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Detection and quantification of human adenovirus (HAdV), JC polyomavirus (JCPyV) and hepatitis A virus (HAV) in recreational waters of Niterói, Rio de Janeiro, Brazil

Juliana Dias, Renan Novaes Pinto, Carmen Baur Vieira, Adriana de Abreu Corrêa*

Laboratory of Environmental Virology, Department of Microbiology and Parasitology, Biomedical Institute, Federal Fluminense University, Niteroi, Rio de Janeiro, Brazil

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ABSTRACT

This study evaluated the impact of sewage discharge in recreational coastal marine environments of Niteroi, Rio de Janeiro, Brazil, over a six-month period by the detection of waterborne enteric viruses. Ten-liter water samples were collected in four beaches from January to July 2017. Viruses were concentrated by an organic flocculation and human adenoviruses (HAdV), polyomavirus (JCPyV), and Hepatitis A virus (HAV) detected by qPCR. Forty-eight water samples were collected, being 43% positive for HAdV and 23% for JCPyV; only one sample was positive for HAV. Viruses were detected in all sampling sites, including in areas suitable for bathing according to the current bacterial standards. The results herein provide an overview of the viral contamination of beaches used for recreational purposes. The viral presence in the sampled areas indicates the need for more rigid effluent discharge controls in these areas, as sewage represents a possible transmission risk for waterborne viral diseases.

1. Introduction

Population growth and socioeconomic development are followed by an increased demand for water, the quantity and quality of which are of fundamental relevance to the health and development of any community (Gerba et al., 2002; Rigotto et al., 2010). Many diseases attributed to unsafe water are caused by enteric microorganisms. Among the pathogens contaminating the aquatic environment, enteric viruses are the most important etiological agents of waterborne diseases causing mild and severe gastroenteritis, meningitis, and hepatitis (Rodríguez-Lázaro et al., 2011).

These viruses are transmitted through the fecal-oral route and primarily infect and replicate in the gastrointestinal tract of the host, being excreted at high concentrations in the feces of infected individuals (Haramoto et al., 2018). Viruses can reach the aquatic environment via the sewage discharge (Calgua et al., 2013; Fumian et al., 2013; Ming et al., 2014) and their presence in water resources is considered a public health concern in many countries.

Microbiological monitoring of recreational water quality in Brazil, and in many other countries, has been based mainly on bacterial indicators, such as thermotolerant coliforms, *Escherichia coli* (*E. coli*) and enterococci (BRAZIL, 2000; EU, 2006; USEPA, 2012). However, it has been established that bacterial contamination does not correlate with the presence or quantification of enteric viruses (Girones et al., 2010; Rigotto et al., 2010; Rusinol et al., 2014).

DNA viruses, such as human adenovirus (HAdV) and JC polyomavirus (JCPyV), have been proposed as possible viral indicators of human fecal contamination in aquatic environments, due to their high prevalence in the population and environment, environmental stability, and human host specificity (Bofill-Mas et al., 2000; Moresco et al., 2012; Rusinol et al., 2014).

HAdVs are non-enveloped double-stranded DNA viruses belonging to the family *Adenoviridae*, genus *Mastadenovirus* (ICTV, 2016). More than 68 types have been described and divided into seven groups (A–G) (Wold and Ison, 2013). They are ubiquitous in the human population and are associated with several diseases, including respiratory infections, conjunctivitis, and gastroenteritis (Berk, 2007; Mena and Gerba, 2009). HAdV species F (HAdV-F, type 40 and 41) are most commonly associated with childhood gastroenteritis (Portes et al., 2016).

JCPyV is a circular double-stranded DNA virus that belongs to the genus *Polyomavirus*, family *Polyomaviridae*. HAdVs are ubiquitous in the population, but usually induce diseases in immunocompromised individuals, with most primary infections being subclinical and occurring early in childhood. Polyomaviruses are emerging viruses contaminating human excreta and are highly prevalent in sewage samples analyzed in many countries (Rachmadi et al., 2016).

* Corresponding author at: R. Prof. Hernani Melo, 101 - São Domingos, Niterói, Rio de Janeiro 24210-130, Brazil.
E-mail address: adrianacorrea@id.uff.br (A. de Abreu Corrêa).

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HAV is a non-enveloped, positive, single-stranded RNA virus that belongs to the genus *Hepatovirus*, family *Picornaviridae*. It is organized into six genotypes (I-VI), although only one serotype has been described. Genotypes I, II, and III are related to human infections (Vaughan et al., 2014). HAV infection occurs globally and is the most common cause of acute viral hepatitis. It is strictly associated with the consumption of contaminated food and water, inadequate sanitation, and poor personal hygiene, being a public health problem worldwide. The endemicity is low in developed regions and high in developing countries (WHO, 2017).

The aim of this study was to assess the impact of sewage discharge in recreational coastal waters from Niteroi, Rio de Janeiro, Brazil, over a six-month period (January to July 2017) by evaluating HAdV, JCPyV, and HAV presence and viral loads.

2. Materials and methods

2.1. Study area

The city of Niterói, Rio de Janeiro, is located 13 km from the State Capital. It has an estimated population of 499,028 inhabitants (IBGE, 2017). The 11 km seashore consists of nine beaches at the Guanabara Bay (Gragoatá, Boa Viagem, Flechas, Icaraí, São Francisco, Charitas, Jurujuba, Adão, and Eva), five oceanic beaches (Piratininga, Sossego, Camboinhas, Itaipú, and Itacoatiara) and two main lagoons (Lagoa de Itaipu and Piratininga). The Guanabara Bay region, more specifically the Jurujuba beach, presents favorable oceanographic conditions for the commercial cultivation of bivalve mollusks that are widely consumed by the population (Dias, 2015).

