Enteric viruses in surface water and sediment samples from the catchment area of Peri Lagoon, Santa Catarina State, Brazil

E. M. Elmahdy, G. Fongaro, C. D. Schissi, M. M. Petrucio and C. R. M. Barardi

ABSTRACT

This paper aims to quantify human adenovirus (HAdV), rotavirus species A (RVA), and hepatitis A virus (HAV) in surface water and sediments and to determine the viability of HAdV in these samples. Water and sediment samples were collected, and HAdV, RVA, and HAV were quantified by real-time polymerase chain reaction (PCR); HAdV was also evaluated for infectivity by a plaque assay (PA). For the water samples, HAdV was detected in 70.8% of the summer collections, with 82.4% containing infectious HAdV; the HAdV incidence in winter was 62.5%. For the sediment samples, the incidence of HAdV was 37.5% in the summer collections, with 66.7% containing infectious HAdV; the HAdV incidence in winter was 37.5%. RVA was detected in 20.8 and 45.8% of surface water samples collected in summer and winter, respectively, and 8.3 and 12.5% of sediment samples collected in summer and winter, respectively. HAV was detected only in surface waters, with 54.8 and 12.5% positivity in summer and winter samples, respectively. This study demonstrated that enteric viruses are present in water and sediments and that the presence of infectious viruses should be investigated whenever possible for quantitative microbial risk assessment studies. Combined analyses of water and sediments are important for reliable public health risk analysis of recreational and lagoon waters.

Key words | coastal lagoon, enteric viruses, infectivity, quantitative PCR (qPCR), sediments, surface water

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INTRODUCTION

The increasing contamination of water resources contributes to the unsustainability of water use worldwide. Climate change has exacerbated this situation, and it is expected that in some countries the predicted reduction in rainfall or alterations in the hydrological system may create or aggravate situations of water scarcity or stress (WATEFCON 2014). Among water resources, coastal lagoons are considered socially and economically important due to their intensive use by humans and animals as water resources, sanitation tools and areas for swimming and recreation. Brazilian coastal lagoons are subjected to several types of anthropogenic uses and influences due to population pressure, contributing to the degradation of their water quality (Branco *et al.* 1998; Petrucio 1998; Hennemann & Petrucio 2011). Indeed, lagoon surface waters, which are used for human consumption and recreational activities, are being contaminated by human and animal pathogens. These pathogens include enteric viruses, which replicate in the gastrointestinal tract, are excreted in feces at extremely high concentrations (ranging from 10^5–10^{13} viral particles per gram of stool), and often enter sewage (Bosch *et al.* 2008). This contamination occurs mainly as a result of unregulated direct or indirect discharge of domestic polluted sewage or its runoff from surrounding populations into lagoons

doi: 10.2166/wh.2015.295

(Donovan *et al.* 2008; Fongaro *et al.* 2014). Viruses are difficult to detect in all aquatic matrices and are often associated with non-specific infections. In addition, viral epidemiology is usually difficult, as many cases of viral infections are not reported to medical authorities, which makes tracing the causes of outbreaks a challenging task (Percival *et al.* 2004). Nevertheless, the contamination of surface water by enteric viruses is a major public health concern related to water resources used for drinking and recreational purposes. This is due to the risks caused by these viruses, which can be acquired by drinking the water, by immersion in recreational water or by skin contact or inhalation if the water has been contaminated with sewage. The fate of these pathogens in the aquatic environment after they have been released into the surface water is controlled by sorption-desorption processes (Wong *et al.* 2012, 2013). Due to their size, colloidal particles appear to be the most important natural vehicle for suspended viruses in the water column, which occurs as a result of this sorption-desorption phenomenon (Gerba *et al.* 1988; Schwartzbrod 1995). Estuarine sediments are considered the only refuge for solid-associated viruses from the water column, and settling may enhance the survival of these pathogens by reducing exposure to various stressors such as sunlight. In fact, it has been proven that viral abundance in sediment exceeds that in the water column by an order of magnitude (Danovaro & Serresi 2000; Danovaro *et al.* 2002; Danovaro & Middelboe 2010). Furthermore, particles from fecal material settled in soil at low temperatures or in underwater sediment will survive for some time and may be detectable for months or years (Percival *et al.* 2004). Recent studies suggest that sediment plays an important role in pathogen contamination by acting as a reservoir from which pathogens can be re-released into the water column as a result of natural or artificial phenomena, such as wind or swimming activity (Bosch 1998; Alm *et al.* 2003; Searcy *et al.* 2006; Salvo & Fabiano 2007). Enteric viruses are the major agents of virus-associated gastroenteritis and hepatitis outbreaks in humans. The following viruses have been detected in water and sediment: the human adenovirus (HAdV), which causes various diseases such as gastroenteritis, upper and lower respiratory system infections, and conjunctivitis (Jiang 2006; Miura *et al.* 2009; Verheyen *et al.* 2009; Calgua *et al.* 2011; Wong *et al.* 2013); rotavirus species A (RVA), the major viral agent causing severe diarrhea in children (Le Guyader *et al.* 1994; Green & Lewis 1999); and hepatitis A virus and enterovirus, which are the most intensively studied with respect to their survival and persistence because they are well-established waterborne pathogens albeit not usually associated with intestinal disease (Le Guyader *et al.* 1994; Jothikumar *et al.* 2000). This study describes the quantification and infectivity of HAdV and the quantification of RVA and hepatitis A virus (HAV) in surface water and sediment samples from a coastal urban lagoon and from the Sangradouro River at different collection points in the city of Florianopolis, Santa Catarina State, Brazil.

