jofre, J., & Lucena, F. (2019). Pathogens, faecal indicators and human-specific microbial source-tracking markers in sewage. *Journal of Applied Microbiology, 126*, 701–717. https://doi.org/10.1111/jam.14112

Girardi, V., Mena, K. D., Albino, S. M., Demoliner, M., Gularte, J. S., de Souza, F. G., Rigotto, C., Quevedo, D. M., Schneider, V. E., Paesi, S. O., Tarwater, P. M., & Spilki, F. R. (2019). Microbial risk assessment in recreational freshwaters from southern Brazil. *Science of the Total Environment, 651*, 298–308. https://doi.org/10.1016/j.scitotenv.2018.09.177

Girones, R., Ferrús, M. A., Alonso, J. L., Rodriguez-Manzano, J., Calgua, B., de Abreu Corrêa, A., Hundesa, A., Carratala, A., & Bofill-Mas, S. (2010). Molecular detection of pathogens in water—The pros and cons of molecular techniques. *Water Research, 44*, 4325–4339. https://doi.org/10.1016/j.watres.2010.06.030

González-Fernández, A., Symonds, E. M., Gallard-Gongora, J. F., Mull, B., Lukasik, J. O., Rivera Navarro, P., Badilla Aguilar, A., Peraud, J., Brown, M. L., Mora Alvarado, D., Breitbart, M., Cairns, M. R., & Harwood, V. J. (2021). Relationships among microbial indicators of fecal pollution, microbial source tracking markers, and pathogens in Costa Rican coastal waters. *Water Research, 188*, 116507. https://doi.org/10.1016/j.watres.2020.116507

Gräfe, D., Ehlers, B., Mäde, D., Ellerbroek, L., Seidler, T., & Johne, R. (2017). Detection and genome characterization of bovine polyomaviruses in beef muscle and ground beef samples from Germany. *International Journal of Food Microbiology, 241*, 168–172. https://doi.org/10.1016/j.ijfoodmicro.2016.10.024

Guerra, S. F. S., Soares, L. S., Lobo, P. S., Penha Júnior, E. T., Sousa Júnior, E. C., Bezerra, D. A. M., Vaz, L. R., Linhares, A. C., & Mascarenhas, J. D. P. (2016). Detection of a novel equine-like G3 rotavirus associated with acute gastroenteritis in Brazil. *Journal of General Virology, 97*, 3131–3138. https://doi.org/10.1099/jgv.0.000626

Gularte, J. S., Girardi, V., Demoliner, M., de Souza, F. G., Filippi, M., Eisen, A. K. A., Mena, K. D., de Quevedo, D. M., Rigotto, C., de Barros, M. P., & Spilki, F. R. (2019). Human mastadenovirus in water, sediment, sea surface microlayer, and bivalve mollusk from southern Brazilian beaches. *Marine Pollution Bulletin, 142*, 335–349. https://doi.org/10.1016/j.marpolbul.2018.12.046

Haack, S. K., Duris, J. W., Kolpin, D. W., Fogarty, L. R., Johnson, H. E., Gibson, K. E., Focazio, M., Schwab, K. J., Hubbard, L. E., & Foreman, W. T. (2015). Genes indicative of zoonotic and swine pathogens are persistent in stream water and sediment following a swine manure spill. *Applied and Environment Microbiology, 81*, 3430–3441. https://doi.org/10.1128/AEM.04195-14

Hernroth, B. E., Conden-Hansson, A. -C., Rehnstam-Holm, A. -S., Girones, R., & Allard, A. K. (2002). Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis : the first scandinavian report. *Applied and Environmental Microbiology*, 68, 4523–4533. https://doi.org/10.1128/AEM.68.9.4523-4533.2002

Hundesa, A., Maluquer de Motes, C., Bofill-Mas, S., Albinana-Gimenez, N., & Girones, R. (2006). Identification of human and animal adenoviruses and polyomaviruses for determination of sources of fecal contamination in the environment. *Applied and Environment Microbiology, 72*, 7886–7893. https://doi.org/10.1128/AEM.01090-06

