ORIGINAL PAPER

Assessment of Water Quality in a Border Region Between the Atlantic Forest and an Urbanised Area in Rio de Janeiro, Brazil

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Abstract The preservation of water resources is one of the goals of the designation of parks that act as natural reservoirs. In order to assess the impact of the presence of humans in an environmental preservation area bordering urban areas, the presence of four pathogenic enteric viruses [group A rotavirus (RV-A), norovirus (NoV), human adenoviruses (HAdV), and hepatitis A virus (HAV)], as well as the physico-chemical parameters, and *Escherichia coli* levels were assessed in riverine water samples. From June 2008 to May 2009, monthly monitoring was performed along the Engenho Novo River. RV-A, NoV, and HAdV were observed in 29 % (31/108) of the water samples, with concentrations of up to 10^3 genome copies/liter. The natural occurrence of infectious HAdV was demonstrated by Integrated Cell Culture-PCR (ICC-PCR). This study confirms the suitability of using the detection of fecal-oral transmitted viruses as a marker of human fecal contamination in water matrices and indicates the spread of pathogenic viruses occurring in an alleged area of environmental protection.

Keywords Environmental virology · Group A rotavirus · Norovirus · Human adenovirus · Hepatitis A virus · Urban forest

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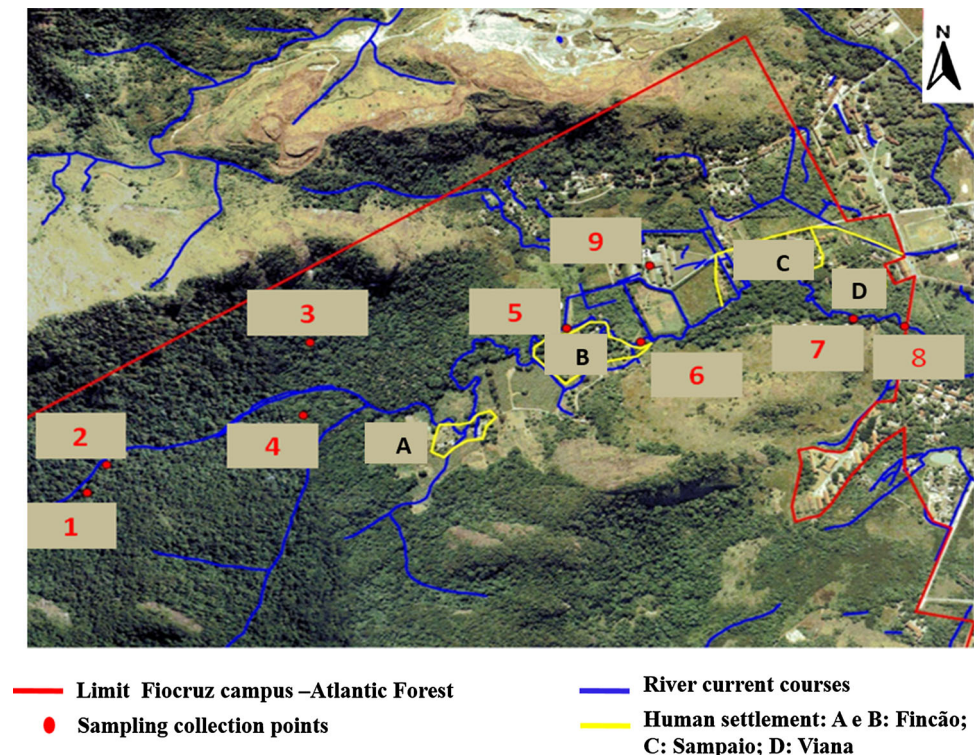Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Introduction

The Atlantic Forest biome is a tropical forest that covers the Eastern, South-eastern, and Southern regions of Brazil. Seven of the nine major river basins of the country are located in these geographic areas. Additionally, the forest supports the quantity and quality of the drinking water that supplies more than 110 million Brazilians in approximately 3,400 municipalities (http://www.ibflorestas.org.br/bioma-mata-atlantica.html).