MATERIAL AND METHODS

Description of the study area

This study was conducted at Peri Lagoon and the Sangradouro River. Peri Lagoon is located in Santa Catarina State (southern Brazil) in the southeastern portion of Florianopolis Island (27°44′S and 48°31′W) (Figure 1).

The Peri Lagoon has a surface area of 5.7 km^2, is surrounded by mountains, and currently constitutes the main source of drinking water for the island inhabitants because it has no direct seawater influence. The waters of the lagoon drain into the Sangradouro River, which extends toward the south of the island, crossing areas of urban occupation and emptying into the ocean to the east between the beaches of Armação and Matadeiro. The river waters are used for the illegal deposition of sewage discharge from homes. The climate in the area is subtropical, with well-distributed rainfall throughout the year (1.85 mm annual rainfall), though rainfall is more frequent during the summer months than during winter (Cecca 1997). The lagoon was the first point of water collection in this study.

The second collection site, comprising points 2–6, was along the Sangradouro River. Points 2 and 3 contained sediment that mainly consisted of sand and sludge, whereas points 4 and 5 had sediment that consisted of sand, silt, gravel, and sludge. Point 6, at the fourth sample collection area located at the end of the Sangradouro River, was the collection point closest to the beach of Armação, and the sediment at this point consisted mainly of sand and sludge (Figure 1).

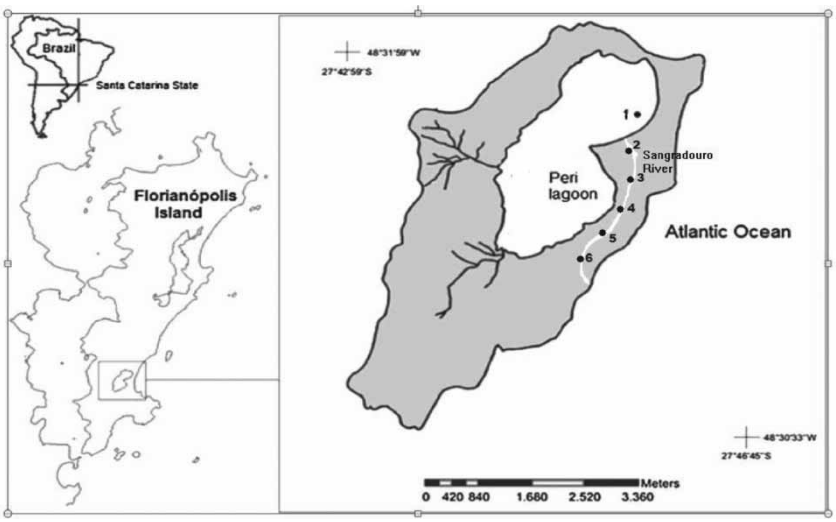

Figure 1 | Location of Peri Lagoon and the sampling sites along the Sangradouro River. The numbers indicate the sampling sites (coordinates for each site: **1**: 27°43 31.9¹S/ 48°30 35.9′W; **2**: 27°43.963′S, 048°30.608′W; **3**: 27°44.540′S, 048°30.596′W; **4**: 27°45.043′S, 048°30.542′W; **5**: 27°45.224′S, 048°30.256′W; **6**: 27°45.117′S, 048°30.143′W) according to Hennemann & Petrucio (2011).

Sampling

Water and sediment

A total of 48 surface water samples (2 L) were collected from the six collection points during the summer and winter seasons of a single year. Four collections were performed for each season, with the summer collections occurring during March/April 2013 and the winter collections occurring during July/August 2013. Additionally, a total of 48 sediment samples (20 g) were collected in parallel with the water samples at each collection point using Petersen grab samplers.

The samples were transported to the laboratory on ice in sterile containers and processed immediately. A total of 96 samples were collected from six sites during 2013.

Viral concentration in water and sediment samples

Viral concentrations in water samples were assessed using the protocol described by Katayama *et al.* (2002). Briefly, the method involves the adsorption of viruses onto an electronegative membrane (Nihon Millipore®, Tokyo, Japan) with a pore size of 0.45 μm and a diameter of 142 mm, followed by elution and then ultrafiltration using a Centriprep Concentrator 50® system (Nihon Millipore®, Tokyo, Japan) to obtain a final volume of approximately 5 mL. The concentrated samples were stored at −80 °C until further analysis. For the sediment samples, the viral concentrations were evaluated according to Environmental Protection Agency (EPA 1992) guidelines, with minor modifications, as described by Schlindwein *et al.* (2009). Briefly, a 0.05 M $AlCl_3$ solution was added to 20 g of wet sediment diluted with an equal volume of phosphate-buffered saline (PBS), and the pH was adjusted to 3.5 with 5 M HCl. To dislodge viruses, the samples were subjected to sonication, 3 times for 30 sec each, on ice. After stirring and centrifugation steps, the viral particles were eluted from the samples using glycine buffer (pH 9.5). The viral concentration was determined by PEG 6000 precipitation, as described by Lewis & Metcalf (1988). The pellet was suspended in 5.0 mL of 0.1 M phosphate buffer (pH 7.2).

