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Spatial distribution of enteric viruses and somatic coliphages in a Lagoon used as drinking water source and recreation in Southern Brazil

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ABSTRACT

This study aimed to evaluate the contamination level of the Peri Lagoon, the main freshwater reservoir of Santa Catarina Island, Southern Brazil, for human adenovirus (HAdV), hepatitis A virus (HAV), rotavirus species A (RVA), and somatic coliphages (SOMCPH). Viruses were also investigated in sediments and their sensitivity against natural sunlight was analysed by studying their spatial distribution in different depths of the water column. A total of 84 water samples and 48 sediment samples were examined by qPCR or RT-qPCR. Infectivity of HAdV and SOMCPH was determined and quantified by plaque assay method. A sum of 64% and 48% of water and sediment samples were positive for HAdV, respectively. RVA was present in 33% and 18% of water and sediment samples, and 25% of water samples were positive for HAV. HAdV were infectious in 76% of water and 83% of sediment samples that were positive by qPCR. SOMCPH could be detected in 42% and 18% of water and sediment samples, respectively. The data pointed a variation of viruses' prevalence according to the different water column depths. These results demonstrated that water sources and sediments contaminated by human wastes could play an important role in the recontamination of water columns harvested for further treatment or used for recreational purposes. These data can be of great value for future risk assessment analysis.

1. Introduction

Enteric viruses enter into the environment by the wastes excreted by infected humans and animals. The aquatic ecosystem may also be contaminated as a result of sewage discharge or run-off from agriculture activities. Soil may be contaminated by agriculture practices using animal manure or pig slurry as fertilizers and/or irrigation with contaminated wastewater. Recreational and drinking waters contaminated by human and animal faecal pollution are a public health concern worldwide. These viruses present in the ecosystems cannot be able to multiply outside a host, but they can persist in the environment for a prolonged time in an infectious state due to their stable non-enveloped nature. Also they can easily adsorb to solid particles, thereby protecting themselves from inactivating factors and may result in unpredictable resuspension to water column due to natural or artificial sediment disruption (Bales et al., 1993; Schwartzbrod, 1995; Hernroth et al., 2002; Alm et al., 2003; Rzezutka and Cook, 2004). Among the enteric viruses, the human adenovirus (HAdV) and rotavirus species A (RVA) represent the major viral agents responsible to cause severe diarrhoea in children, and hepatitis A virus (HAV), which is often associated with ingestion of contaminated vegetables and food, causes an acute form of hepatitis. These viruses replicate in the gastrointestinal tract and can be excreted by infected persons in high concentrations (10^8–10^{11} particles/g of faeces). Ingestion of sewage contaminated water or food even at low concentrations are able to cause human illness as paralysis, myocarditis, meningitis, diarrhoea, and hepatitis (Bosch et al., 2008, 1991). Outbreaks of waterborne enteric viruses in recreational and drinking water have increased in the past years, unlikely these outbreaks are unreported due to self-limiting nature of many common infections and lack of well trained staff to perform the correct diagnosis. Presence of these viruses in

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the aquatic system is well documented in the literature (Haramoto et al., 2010; Hamza et al., 2009; Rigotto et al., 2010).

For many years, microbial indicators such as total coliforms, faecal coliforms and *Escherichia coli* were used for predicting levels of microbiological water quality. One of the major drawbacks in using these indicators is the die off quickly in comparison to enteric viruses (Bordalo et al., 2002; Rzezutka and Cook, 2004). Many studies advocated phages infecting enteric bacteria as a potential viral indicator to estimate the microbial contamination in the water environment, also as indicators of water treatment process efficacy (Sobsey et al., 1994). Somatic coliphage (SOMCPH) are indicated by many studies to fill in this gap depending on their correlation with enteric viruses' presence (Stetler, 1984; Payment and Franco, 1993; Wiedenmann et al., 2006). There are many arguments to propose coliphage as a suitable indicator for waterborne viruses due to their possible replication outside the gut in the aquatic environment; however this replication is negligible due to low density of phage and host bacteria (Jofre, 2008).

Different environmental factors such as pH, temperature, microbial predators, and sunlight that affecting the survival of these faecal pathogens in the aquatic environment are well documented in the literature. However few studies are available related to the effect of natural sunlight on these enteric viruses (Watts et al., 1995) and this also depended on sunlight spectrum, intensity and depths of water column (Silverman et al., 2013).

This study aimed to quantify the presence of HAdV, RVA, HAV and SOMCPH both in waters or sediment samples. In addition we evaluated the spatial distribution of viruses in water column in different depths and verified the possible correlation between the presence of coliphages and the studied human enteric viruses.