The State of Rio de Janeiro (Southeast region) is fully located in the Atlantic Forest Biome. However, it is currently estimated that less than 20 % of the state is covered by forest that is part of an environmental preservation area (http://www.sosma.org.br). The Pedra Branca State Park is an area located in west Rio de Janeiro and is 12,500 ha of area, making it one of the largest urban parks in the world. The geographical position of the park, which is surrounded by areas of increasing urbanization, is a conflicting factor for environmental conservation. In addition to the concentration of housings, the discharge of sewage, and soil degradation, the reduction of riparian vegetation along the basins might be responsible for the contamination of reservoirs that supply many surrounding neighborhoods (http://www.parquepedrabranca.com/p/macico-da-pedra-branca.html).

Fecal pollution is a primary health concern in the environment, with contaminated water being a potential source of gastrointestinal diseases, especially those associated with viral infections. Hepatitis is another water-related disease with a significant public health impact, especially in developing countries (Bosch et al. 2008; Gentile 2008).

Currently, some studies have proposed the detection of enteric viruses as an indicator of human contamination,

Fig. 1 Satellite photo of Fiocruz, Atlantic forest campus. *Red points* represent the location of the collection points (Color figure online)

Legend:
— Limit Fiocruz campus –Atlantic Forest
● Sampling collection points
— River current courses
— Human settlement: A e B: Fincão; C: Sampaio; D: Viana

since the lack of association between virus presence and classical bacterial markers routinely used for water quality assessment is well recognized (Pina et al. 1998, Pusch et al. 2005; Wyer et al. 2012). In this study, the presence of *Escherichia coli* (*E. coli)* and four enteric viruses [group A rotavirus (RV-A), norovirus (NoV), human adenovirus (HAdV), and hepatitis A virus (HAV)], as well as physico-chemical parameters were investigated in riverine water samples in order to assess the impact of humans in an area of environmental preservation.

Materials and Methods

Study Area

Monitoring of samples was performed along the Engenho Novo River located in a unit of environmental preservation that is adjacent to and partially overlaps the Pedra Branca State Park in the Metropolitan Region of Rio de Janeiro. This bordering region contains 200 houses and approximately 1,000 residents, with many of them getting food from family farms. The whole river is used for recreational purposes.

Nine sampling collection points (P1–P9) were determined using a global positioning system (GPS, Garmin Etrex Legend®, USA). Eight of them (P1–P8) were located along the river and grouped into two sets defined by

demographic characteristics. P1–P4 were located in a nonoccupied area, and P5–P8 were in an inhabited area. P9 was defined as a pickup point for river water that was piped to be used for consumption (Fig. 1).

Environmental Sample Collection

From June 2008 to May 2009, surface water samples (2 L each) were collected monthly in sterile bottles and transported to the laboratory, where they were immediately processed to investigate the presence of viruses and bacteria. Physico-chemical parameters were measured *in loco*. Extra samples from P3 to P8, representing points of collection from locations with different demographic characteristics, were obtained to evaluate the recovery rate of RV-A and NoV GII through spiking experiments.

Physico-Chemical Parameters

Temperature (°C), conductivity (µS/cm), dissolved oxygen (DO) (mg/L), turbidity (NTU), and pH were measured using the Water Quality Checker® U-10 (Horiba Ltd, Kyoto, Japan). Statistical analyses were performed using GraphPad Prism® software, v 5. A Mann–Whitney U test was used to assess significant differences between values recorded from the two areas (P1–P8). A *p* value less than 0.05 was considered to be statistically significant.

Bacteriological Analysis

To quantify the microbial indicator *E. coli*, we used the Colilert®-18 Quanti-Tray®/2000 Kit (IDEXX Laboratories, Connecticut, USA) following the manufacturer's instructions. This method was performed within 2 h of collection on 10^{-1}–10^{-2} dilutions, and results were expressed as the Most Probable Number (MPN)/100 mL.

Virus Concentration Method

Viruses were concentrated from the 2 L water samples by an adsorption-elution method using a negatively charged membrane, as described by Katayama et al. (2002). Prior to the adsorption step, the residue was removed by filtration with a pre-filter AP-20® (Millipore, Brazil). After the steps of concentration (adsorption/elution, and ultrafiltration) a final volume of 2 mL was obtained. For cleaning, the system was soaked briefly in a 10 % solution of bleach and then rinsed in deionised water prior to each use.