Physicochemical analysis

Water temperature (WT), conductivity (Cond.), pH, salinity (Sal.) and dissolved oxygen (DO) were measured *in situ* with specific probes (WTW-Multi350i) after collection, as outlined in *Standard Methods for the Examination of Water and Wastewater* described by the American Public Health Association (APHA 1998).

For sediment samples, total solids, volatile solids, suspended solids and humidity were measured according to APHA (2012).

Extraction of viral nucleic acids

Nucleic acid extraction was performed using a QIAmp MinElute Virus Spin Kit (Qiagen, Brazil), following the manufacturer's instructions. In this method, total nucleic acids were eluted in 60 μL of elution buffer, collected in sterile nuclease-free centrifuge tubes and stored at −80 °C prior to analysis.

Quantitative PCR assay (qPCR)

For HAdV amplification, quantitative polymerase chain reaction (qPCR) was performed using the Taqman Universal PCR Master Mix Kit (Applied Biosystems) in a final volume of 15 μL using 10 μL of extracted nucleic acids (diluted 1:10) under the following conditions: 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. For the amplification of HAV and RVA, the same final volumes and nucleic acid dilutions were employed using the QuantiTect Probe RT-PCR kit (Qiagen), which contains reverse transcriptase (RT) and a mixture of HotStartTaq DNA polymerase, dNTPs and the appropriate saline concentrations. The cycling protocol used for HAV and RVA qRT-PCR was as follows: an initial temperature of 50 °C for 30 min for reverse transcription, followed by denaturation at 95 °C for 15 min and 45 cycles of amplification at 95 °C for 10 sec (denaturation), 55 °C for 20 sec (annealing) and extension at 72 °C for 15 sec. All primers and probes as well as their respective final concentrations are described in Table 1.

All amplifications were performed using a StepOne Plus® Real-Time qPCR System (Applied Biosystems, USA). Each sample was analyzed in duplicate. To evaluate the effect of qPCR inhibitors and to determine the optimal DNA dilutions for avoiding inhibitor interference, RV-SA11 in known quantities was inoculated into samples determined to be negative for RVA by qPCR, which were then used for nucleic acid extraction followed by qPCR using the TaqMan assay according to Zeng *et al.* (2008), as described above. A 1:10 dilution of the samples was considered sufficient to avoid the influence of qPCR inhibitors; therefore, the nucleic acids in all of the analyzed samples were diluted in the same way. A standard curve was generated for all of the viruses using a 10-fold serial dilution of the pCR2.1 vector (Invitrogen, USA) containing the target region appropriate for the type of virus. Triplicate samples were used for each dilution point, and a standard curve was prepared for each set of assays. Ultrapure water was used as the non-template control, and non-amplification controls were included in each run.

Viral recovery assay

Two experiments were performed in duplicate to evaluate viral recovery rates from the different types of samples used in this study. The water and sediment samples from all sites of collection were tested for viral recovery efficiency via spiking experiments, as follows. Water samples (2 L), sediment (20 g) and ultrapure water were inoculated with 8.0×10^6 GC mL^{-1} (genome copies per milliliter) of simian rotavirus-SA11 (RVA-SA11) and then concentrated and clarified using exactly the same protocols described above for the actual samples. Rotavirus recovery from the respective samples was then quantified by real-time PCR using the

Table 1 | Primers and probes used for detection of HAdV, RVA, and HAV by qPCR

Virus	Primers and probes	Sequence 5′-3′	Final conc. of Primers	Reference
HAdV	HAdV F	C(AT)TACATGCACATC(GT)C(CG)GG	900 nM	Hernroth *et al.* (2002)
	HAdV R	C(AG)CGGGC(GA)AA(CT)TGCACCAG	900 nM	
	HAdV probe	**FAM**-CCGGGCTCAGGTACTCCGAGGCGTCCT-**TAMRA**	225 nM	
RVA	RVA F	ACCATCTWCACRTRACCCTCTATGAG	0.25 μM	Zeng *et al.* (2008)
	RVA R	GGTCACATAACGCCCCTATAGC	0.25 μM	
	RVA probe	**VIC**-AGTTAAAAGCTAACACTGTCAAA-**MGB**	0.15 μM	
HAV	HAV F	GGTAGGCTACGGGTGAAAC	0.25 μM	Jothikumar *et al.* (2005)
	HAV R	GCGGATATTGGTGAGTTGTT	0.25 μM	
	HAV probe	**FAM**-CTTAGGCTAATACTTTATGAAGAGATGC-**TAMRA**	0.15 μM	

TaqMan technique, as previously described by Zeng *et al.* (2008).

The calculation of the RVA-SA11 recovery percentage (in number of genome copies) accounted for the original number seeded.

