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Enteric viruses and adenovirus diversity in waters from 2016 Olympic venues

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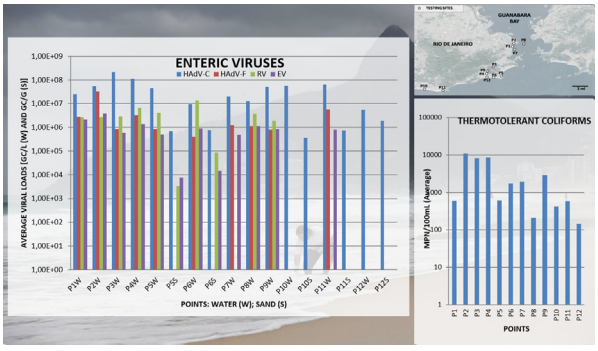
HIGHLIGHTS

- Hundred-forty out of 146 (95.9%) of water samples showed contamination with at least one type of virus.
- Diverse adenovirus species were found in water samples from 2016 Olympic city.
- Viral loads found in the water were similar to those commonly found in sewage effluents.

GRAPHICAL ABSTRACT



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ABSTRACT

Rio de Janeiro's inner and coastal waters are heavily impacted by human sewage pollution for decades. Enteric viruses, including human adenoviruses (HAdV), human enterovirus (EV), group A rotavirus (RV) and hepatitis A virus (HAV) are more likely to be found in contaminated surface waters. The present work aimed to assess the frequency and loads of EV, HAdV-C and -F species, RV and HAV in sand and water samples from venues used during the 2016 Summer Olympics and by tourists attending the event. Sixteen monthly collections were carried out from March 2015 to July 2016 in 12 different sites from Rio de Janeiro, Brazil. Total and thermotolerant coliform counting was performed along molecular detection of virus was performed using quantitative polymerase chain reaction (qPCR). Analyses of all samples were further investigated by integrated cell culture PCR to check about the presence of HAdV infectious virus particles. The results show that 95.9% of water samples showed contamination with at least one type of virus. Regarding the viruses individually (% for water and sand respectively): HAdV-C (93.1%–57.8%), HAdV-F (25.3%–0%), RV (12.3%–4.4%), EV (26.7%–8.8%) and HAV (0%). The viral loads ranged from 10^3 gc/L up to 10^9 gc/L (water), and 10^3 gc/g to 10^6 gc/g (sand). In the phylogenetic tree, were classified into four main clusters, referring to species C, D, F and BAdV. And up to 90% of sites studied presented at least once presence of infectious HAdV-C. The most contaminated points were the Rodrigo de Freitas Lagoon, where Olympic rowing took place, and the Marina da Glória, the starting point for the sailing races, demonstrating serious problem of fecal contamination of water resources and threatens the health of Olympic athletes, tourists and residents.

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1. Introduction

Viral contamination in aquatic ecosystems highlights the problem of water pollution due to continuous discharge of domestic sewage (Vieira et al., 2012). The lack or ineffectiveness of sanitary sewage services contributes to exacerbate environmental degradation levels and the viral spread in the environment. (Prado and Miagostovich, 2014). Water is essential for life and maintaining the quality of water resources has been one of the biggest challenges of this century (Wimalawansa and Wimalawansa, 2014). Sources of fecal pollution may include discharge of raw sewage, surface runoff, slaughterhouses, and industrial activities (Oliveira and Cunha, 2014). In addition to water, the soil (especially sediments) acts as an important reservoir for several microorganisms (Santamaría and Toranzos, 2003). This contamination interferes in the global environment of the affected area, and may be the cause of public health problems (Alm et al., 2003). Viruses can percolate through the soil by adsorption-desorption phenomenon reaching groundwater (Keeley et al., 2003) or return to the water column from the sediment (Alm et al., 2003).

Recreational water users can be exposed to a range of disease-causing microorganisms. Waters contaminated with human feces are regarded as a greater risk to human health, as they are more likely to contain human-specific enteric pathogens (Oliveira et al., 2016). Among other microorganisms, enteric viruses are associated with environmental contamination and the occurrence of waterborne diseases, being excreted in the feces of infected individuals in large quantities (10^5–10^{13} viral particles per gram of stool) (Bosch et al., 2008). These viruses may resist as contaminants from the environment (soil and water) for long periods (Katayama et al., 2002). Human enteric viruses such as enterovirus (EV), adenovirus (HAdV) and rotavirus (RV) (Vecchia et al., 2012) are characterized by their stability, both in the gastrointestinal tract and in the environment (Katayama et al., 2002). Frequently detected in rivers, groundwater, recreational water, drinking water, wastewater and sewage (Rigotto et al., 2010; Staggemeier et al., 2015a). They are important indicators of human fecal contamination (Jiang et al., 2001; Katayama et al., 2008; De Oliveira et al., 2012). Enteric viruses are mainly resistant to water and sewage treatment, have the adsorbing capacity more quickly to solid particles, ability to confer protection to inactivation factors (Hernroth, 2002). Several studies related to viral presence in the water resources in different countries have reported high rates of contamination in several areas, further show the involvement of these enteric viruses in waterborne viral outbreaks (Lee and Kim, 2002; Lopman et al., 2003; Rodríguez-Díaz et al., 2009; Apostol et al., 2012). These pathogens cause to human gastroenteritis, respiratory infections, conjunctivitis, hepatitis and diseases that have high mortality rate as meningitis and encephalitis (Kocwa-Haluch, 2001). Brazil is ranked in the 112th position among 200 countries in the basic sanitation international ranking (SNIS, 2013). Only 37.9% of sewage produced undergo any treatment process prior to being discarded in the environment (Prado and Miagostovich, 2014), revealing the lack of basic sanitation in the country as a chronic problem.