The efficiency of the viral concentration method was evaluated by spiking experiments with known concentrations of RV-A [3.0×10^8–1.5×10^9 genome copies (GC)/L] and NoV genogroup (G) II (1.6×10^5–1.6×10^6 GC/L). Samples were processed following the same method as described above. Virus recovery rates obtained from water samples collected in P3 were 6.2 % and 6.7 % for RV-A and NoV GII, respectively. For water samples collected from P8, recovery rates were 0.5 and 3.3 % for RV-A and NoV GII, respectively.

Genome Purification, Reverse Transcription, and Virus Detection

The viral nucleic acids were extracted from the concentrated water using the QIAamp® viral RNA Mini kit (QIAGEN, Valencia, Spain) following the manufacturer's instructions. The cDNA synthesis was performed using random primer pd(N)6™ (Amersham Biosciences, USA) and the SuperScript® III Reverse Transcriptase (Invitrogen, USA). The detection of RV-A, HAdV, NoV, and HAV were performed using quantitative polymerase chain reaction (qPCR), using TaqMan® Universal PCR Master Mix kit (Applied Biosystems, CA, USA), and specific primers and probes as previously described (Heim et al. 2003; Kageyama et al. 2003, Villar et al. 2006; Zeng et al. 2008). Table 1 shows primers, probe sequences, and the target genome region of qPCR amplification. Reactions were performed in duplicate using the ABI 7500® Real-Time PCR System (Applied Biosystems). Specific positive and negative controls (positive clinical samples previously sequenced for each virus type and RNA/DNA-free water) were used in all procedures.

Integrated Cell Culture/PCR (ICC-PCR)

To assess the potential for virus infectivity, samples that were HAdV-positive by qPCR were inoculated onto HEK 293 cells (human embryonic kidney cells transformed by HAdV-5) for virus isolation. Prior to this step, cytotoxicity assays were performed according to Rigotto et al. (2010). Briefly, autoclaved water samples were tested in serial two-fold dilutions in serum-free Dulbecco's MEM medium (Gibco® Invitrogen, MA, USA). The cells were analyzed to establish the noncytotoxic dilutions to be used for ICC-PCR. Water sample dilutions of 1:2 and 1:4 (noncytotoxic condition) were used in HEK 293 cells when ICC-PCR was applied. For the HAdV infectivity, 200 μL of each dilution selected was inoculated in duplicate in tubes containing monolayers of a HEK 293 cells (10^5–10^6 cell/mL) and incubated for 1 h at 37 °C to allow for viral adsorption. Afterward, the inoculum was removed, and the cells were washed twice with 1x PBS in order to remove virus particles that had not penetrated the cells. The tubes were incubated at 37 °C for 48 h in maintenance medium. After this period, cells were removed from the surface of the tubes and lysed by freezing/thawing (−70 °C/37 °C) three times to release the virus particles. The samples were then centrifuged at $5,000 \times g$ for 15 min at 4 °C, and the supernatant was recovered for HAdV DNA purification, as described above. To confirm virus replication, a nested-PCR was performed according to the protocol described by Allard et al. (1992).

Results

A total of 108 water samples were obtained: 96 from along the Engenho Novo stream (P1–P8) and 12 from the point of water abstraction for producing tap water (P9). Physicochemical parameter analysis revealed no variation in the mean values for all of the points studied, except for turbidity, which increased significantly ($p = 0.028$) in the inhabited area of the campus (P5–P8; Table 2). Water samples from this specific area had *E.coli* concentrations ranging from 2.2×10^3–1.3×10^4 MPN/100 mL in 98 % (47/48) of them. P1–P4 showed values ranging from 3.0–4.0×10^2 MPN/100 mL, and in P9, the values were less than 2.9×10^1 MPN/100 mL in all of the samples analyzed.