Plaque assay for HAdV

The A549 cell line (kindly provided by Dr Rosina Gironès from the University of Barcelona, Spain) was used for the propagation of HAdV and for the viral viability assay. To quantify the presence of infectious HAdV in the environmental samples, all of the water and sediment samples that tested positive for viruses (as previously detected by qPCR) were treated with antibiotics (10 U mL^{-1} penicillin, 10 µg mL^{-1} streptomycin and 0.025 µg mL^{-1} amphotericin B). For all tested samples, a non-cytotoxic dilution was then selected and inoculated (0.2 mL) in triplicate into A549 cells. These cells were previously cultivated in 24-well tissue culture plates at a density of 3.0×10^6 cells $well^{-1}$ and were incubated at 37 °C in 5% CO_2 for 24 h, as described by Cromeans *et al.* (2008), Rigotto *et al.* (2010), and Fongaro *et al.* (2013).

Briefly, the cells were incubated for 1 h at 37 °C in 5% CO_2 and gently rocked every 15 min for viral adsorption. The inoculated cells were then carefully washed once with pre-warmed PBS and overlaid with warm high-glucose Dulbecco's modified Eagle's medium (2×), 0.6% warm Bacto-agar containing 5% FBS, 0.1 mM sodium pyruvate, 10 U mL^{-1} penicillin, 10 µg mL^{-1} streptomycin and 26 mM $MgCl_2$. The cells were incubated at 37 °C with 5% CO_2 for 7 days. At 1 week post-infection, the agar overlay was gently removed, and the cells were stained with 20% Gram's crystal violet. The cell monolayer was examined using a microscope, and the plaques were counted and expressed as plaque forming units per mL (PFU mL^{-1}).

Viral integrity test

To determine the presence of undamaged HAdV particles, the samples were treated with DNaseI to assess the integrity of the viral capsid (genetic material not protected by the viral capsid would be degraded by this nuclease) (Nuanualsuwan & Cliver 2002). All samples that were positive for HAdV in the real-time PCR assay were subjected to DNase I digestion following the manufacturer's instructions (Sigma–Aldrich, Steinheim, Germany) to degrade all free viral DNA present in the samples, as described by Viancelli *et al.* (2012). Briefly, the reaction was performed by adding 1 U of DNase I (a quantity sufficient to degrade 100% of the DNA added), 1× buffer and 170 µL of nuclease-free water as a negative control for each concentrated sample; the reaction was then incubated for 15 min at room temperature. Enzyme activity was blocked with EDTA (25 mM), and the samples were incubated for 10 min at 70 °C. All DNase-treated samples were further subjected to nucleic acid extraction, and qPCR was performed as previously described (Hernroth *et al.* 2002).

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 5.0 (USA) software. A Pearson correlation, two-way analysis of variance (ANOVA) and Student's *t*-test were performed ($P < 0.05$). The Pearson correlation was applied to evaluate positive or negative correlations between viral distribution and physicochemical parameters. Two-way ANOVA and Student's *t*-tests were performed to evaluate differences between the points evaluated and their variables (viral quantity and physicochemical parameters) during the collection period. The critical *P*-value for the test was set at <0.05.

RESULTS

Physicochemical analysis

The mean values of the physicochemical parameters measured for all samples from each collection point in both summer and winter are presented in Table 2. The analyses were performed using Student's *t*-test**.** According to these data, the average sample temperature, pH, salinity, and conductivity during summer and winter were not significantly different among the collection points ($P \leq 0.05$). In contrast, the mean values of dissolved oxygen during summer and winter were significantly different among the collection points ($P \leq 0.01$). Regarding the sediment analysis, the average total solids, fixed solids, volatile solids and

Table 2 | Physicochemical analysis for surface water and sediment analysis in each point of collection during the summer (sum.) and winter (win.) seasons, statistically significant differences noted by (*), (#)

Parameter	Point 1 Sum.	Win.	Point 2 Sum.	Win.	Point 3 Sum.	Win.	Point 4 Sum.	Win.	Point 5 Sum.	Win.	Point 6 Sum.	Win.
Surface water												
Sample temp. (°C)	24.95 ± 1.00	18.75 ± 1.46	24.88 ± 1.62	17.78 ± 1.39	24.75 ± 1.94	18.83 ± 2.60	24.58 ± 2.08	18.25 ± 2.06	25.18 ± 2.88	18.13 ± 2.62	26.2 ± 3.52	18.78 ± 2.4
pH	6.05 ± 0.04	6.28 ± 0.24	6.66 ± 0.16	6.25 ± 0.34	6.18 ± 0.30	6.48 ± 0.26	6.4 ± 0.21	6.58 ± 0.35	6.75 ± 0.15	6.33 ± 0.39	6.25 ± 0.43	6.45 ± 0.34
Conductivity (μS/cm)	39.88 ± 5.02	43.85 ± 10.9	38.7 ± 5.91	47.9 ± 12.7	46.8 ± 19.69	46.1 ± 12.6	49.5 ± 15.35	46.93 ± 20.1	54 ± 11.66	41 ± 15.7	43.28 ± 20.52	42.92 ± 10.11
Dissolved oxygen (mg/L)	6.75* ± 0.96	7.98* ± 0.39	5.75* ± 2.62	8.71* ± 3.77	2.24* ± 0.46	4.82* ± 0.38	1.89* ± 0.41	4.61* ± 0.35	1.85* ± 0.47	3.89* ± 0.55	2.76* ± 1.46	6.7* ± 4.0
Salinity (ppt)	–	–	–	–	–	–	–	–	0.1 ± 0.07	0.73 ± 0.82	0.37 ± 0.3	0.75 ± 0.83
Sediment												
Total solids (mg/L)	72.84#		122.25		119.07		121.61		98.8#		135.36	
Fixed solids (mg/L)	98.6#		165.51		162.20		165.45		134.38#		184.11	
Volatile solids (mg/L)	1.35#		2.26		3.71		3.77		3.06#		4.19	
Humidity (%)	27#		45		44		44.7		36.3#		49.7	

±Standard deviation.

humidity of the samples were not significantly different among the points of collection in either the summer or winter months ($P > 0.05$), as shown in Table 2.