2. Material and methods

2.1. Water and sediment samples

Peri Lagoon is located in a protected area of the Municipal Park of Lagoa do Peri, in south-eastern island of Santa Catarina, city of Florianópolis, Brazil (27°43′30″S 48°32′18″W). It has a water surface of 5.7 km^2, with an average depth of 4.2 m at the margins and 10 m at the center; This Lagoon is widely used for recreational activities (swimming and fishing) and as an important water supply for approximately 110,000 inhabitants of the south and east portions of the island.

Due to the importance of Peri Lagoon, four sites were selected for water (2 L) and sediment (20 g) sampling collection: Site 1) located at the center of Lagoon – this site was composed by 4 collection points for water sampling characterized by different depths [surface water, 0.9 m, 5.5 m and 8 m in deep] and 1 for sediment sample; site 2) located in the waterfall Cachoeira Grande; site 3) located in the waterfall Ribeirão Grande; site 4) located in the shore of the Lagoon. Water and sediment samples were collected monthly along one year, from January 2014 to December 2014, 7 water and 4 sediment sampling (totalizing 132 samples). Sediment samples were collected using a Petersen grab sampler. The samples were transported to the laboratory on ice in sterile containers and processed immediately.

2.2. Physicochemical analysis

Water temperature (WT), conductivity (Cond), pH and dissolved oxygen (DO) were measured *in situ* with specific probes (WTW-Multi350i) for all of samples after collection, as outlined in the standard methods for the examination of water and wastewater described by the American Public Health Association (APHA, 1998). Analysis of nutrients was carried out only for samples taken from site 1 (center of Lagoon). Water samples were taken to the laboratory to quantify Ortho-Phosphate (PO_4), Total Phosphate (TP), Nitrite (NO_2·N), nitrate (NO_3·N), ammonium nitrogen (NH_4·N), and total nitrogen (TN) were determined in filtered water samples using a Millipore AP40–47 mm glass fiber (APHA, 2005).

2.3. Detection and enumeration of SOMCPH in surface water and sediment samples

SOMCPH were quantified by a double agar layer technique following the ISO 10705-2 standard (ISO, 2000). *E. coli* (ATCC13706) was used as the host for SOMCPH detection according to ISO/FDIS 10705-2:2000.

The volume of water tested for each phage was 10 mL of water and 10 g of sediment. Briefly, the water sample was filtered through a cellulose ester membrane filter, with 0.22 μm pore size and 47 mm diameter. In case of sediment, the samples were diluted first with peptone water (1:10) and then filtered through a cellulose ester membrane filter. *E. coli* C strain ATCC 13706 were grown in modified Scholten's broth (MSB). To each culture tube, 1 mL of the original water sample (diluted in case of sediment) was added to 1 mL of host culture and 2.5 mL of semi solid Modified Scholten's Agar (ssMSA) heated at 47 °C. The solution was mixed carefully avoiding the formation of air bubbles and poured on a layer of complete solid Modified Scholten's Agar (MSA) prepared previously in a 9 cm Petri dish pre-warmed at room temperature. After solidification on a horizontal, cool surface, the plates were incubated upside-down at 36 ± 2 °C for 18 ± 2 h. The bacteriophage PhiX-174 (*Microviridae*) was utilized as positive control in all the analyses. The detection limit (DL) for phage was 10 PFU/100 mL or gram of water or sediment, respectively. The results for phages were expressed in PFU/L.

2.4. Viral concentration in water and sediment samples

Viral concentrations in water samples were assessed based on the method proposed by Katayama et al. (2002). Briefly, the method involves the adsorption of viruses onto an electronegative membrane (Nihon Millipore®) with a pore size of 0.45 μm and a diameter of 142 mm, followed by elution of viral particles adsorbed to the membrane with 2.5 mL of 1 mM NaOH (pH 10.5). The filtrate was then neutralized with 12.5 μL of 50 mM H_2SO_4 and 12.5 μL in 100 × Tris-EDTA (TE) buffer. The resulting mixture was aliquoted and stored at −80 °C until further processing. For each sediment sample (20 g), 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 buffer (PBS), and the pH was adjusted to 3.5 with 5 M HCl. After, the viral particles were eluted from the samples using glycine buffer (pH 9.5). The viral concentration was performed by PEG 6000 precipitation, as described by Lewis and Metcalf (1988), Elmahdy et al. (2015). The pellet was suspended in 5.0 mL of 0.1 M PBS (pH 7.2) and keep at −20 °C to further detection.

2.5. 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.