Concerning viral contamination, 29 % (31/108) of the water samples studied had at least one virus detected, totaling 37 strains. RV-A ($n = 17$), NoV GII ($n = 10$), NoV GI ($n = 3$), and HAdV ($n = 7$) were detected at logarithmic concentrations of 10^3. The HAV genome was not detected in any sample. The inhibited area (P5–P8) represented 74.2 % of the virus-positive water samples,

Table 1 Oligonucleotide primer and probe sequences and genome regions used for virus detection and quantification in water samples

[a] 6-Carboxyfluorescein (FAM) or 4,7,2′-trichloro-7′-phenyl-6-carboxyfluorescein (VIC) used as reporter dye and was coupled to the 5′end of the oligonucleotide probe, and 6-carboxy-tetramethylrhodamine (TAMRA) was used as the quencher dye and was coupled at the 3′ end

ORF Open reading frame, NCR noncoding region, and mixed bases in the primers/probes: Y C/T, N A/T/C/G, R A/G, W A/T, and B C/G/T

Viruses	Primers/ Probe	Nucleotide sequence	Genome region	References
RV-A	NSP3F	ACCATCTWCACRTRACCCTCTATGAG	NSP3	Zeng et al.
	NSP3R	GGTCACATAACGCCCCTATAGC	Gene segment	(2008)
	Probe	AGTTAAAAGCTAACACTGTCAAA		
HAdV	AQ1 F	GCCACGGTGGGGTTTCTAAACTT	Hexon gene	Heim et al.
	AQ2 R	GCCCCAGTGGTCTTACATGCACATC		(2003)
	Probe[a]	TGCACCAGACCCGGGCTCAGGTACTCCGA		
NoV GI	COG1F	CGYTGGATGCGNTTYCATGA	ORF1-ORF2 junction	Kageyama et al. (2003)
	COG1R	CTTAGACGCCATCATCATTYAC		
	Probe[a]	AGATYGCGATCYCCTGTCCA		
NoV GII	COG2F	CARGARBCNATGTTYAGRTGGATGAG	ORF1-ORF2 junction	Kageyama et al. (2003)
	COG2R	TCGACGCCATCTTCATTCACA		
	Probe[a]	TGGGAGGGCGATCGCAATCT		
HAV	Forward	CTGCAGGTTCAGGGTTCTTAAATC	5′ NCR	Villar et al. (2006)
	Reverse	GAGAGCCCTGGAAGAAAGAAGA		
	Probe[a]	ACTCATTTTTCACGCTTTCTG		

Table 2 Mean values of physico-chemical parameters grouped according to demographic characteristics

Sampling collection points (no. of samples)	Physico-chemical parameters				
	pH	Conductivity (mS/cm)	Turbidity* (NTU)	Dissolved oxygen (mg/L)	Temperature (°C)
P1–4 (48)	7.0	0.083	0.3	8.2	21.6
P5–8 (48)	6.7	0.100	3.0	7.8	22.5
P9 (12)	7.0	0.081	0.8	7.4	23.5

* $p < 0.05$, Mann–Whitney U test

mS/cm milliSiemens per centimeters, NTU nephelometric turbidity units, mg/L milligram per liter, C Celsius

Table 3 Detection of group A rotavirus (RV-A), norovirus genogroup I and II (NoV GI and GII), human adenovirus (HAdV), and hepatitis A viruses (HAV)

Sampling collection points	Positive samples/ analyzed samples	Number of positive sample for any/each virus tested					
		Any virus	RV-A	NoV GI	NoV GII	HuAdV	HAV
P1–4	7/48 (15 %)	7	5	0	0	2	0
P5–8	23/48 (48 %)	29	12	3	10	4	0
P9	1/12 (8 %)	1	0	0	0	1	0
Total	31/108 (29 %)	37	7	3	10	7	0

78.4 % (29/37) of the individual viruses detected, and 100 % of the NoV-positive samples (Table 3).

The microbiological parameters (*E.coli* and gastroenteric viruses) revealed RV-A and HAdV detection in P1–P4, and in P9 where *E.coli* counts presented values lower than 2,000 MPN/mL (Fig. 2). According to Brazilian Legislation (BRASIL 2000), 43.5 % (47/108) of environmental samples (P1–P9) were deemed inappropriate. This *E.coli* value is used as a cut off for classifying primary contact recreational water in the categories acceptable and unacceptable.

The cytotoxicity assay demonstrated a natural occurrence of infectious HAdV in two (P6–P9) of seven positive samples, as was determined by the observation of bands of the expected size (171 bp) by gel electrophoresis (data not shown).