Viral recovery assay

The mean viral recovery rates, as determined by qPCR (GC units), were approximately 10% (8×10^5 GC mL^{-1}) for the water and 46% (3.7×10^6 GC g^{-1}) for the sediment samples.

Viral analysis

For the surface waters collected during summer, the incidence of HAdV was 70.8% (17/24), ranging from 6.9×10^5 to 2.4×10^8 GC L^{-1}. All 17 positive samples were found to have undamaged particles after nuclease treatment at concentrations ranging from 1.2×10^4 to 8.9×10^4 GC L^{-1}; of these, 82.4% (14/17) contained infectious particles at concentrations ranging from 4×10^3 to 1.07×10^4 PFU L^{-1}. For the winter collections, the HAdV incidence was 62.5% (15/24), ranging from 8.9×10^5 to 1.7×10^8 GC L^{-1}. Of these positive samples, 93.3% (14/15) had undamaged particles after nuclease treatment at concentrations ranging from 1.3×10^3 to 2.5×10^4 GC L^{-1}. No infectious units could be measured by PFU during the winter collections. A two-way ANOVA was performed, and the mean results for HAdV GC for each point were statistically significant for both the summer and winter collections ($P < 0.05$), as shown in Figure 2.

With regard to the sediment samples collected in summer, the HAdV incidence was 37.5% (9/24), ranging from 4.2×10^9 to 1.9×10^{10} GC kg^{-1}. All positive samples were found to contain undamaged particles after nuclease treatment at concentrations ranging from 1.8×10^4 to 8.6×10^5 GC L^{-1}. Of these samples, 66.7% (6/9) contained infectious HAdV at concentrations ranging from 1.3×10^3 to 8×10^4 PFU kg^{-1}. The results of the winter collections revealed an HAdV incidence of 37.5% (9/24), ranging from 3.1×10^8 to 6.01×10^9 GC kg^{-1} with 100% (9/9) undamaged particles, ranging from 4.7×10^4 to 3.3×10^5 GC L^{-1}. No infectious units could be measured by PFU. The difference between the infectious and undamaged HAdV particles was statistically significant (Student's t-test, $P < 0.05$), as shown in Figure 2.

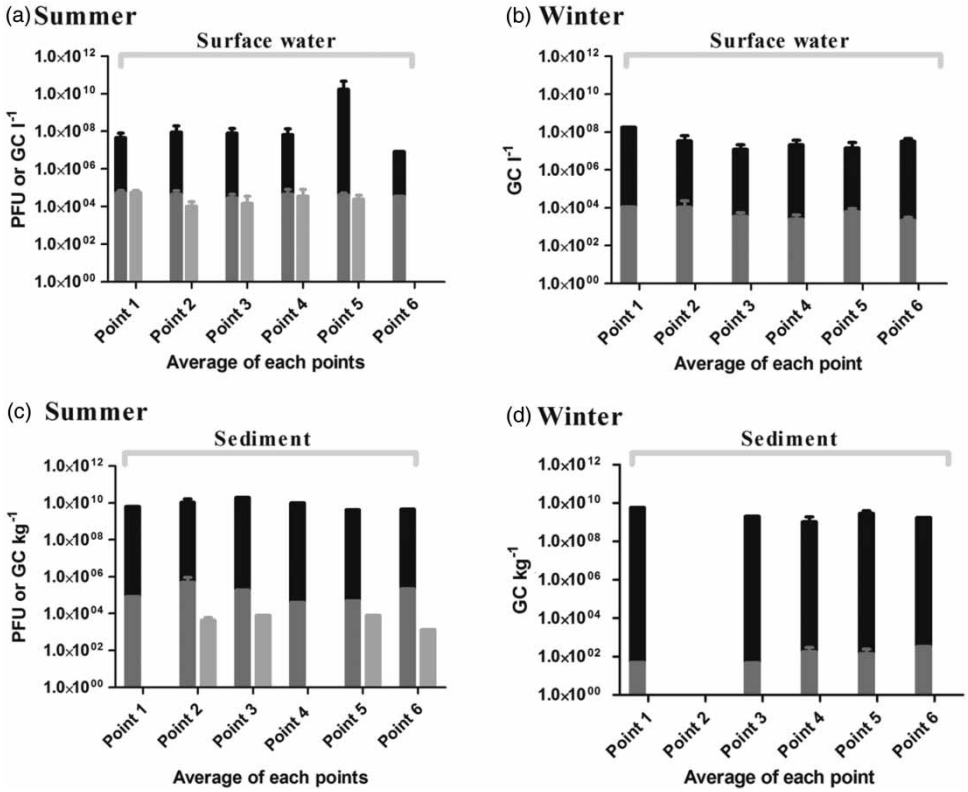

Figure 2 | Average number of HAdV genome copies before and after treatment with DNase I and the number of infectious particles in surface water and sediment samples during summer and winter in Peri Lagoon and the Sangradouro River ($n = 24$ for surface water and $n = 24$ for sediment); GC (■), PFU (■), GC-DNase I treatment (■).