Hundesa, A., Maluquer de Motes, C., Albinana-Gimenez, N., Rodriguez-Manzano, J., Bofill-Mas, S., Suñen, E., & Rosina Girones, R. (2009). Development of a qPCR assay for the quantification of porcine adenoviruses as an MST tool for swine fecal contamination in the environment. *Journal of Virological Methods, 158*, 130–135. https://doi.org/10.1016/j.jviromet.2009.02.006

Hundesa, A., Bofill-Mas, S., Maluquer de Motes, C., Rodriguez-Manzano, J., Bach, A., Casas, M., & Girones, R. (2010). Development of a quantitative PCR assay for the quantitation of bovine polyomavirus as a microbial sourcetracking tool. *Journal of Virological Methods, 163*, 385–389. https://doi.org/10.1016/j.jviromet.2009.10.029

Iturriza Gómara, M., Wong, C., Blome, S., Desselberger, U., & Gray, J. (2002). Rotavirus subgroup characterisation by restriction endonuclease digestion of a cDNA fragment of the VP6 gene. *Journal of Virological Methods, 105*, 99–103. https://doi.org/10.1016/S0166-0934(02)00087-3

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution, 16*, 111–120. https://doi.org/10.1007/BF01731581

Korajkic, A., Wanjugi, P., Brooks, L., Cao, Y., & Harwood, V. J. (2019). Persistence and decay of fecal microbiota in aquatic habitats. *Microbiology and Molecular Biology Reviews, 83*, e00005-19. https://doi.org/10.1128/MMBR.00005-19

Kumthip, K., Khamrin, P., Kongkaew, A., Vachirachewin, R., Malasao, R., Ushijima, H., & Maneekarn, N. (2019). Molecular epidemiology and characterization of porcine adenoviruses in pigs with diarrhea in Thailand. *Infection, Genetics and Evolution, Journal of Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases, 67*, 73–77. https://doi.org/10.1016/j.meegid.2018.10.026

Luchs, A., da Costa, A. C., Cilli, A., Komninakis, S. C. V., Carmona, R. C. C., Morillo, S. G., Sabino, E. C., & da Carmo Sampaio Tavares Timenetsky, M. D. (2019). First detection of DS-1-like G1P[8] double-gene reassortant rotavirus strains on The American Continent, Brazil, 2013. *Scientific Reports, 9*, 2210. https://doi.org/10.1038/s41598-019-38703-7

Miagostovich, M. P., Rocha, M. S., Dos Reis, F. B., Sampaio, M. S., de Saldanha da Gama Gracie, C. R., Malta, F. C., Rodrigues, J., Genuino, A., da Silva Assis, M. R., Fumian, T. M., & Barrocas, P. R. G. (2020). Gastroenteric viruses detection in a drinking water distribution-to-consumption system in a low-income community in Rio de Janeiro. *Food and Environmental Virology, 12*, 130–136. https://doi.org/10.1007/s12560-020-09423-3

Nagarajan, V., Chen, J. S., Hsu, B. M., Hsu, G. J., Wang, J. L., & Hussain, B. (2021). Prevalence, distribution, and genotypes of adenovirus and norovirus in the puzi river and its tributaries and the surrounding areas in Taiwan. *GeoHealth*. https://doi.org/10.1029/2021GH000465

Oliveira Júnior, J. F., Delgado, R. C., Gois, G., Lannes, A., Dias, F. O., Souza, J. C., & Souza, M. (2014). Análise da Precipitação e sua Relação com Sistemas Meteorológicos em Seropédica, Rio de Janeiro. *Floresta E Ambiente, 21*, 140–149. https://doi.org/10.4322/floram.2014.030

Peretti, A., FitzGerald, P. C., Bliskovsky, V., Buck, C. B., & Pastrana, D. V. (2015). Hamburger polyomaviruses. *Journal of General Virology, 96*, 833–839. https://doi.org/10.1099/vir.0.000033

Pina, S., Jofre, J., Emerson, S. U., Purcell, R. H., & Girones, R. (1998). Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. *Applied and Environment Microbiology, 64*, 4485–4488. https://doi.org/10.1128/AEM.64.11.4485-4488.1998

Rafie, K., Lenman, A., Fuchs, J., Rajan, A., Arnberg, N., & Carlson, L.-A. (2021). The structure of enteric human adenovirus 41—A leading cause of diarrhea in children. *Science Advances, 7*, eabe0974. https://doi.org/10.1126/sciadv.abe0974