The State Environmental Institute (INEA) monitors the recreational water quality based on national bacteriological indexes (CONAMA resolution 274/2000), and there is no additional data on viral contamination in these waters. The bathing conditions of the beaches of the State of Rio de Janeiro are regularly disseminated through bulletins, which are made available periodically on the INEA website (INEA, 2017). According to INEA, for the last 17 years, the majority of the beaches in the Guanabara Bay region have been classified as not suitable for recreational activities. In the oceanic region, four beaches have been recommended for bathing (Piratininga, Sossego, Camboinhas, and Itacoatiara) and Itaipu beach has been recommended with restrictions in the rainy season.

Water samples were collected from four beaches representing areas used for recreational activities and with different water qualities (Fig. 1): Icaraí, São Francisco, Charitas (located in Guanabara Bay) and Piratininga (located in the Oceanic region).

2.2. Sampling schedule, physicochemical parameters and water quality conditions

Seawater samples were collected from January to July 2017. Ten-liter water samples were obtained biweekly, with a total of 48 samples (12 samples per site). They were collected in plastic bottles and brought to the laboratory to assess viral concentrations.

External Process Controls (EPC) were included as positive controls for viral concentration and detection analyses. For the controls an extra 10 L sample of one site at each sampling event (randomly selected) was collected and spiked with a known concentration of human adenovirus 35 (HAdV35 $\sim 10^6$ GC/mL), these samples were also used to calculate viral recovery.

Physicochemical parameters, such as pH, salinity (ppt), temperature (°C), conductivity (mS), and turbidity (ppt) were determined in loco using the multi-parameter equipment COMBO 5 (AKSO, São Leopoldo, Rio Grande do Sul, Brazil).

Data on the water quality of each sampling site were obtained from the environmental monitoring bulletins available at the INEA website (INEA, 2017). Recreational water quality conditions for primary contact activities, such as skiing, swimming, and diving, are based on the quantification of thermotolerant coliforms, *E. coli*, and enterococci (CONAMA Resolution No. 274/2000). According to this resolution, the beaches are classified into two distinct categories based on the concentration of fecal coliforms or *E. coli* from analyses of five consecutive samplings. When fecal coliform densities > 1000 MPN/100 mL are detected in two or more samples from a set of five consecutive samplings over a period of five weeks or less, the beach is characterized as unsuitable for primary recreation contact. This indicates a compromise in the sanitary quality of the water, which implies an increased the risk of contamination to the bather. Waters are considered suitable when the evaluated samples do not meet the criteria established for unsuitable waters. Beaches compliant with the regulation can vary from satisfactory to excellent. Thus, when ≥80% of a set of samples from each of the prior five weeks contains a maximum of 1000 fecal coliforms (thermotolerant) or 800 *E. coli* or 100 enterococci per 100 mL, the water is considered satisfactory. When ≥80% of a set of samples obtained in each of the prior five weeks contains at most 250 fecal coliforms (thermotolerant) or 200 *E. coli* or 25 enterococci per 1000 mL, the water samples are classified as excellent.

2.3. Virus concentration

Viral concentration analysis was performed using the organic flocculation method as described by Calgua et al. (2008), which is based on the adsorption of viruses to pre-flocculated skimmed milk proteins. The 10 L seawater samples were acidified to pH 3.5 using 0.1 N HCl, and the solution of acidified (pH 3.5) skimmed milk was added to reach a final 0.01% concentration. Samples were stirred for 8 h at room temperature and left to sediment for 8 h. The supernatant was removed, and the precipitate (approximately 0.5 L) was centrifuged at 7000 × *g* for 30 min at 4 °C. The pellet was resuspended in 10 mL of phosphate buffer pH 7.5, 0.2 M (1:2 *v*/v 0.2 M Na2HPO4 and 1:2 v/v 0.2 M NaH2PO4) and stored at −80 °C until further analysis.

2.4. Viral detection and quantification

Viral nucleic acids were extracted from 400 μL of each concentrate as described by Boom et al. (1990). This procedure is based on the lysis of viral particles with guanidinium thiocyanate followed by the adsorption of nucleic acids to silica particles, and resuspension at a final volume of 40 μL.

Virus detection and quantification were carried out by absolute quantitative PCR (qPCR) and synthetic standard curves containing the DNA sequences of the three viruses analyzed (gBlock® Gene Fragments, IDT Integrated DNA Technologies, Coralville, Iowa, USA). Obtained DNA was quantified by spectrophotometry on a Nanodrop 2000® (ThermoScientific, USA) and ten-fold dilutions of DNA molecules per one mL were made in TE buffer, pH 8. Standard dilutions were then aliquoted and stored at −80 °C until use. The qPCR reactions for all viruses presented detection limits below 100 genomic copies per reaction. The slope ranged from −3.306 to −3.495, square regression coefficient (r^2) value was 0.997, and the reaction efficiencies were 93–100%. The assays were performed with the StepOne Plus TM Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). For HAV quantification, a reverse transcription reaction using random primers and Superscript III Enzyme (Invitrogen, Carlsbad, California, USA) was performed according to the manufacturer's specifications prior to the qPCR assay. The qPCR protocols for HAdV, JCPyV and HAV detection and quantification were performed as previously described by Hernroth et al. (2002), Pal et al. (2006) and De Paula et al. (2007), respectively.

All samples were tested in duplicate and in ten-fold dilutions (undiluted and 1:10 diluted DNA/cDNA), totaling four qPCR reactions per sample. The samples were considered positive when at least one replicate was detected at the cycle threshold (CT) ≤ 38.