The difference between the total genomic DNA and intact HAdV DNA was also statistically significant (Student's *t*-test, $P < 0.05$) in all cases. The log reduction was calculated by $[\log_{10} (GC_{total} \times GC_{undamaged}{}^{-1})]$, and the general means of the log difference during summer and winter for the surface water samples and sediment samples were 0.81 ± 0.56 and 1.27 ± 1.59, respectively, as shown in Figure 3. This log reduction was calculated using the following formula: $\log_{10}$ reduction $= \log_{10}(GC_t GC_u{}^{-1})$, where GC_t is the total number of HAdV particles (GC L^{-1}) and GC_u is the number of undamaged HAdV particles (GC L^{-1}) (Student's *t*-test, $P < 0.05$).

The RVA incidence in the surface water samples collected during summer was 20.8% (5/24), ranging from 1.94×10^5 to 4.08×10^5 GC L^{-1}. In winter, this incidence was 45.8% (11/24), ranging from 3.09×10^4 to 5.4×10^5 GC L^{-1}. For the sediment samples collected during summer, the RVA incidence was 8.3% (2/24), ranging from 1.73×10^2 to 2.12×10^2 GC kg^{-1}; in winter, this incidence was 12.5% (3/24), ranging from 2.89×10^2 to 3.39×10^2 GC kg^{-1} (Figure 4(a) and (b)).

The HAV incidence in the water samples was 45.8% (11/24), ranging from 5.45×10^1 to 1.11×10^3 GC L^{-1}, and 12.5% (3/24), ranging from 1.61×10^2 to 2.75×10^2 GC L^{-1}, for the summer and winter collections, respectively. No HAV was detected in the sediment samples, as shown in Figure 4(a) and (b).

The overall mean percentages of enteric viruses detected in the surface water and sediment samples from summer and winter collections showed that HAdV was the most frequently detected enteric virus, followed by RVA and HAV, as shown in Table 3.

As presented in Figure 5, the overall mean percentages for the seasonal occurrence of the three viruses showed that RVA was more frequently detected in both surface water and sediment during the winter season. In contrast, the frequency of HAV detected in the summer samples was higher than that in the winter samples, but only for surface water. HAdV was the most prevalent virus and showed no difference in seasonal prevalence. However, the seasonal

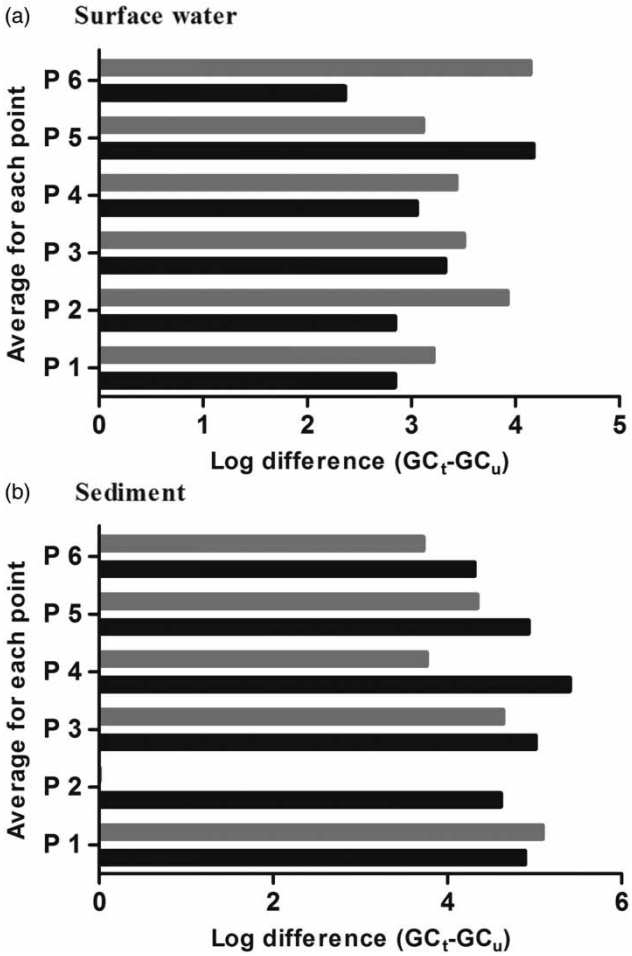

Figure 3 | Log difference (GC_{total}–$GC_{undamaged}$) between total and undamaged viral GC during the summer and winter collections ($P < 0.05$); summer (■), winter (■).