Discussion

The main approach of this study was to demonstrate the impact of human presence in an Atlantic forest biome with
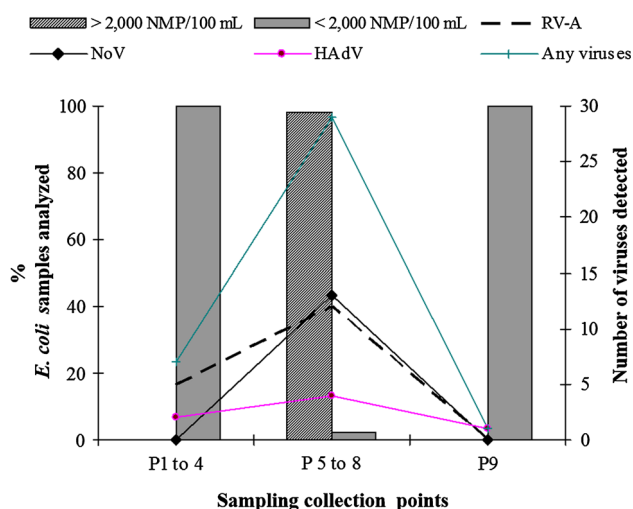
Fig. 2 Detection of rotavirus A (RV-A), norovirus genogroup I, II (NoV GI and GII), and human adenovirus (HAdV) according to *E.coli* parameters based on Brazilian regulation (< 2,000 MPN/100 mL) and (> 2,000 MPN/100 mL)

fresh water resources using physico-chemical and microbiological criteria, with an emphasis on enteric viruses. Our results showed that virus detection was a useful tool for the characterization of human contamination, revealing the lack of environmental protection measures, such as a proper treatment of sewage, on a small scale.

For the physico-chemical analysis, all parameters showed similar results along the river, except for the turbidity, which showed statistically significant different values for the two areas studied (P1–P8). Here, turbidity mean values of 3 NTU were associated with virus detection, as well as *E.coli* values above 2,000 MPN/100 mL were found in inhabited area P5–P8 area. The lack of a correlation among the physico-chemical, bacteriological, and virological parameters are well documented and many studies pointed out the need to consider viral parameters to assess water quality (Pina et al. 1998; Pusch et al. 2005; Rodriguez-Manzano et al. 2013).

The present study also showed a natural occurrence of infectious HAdV, as demonstrated by ICC-PCR, emphasizing that molecular methods used for virus detection should be considered even though such methods do not provide information on the infectivity of the viral particle (Haramoto et al. 2007; Hamza et al. 2009; Girones et al. 2010). In fact, the low stability of free nucleic acids in the environment suggests that molecular methods detect intact viral particles and not the particle-free viral genome (Meleg et al. 2006; Carducci et al. 2008), and ICC-PCR has been described as a useful tool, providing more reliable data regarding the infectious potential of viral particles (Rigotto et al. 2005; 2010; Dong et al. 2010). In the present study, the risk of using water for human consumption (P9)

obtained from natural resources that impacted by human presence without previous treatment was demonstrated.

As previously observed by Miagostovich et al. (2008), RV-A and HAdV were detected in remote areas, where the forest and the river water have not undergone heavy anthropogenic influence. The use of the water for recreational purposes, the resistance of these viruses, the high prevalence of RV-A in Brazil (Leite et al. 2008; Carvalho-Costa et al. 2009), and the fact that all HAdV is excreted in feces (Mena and Gerba 2009) may explain their occurrence in these areas. Although HAV was not detected in this study, its presence in the Metropolitan Region of the Rio de Janeiro was demonstrated by molecular detection in 58 % (14/24) of raw sewage samples obtained from an urban wastewater treatment plant located in the city (Prado et al. 2012).

The ability to detect infectious viral particles in water and other environmental samples is of great importance in predicting public health risks (Ko et al. 2003). However, developing countries, such as Brazil, have not yet established a standard for determining the risk of viral infection from environmental water meant for consumption or recreational purposes in the current legislation. This study confirms the suitability of using the detection of enteric transmitted viruses as an alternative marker of human fecal contamination in water matrices and indicates that the spread of pathogenic viruses is occurring in an alleged area of environmental protection. The applicability of viral detection can assist studies of environmental impact assessments, helping with effective solutions for the prevention and control of water-related diseases in areas with varying degrees of human occupation and environmental disturbance.

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