Rusiñol, M., Fernandez-Cassi, X., Hundesa, A., Vieira, C., Kern, A., Eriksson, I., Ziros, P., Kay, D., Miagostovich, M., Vargha, M., Allard, A., Vantarakis, A., Wyn-Jones, P., Bofill-Mas, S., & Girones, R. (2014). Application of human and animal viral microbial source tracking tools in fresh and marine waters from five different geographical areas. *Water Research, 59*, 119–129. https://doi.org/10.1016/j.watres.2014.04.013

Rusiñol, M., Hundesa, A., Cárdenas-Youngs, Y., Fernández-Bravo, A., Pérez-Cataluña, A., Moreno-Mesonero, L., Moreno, Y., Calvo, M., Alonso, J. L., Figueras, M. J., Araujo, R., Bofill-Mas, S., & Girones, R. (2020). Microbiological contamination of conventional and reclaimed irrigation water: Evaluation and management measures. *Science of the Total Environment, 710*, 136298. https://doi.org/10.1016/j.scitotenv.2019.136298

Silva-Sales, M., Leal, E., de Milagres, F. A. P., Brustulin, R., Morais, V. D. S., Marcatti, R., Araújo, E. L. L., Witkin, S. S., Deng, X., Sabino, E. C., Delwart, E., Luchs, A., & da Costa, A. C. (2020). Genomic constellation of human Rotavirus A strains identified in Northern Brazil: a 6-year follow-up (2010–2016). *Revista Do Instituto De Medicina Tropical De São Paulo, 62*, e98. https://doi.org/10.1590/S1678-9946202062098

Tahmasebi, R., Luchs, A., Tardy, K., Hefford, P. M., Tinker, R. J., Eilami, O., de Padua Milagres, F. A., Brustulin, R., Teles, M.

D. A. R., Dos Santos, M. V., Moreira, C. H. V., Buccheri, R., Araújo, E. L. L., Villanova, F., Deng, X., Sabino, E. C., Delwart, E., Leal, É., & Charlys da Costa, A. (2020). Viral gastroenteritis in Tocantins, Brazil: characterizing the diversity of human adenovirus F through next-generation sequencing and bioinformatics. *Journal of General Virology, 101*, 1280–1288. https://doi.org/10.1099/jgv.0.001500

Tubbs Filho, D., Antunes, J. C. O., & Vettorazzi, J. S. (2012). *Bacia Hidrográfica dos Rios Guandu, da Guarda e Guandu-Mirim. Experiências para a gestão dos recursos hídricos*. Rio de Janeiro: INEA.

Van Borm, S., Rosseel, T., Behaeghel, I., Saulmont, M., Delooz, L., Petitjean, T., Mathijs, E., & Vandenbussche, F. (2016). Complete genome sequence of bovine polyomavirus Type 1 from aborted cattle, isolated in Belgium in 2014. *Genome Announcements, 4*, e01646-e1715. https://doi.org/10.1128/genomeA.01646-15

Viancelli, A., Garcia, L. A. T., Kunz, A., Steinmetz, R., Esteves, P. A., & Barardi, C. R. M. (2012). Detection of circoviruses and porcine adenoviruses in water samples collected from swine manure treatment systems. *Research in Veterinary Science, 93*, 538–543. https://doi.org/10.1016/j.rvsc.2011.07.022

Wang, J., Horner, G. W., & O'Keefe, J. S. (2005). Detection and molecular characterisation of bovine polyomavirus in bovine sera in New Zealand. *New Zealand Veterinary Journal, 53*, 26–30. https://doi.org/10.1080/00480169.2005.36465

Wong, K., Fong, T.-T., Bibby, K., & Molina, M. (2012). Application of enteric viruses for fecal pollution source tracking in environmental waters. *Environment International, 45*, 151–164. https://doi.org/10.1016/j.envint.2012.02.009

Zeng, S. -Q., Halkosalo, A., Salminen, M., Szakal, E. D., Puustinen, L., & Vesikari, T. (2008). One-step quantitative RTPCR for the detection of rotavirus in acute gastroenteritis. *Journal of Virological Methods, 153*, 238–240. https://doi.org/10.1016/j.jviromet.2008.08.004