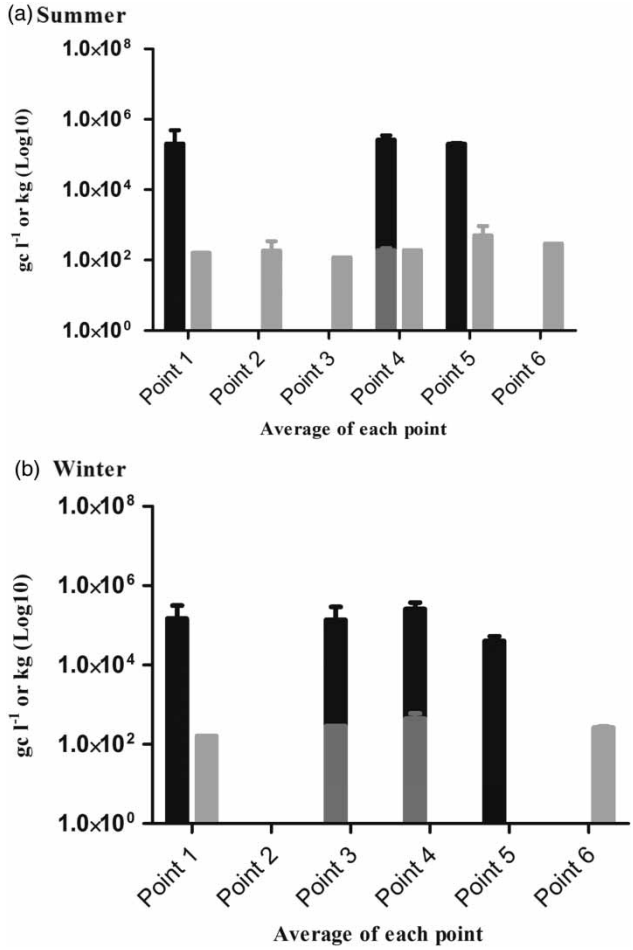

Figure 4 | Average number of RVA and HAV GC occurring in surface water and sediment samples during summer and winter at the six collection points ($n = 24$ for surface water and sediment) ($P > 0.05$), surface water (■), sediment (■), HAV: surface water (■).

distributions of these viruses were not statistically significant (Student's *t*-test, $P > 0.05$).

DISCUSSION

Coastal lagoons are important for water suppliers and sanitation processes and are considered to be important contributors to the groundwater supply. Contamination of lagoons with enteric viruses due to the illegal discharge of domestic sewage represents a significant risk to public health (Aslan *et al.* 2011; Moresco *et al.* 2012). In this study, we investigated the presence of human viral pollution (in the form of HAdV, RVA and HAV) in surface water and sediment over a period of one year, concentrating our collections during the summer and winter seasons, to investigate the role of sediments as reservoirs for these pathogens and to ascertain the possible influence of seasonality on the presence of these viruses.

HAdV proved to be the most prevalent virus in this study during the two seasons of collection, both in surface water and sediment. These results corroborated those of other studies, which found that HAdV is the most prevalent virus in different environmental matrices. Indeed, HAdV was detected using nested PCR in 75% of lagoon water samples tested by Rigotto *et al.* (2010). Additionally, other studies have reported a high incidence of HAdV in different aquatic environments (Choo & Kim 2006; Hundesa *et al.* 2006; Miagostovich *et al.* 2008; Moresco *et al.* 2012; Fongaro

Table 3 | Occurrence percentages for each virus in collected samples for each point of collection regardless of season

Points of collection		HAdV	RVH	HAV
Point 1	Wat.	4/8 (50%)	4/8 (50%)	2/8 (25%)
	Sed.	2/8 (25%)		
Point 2	Wat.	7/8 (88%)	ND	3/8 (38%)
	Sed.	4/8 (50%)		
Point 3	Wat.	6/8 (75%)	3/8 (38%)	1/8 (13%)
	Sed.	2/8 (25%)	1/8 (13%)	
Point 4	Wat.	5/8 (63%)	4/8 (50%)	1/8 (13%)
	Sed.	4/8 (50%)	4/8 (50%)	
Point 5	Wat.	6/8 (75%)	4/8 (50%)	4/8 (50%)
	Sed.	4/8 (50%)		
Point 6	Wat.	4/8 (50%)	ND	3/8 (38%)
	Sed.	2/8 (25%)		

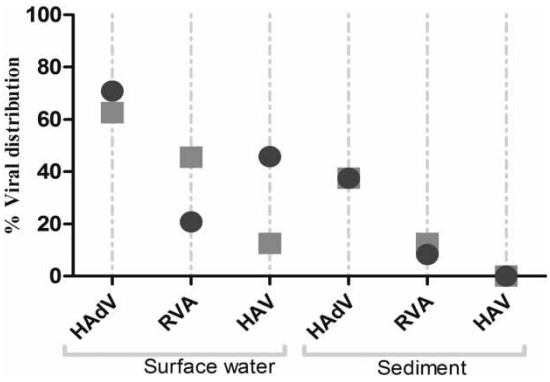

Figure 5 | Viral distribution (percentages) in surface water and sediment samples during the summer and winter collections ($P > 0.05$); summer (●), winter (■).

et al. 2012, 2013). The lower number of HAdV-positive sediment samples may be due to limitations of the method employed for viral elution from such matrices, the presence of inhibitors related to the nature of the samples or the strong association of this virus with sediment, which cannot be disrupted by the virus recovery procedure employed (Sano *et al.* 2003; Albinana-Gimenez *et al.* 2009; Sidhu & Toze 2009; Rock *et al.* 2010).

However, the number of HAdV particles (GC) in the positive sediment samples was two or three log values higher than that in the water samples. HAdV tends to adsorb onto suspended particles due to its high sorption capacity and size, which has an effect on interactions with environmental colloidal particles (Dowd *et al.* 1998; Wong *et al.* 2013).