The Guanabara Bay is the second largest bay on the coast of Brazil, it is an important source of water resources is also the largest recipient of domestic and industrial effluents along the basin, draining the total or partial sewage from 16 municipalities, with about 11.8 million inhabitants in the metropolitan area of the city of Rio de Janeiro (Fistarol et al., 2015). These effluents are discharged into the Bay or ocean (Ipanema and Barra da Tijuca Beaches), about 50 rivers and streams discharging its waters in these places (SEMADS, 2001). On average 50.4% of households are connected to sewage treatment systems, and the city of Rio de Janeiro with 78% of connections (Fistarol et al., 2015). According to Coelho (2007) there is a deficit of 13 m^3 s between production (20 m^3 s) and sewage treatment (7 m^3 s).

Rio de Janeiro city was the venue for the 2016 Olympics Games, 10,000 athletes participated in the competition and almost 1400 were directly exposed to the polluted waters of Guanabara Bay. These athletes engage in sailing competitions, swimming, canoeing and rowing. Rio de Janeiro's inner and coastal waters are heavily impacted by human sewage pollution for decades. Even though authorities promised in their 2009 winning Olympic bid that cleaning the waterways would be a major legacy of the event, the infrastructure needed to accomplish this task was not constructed completely.

The present work aimed to assess the frequency and loads of EV, HAdV-C and -F species, RV and Hepatitis A virus (HAV) in sand and water samples from Guanabara Bay, Rodrigo de Freitas Lagoon, and beaches of Ipanema, Copacabana, Marina da Glória, Leblon and Barra da Tijuca, in venues used during the 2016 Summer Olympics, by athletes and tourists attending the event, as well as for inhabitants.

2. Material and methods

2.1. Sampling points

Sampling sites included the following: Marina da Glória (P1 and P2; where sailors enter water with boats for some sailing classes), Rodrigo de Freitas Lagoon (P3, near starting point of races; P4, near finish line of races; P9, inside the race course), Copacabana Beach (P5, where athletes enter the water for marathon and triathlon swimming), Ipanema Beach (P6, where athletes leave the water for marathon and triathlon swimming), Guanabara bay (P7, offshore inside Sugarloaf sailing course; P8, offshore inside the naval school sailing course), Barra da Tijuca Beach (P10 and P11) and Leblon Beach (P12) (Fig. 1). Samples from Points 1–9 were collected monthly for 16 months. Points 10–12 were collected from March to July 2016. Each collection point had its location demarcated by Global Positioning System and its UTM coordinates annotated and plotted. Water (500 mL each) and sediment samples (100 g each) were collected aseptically from each point in sterilized glass bottles, as well as 100 mL of water for coliform detection, totaling 146 water and 45 sediment samples. Additional 50 mL samples were collected in Falcon conical tubes. They were transported to the laboratory under refrigeration, and were kept at 4 °C until its concentration. All of them were processed in no >24 h after collection. All laboratory procedures from the arrival of the samples to laboratory until final processing were made under strict measures to avoid contamination and blank controls of ultrapure water were used to monitor adventitious contaminants.

2.2. Positive controls

All experiments were made using prototype viral strains from HAdV-5, HAdV-2, HAdV-41, poliovirus and rotavirus and hepatitis A as positive controls. Viruses were cultivated in A549 (for HAdV), Vero (poliovirus), MA-104 (rotavirus) and FRhK-4 (hepatitis A) cells following standard procedures.

2.3. Coliform detection

Total and thermotolerant coliforms most probable numbers per 100 mL (MPN/100 mL) were determined using Colilert® test kit (IDDEX®) following the manufacturer's instructions within 24 h after collection. The specific nutrient indicators that make up the Colilert® are the substrate ONPG (ortho-nitrophenol-galactopiranoside) and MUG (4-methyl-umbeliferil-D-glucuronic). The test was considered positive for fecal coliforms when staining showed blue fluorescence when exposed to UV light. The test was considered negative in the absence of development of color. The results were expressed in MPN (most probable number in 100 mL of water) according to the reference chart provided by the manufacturer.

2.4. Virus concentration

In order to detect viruses from sand samples, 1 g of the sediments were diluted onto 1 mL of Eagle's minimum essential medium (E-MEM, Nutricell; pH 11.5). The solution was homogenized by vortexing

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