The detection of infectious viruses in environmental samples requires the use of adequate susceptible cell lines in which the viruses can propagate and produce cytopathic effects (CPE) (Rodríguez *et al.* 2009). As the presence of the viral genome does not provide information on infectivity, a viability assay was performed to detect infectious HAdV particles in the samples containing HAdV nucleic acid. The majority of HAdV-positive samples from the summer collection contained infectious particles; however, we did not find any infectious units in either the surface water or sediment samples from the winter collection. Factors such as temperature, pH and UV radiation are known to cause conformational changes in the viral capsid, resulting in inactivation; nonetheless, enteric viruses can be adsorbed onto organic particles, protecting them from inactivation factors (Ward *et al.* 1986; Battigelli *et al.* 1993; Carter 2005; Choi & Jiang 2005; Fong & Lipp 2005; Fongaro *et al.* 2013). Infectivity testing is important when quantitative microbial risk assessment is the goal. To assess possible damage to the HAdV capsid, which results in a lack of protection for nucleic acids, DNase pre-treatment prior to nucleic acid extraction was applied to all the samples analyzed in the present work. Previous studies have reported that this enzymatic technique is a simple alternative method that can provide information about potential viral infectivity without the need for laborious cell culture techniques (Rodríguez *et al.* 2009; Viancelli *et al.* 2012; Fongaro *et al.* 2013).

Although this assay allows for the differentiation of undamaged viruses (with protected genomes) from damaged viruses (with unprotected genomes) or even free viral genomes, it nevertheless is unable to differentiate inactivated viruses with genetic material that is still protected by the viral capsid (Rodríguez *et al.* 2009; Fongaro *et al.* 2013). This limitation was revealed when we found that not all samples containing undamaged HAdV, as detected by qPCR, were infectious by the plaque assay. The notable differences among these results highlight the critical importance of combining, whenever possible, molecular and cell culture techniques during the monitoring of viruses in the aquatic environment. The high prevalence of HAdV in the surface water and sediment samples during both seasons indicates a lack of seasonal difference in prevalence, a result that has been confirmed by other studies performed by Formiga-Cruz *et al.* (2005) and Rigotto *et al.* (2010), in

which this virus was detected throughout the year in high numbers. This high incidence may be due to the high stability of HAdV in the aquatic environment and its sporadic shedding in the feces of most adults (Wyn-Jones *et al.* 2011). In addition, the statistical analyses that we performed could not establish a correlation between HAdV detection and seasonal changes.

RT-qPCR analysis of RVA and HAV revealed that these viruses were frequently present in surface water (prevalences of 33.2% and 29.2%, respectively). However, the low incidence of these RNA viruses in sediment samples (10.4% and ND, respectively) may be due to their weak adsorption onto sediment particles or their rapid inactivation in sediments. Previous studies performed by Chung & Sobsey (1993) and Green & Lewis (1999) reported a low incidence of rotavirus in sediments, and indicated that adsorption onto sediment may inactivate this virus. Other studies exploring HAV incidence in Brazil have also shown a low incidence in environmental samples (Rigotto *et al.* 2010). However, Green & Lewis (1999) reported a high incidence of HAV in sediment samples contaminated by wastewater (87.5%). The high incidence of these viruses in water samples collected from the collection points along the Sangradouro River indicate that these viruses may play an important role in the contamination of Peri Lagoon and alterations in this ecosystem.

RVA showed a marked winter seasonal peak (45.5% prevalence), which has also been reported in previous studies (Cook *et al.* 1990; Levy *et al.* 2009; Payne *et al.* 2011; Chigor & Okoh 2012). Some authors have demonstrated higher levels of RVA during cold, dry months (Steele & Glass 2011), whereas others have suggested that this seasonal pattern may vary according to the RVA serotype (Sarkar *et al.* 2008; Jagai *et al.* 2012).

In the present study, HAV displayed a higher incidence during summer (45.8%). However, Chigor & Okoh (2012) reported higher detection of HAV in South Africa during the winter and spring seasons (43%). Another study performed in Brazil by Rigotto *et al.* (2010) documented a seasonal pattern for HAV during the spring and winter seasons, with a high incidence during October and July. Additionally, a study performed in Rio de Janeiro by Villar *et al.* (2002) reported a peak HAV incidence during hot and rainy months. However, it is important to consider the fact that HAV infections are underreported, and many cases are asymptomatic. None of the physicochemical parameters or sediments showed sufficiently consistent significant correlations with the presence of viruses or with HAdV infectivity. The lack of correlation between physicochemical and virological parameters has been well documented by other studies (Lee *et al.* 2013; Vecchia *et al.* 2015).

This study also highlighted the importance of evaluating the ecosystems interconnected with the Sangradouro River to enhance the safety of drinking water and recreational water sources in this environment. This water may otherwise pose a threat to the population that is mainly responsible for the degradation of its quality in this lagoon and neighboring waters.

ACKNOWLEDGEMENTS

This work was financially supported by CNPq Project number 556276/2009-4-CT Hidro n. 22/2009 (process n. 161663/2012-5), CNPq-PDJ, CNPq-TWAS and CAPES/PNPD.

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First received 9 December 2014; accepted in revised form 9 July 2015. Available online 22 August 2015