



Microbial risk assessment in recreational freshwaters from southern Brazil



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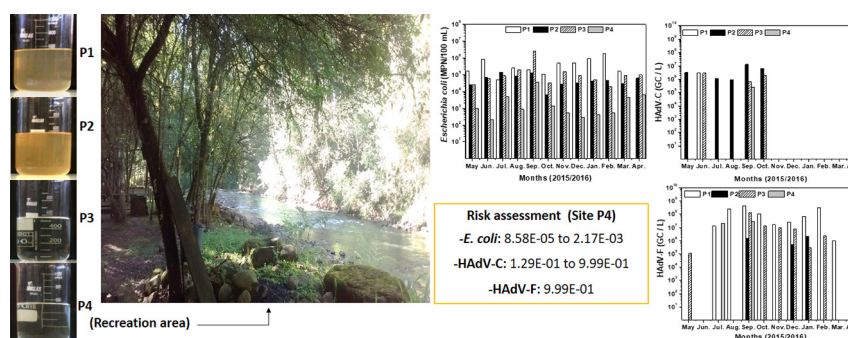
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HIGHLIGHTS

- HAdV-F was more prevalent in freshwater samples than HAdV-C.
- The highest number of the samples positive for infectivity was found for HAdV-C.
- Freshwaters used for recreation may pose a high risk of infection to HAdV.
- Risk of infection for HAdV was much higher than for *Escherichia coli*.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, total coliforms (TC), *Escherichia coli*, enterovirus (EV), rotavirus (RV), and human mastadenovirus species C and F (HAdV-C and HAdV-F) were evaluated in water samples from Belo Stream. For HAdV-C and F, the infectivity was assessed by integrated cell culture quantitative real-time polymerase chain reaction (ICC-qPCR). Samples were collected monthly (May/2015 to April/2016) at four sites. Viral analyses were performed for both ultracentrifuge-concentrated and unconcentrated samples. For site P4 (used for recreational purposes), QMRA was applied to estimate health risks associated with exposure to *E. coli* and HAdV-C and F. TC and *E. coli* were present throughout the collection period. EV and RV were not detected. HAdV-C were present in 8.51% (1.89E + 06 to 2.28E + 07 GC (Genomic Copies)/L) and 21.27% (2.36E + 05 to 1.29E + 07 GC/L) for unconcentrated and concentrated samples, respectively. For HAdV-F were 12.76% (2.77E + 07 to 3.31E + 08 GC/L) and 48.93% (1.10E + 05 to 4.50E + 08 GC/L) for unconcentrated and concentrated samples, respectively. For unconcentrated samples, infectivity for HAdV-C was detected in 37.20% (1st ICC-qPCR) and 25.58% (2nd ICC-qPCR). For HAdV-F, infectivity was detected in 6.97% (1st ICC-qPCR) and 6.97% (2nd ICC-qPCR). For concentrated samples, HAdV-C infectious was observed in 17.02% (1st ICC-qPCR) and in 8.51% (2nd ICC-qPCR). For

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HAdV-F, were present in 8.51% for both 1st and 2nd ICC-qPCR. Statistical analyzes showed significant difference between the collection sites when analyzed the molecular data of HAdV-F, data of TC and *E. coli*. Correlation tests showed direct correlation between HAdV-F with *E. coli* and TC. *E. coli* concentrations translated to the lowest estimates of infection risks (8.58E-05 to 2.17E-03). HAdV-F concentrations were associated with the highest infection risks at 9.99E-01 and for group C, 1.29E-01 to 9.99E-01. These results show that commonly used bacterial indicators for water quality may not infer health risks associated with viruses in recreational freshwaters.

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1. Introduction

Monitoring water quality is an important mechanism for tracking sources of contamination and preventing possible waterborne diseases, since it provides information on possible human exposures associated with agricultural and recreational uses (Abia et al., 2017; Klove et al., 2017; Fumian et al., 2018; Rajendran et al., 2018; Zhu et al., 2018). Urbanization in resource-poor areas, like Latin America, has been linked to detrimental impacts on aquatic ecosystems (Spilki et al., 2016). Domestic sewage is one of the main sources of pollution in these environments, since when released without proper treatment, may transmit a number of pathogens associated with illnesses like gastroenteritis (Gu et al., 2018; Mackowiak et al., 2018). Enteric viruses, such as human mastadenovirus (HAdV), enterovirus (EV) and rotavirus (RV), are excreted in the feces of infected individuals and are considered important pathogens of diarrheal disease (Biscaro et al., 2018; Mackowiak et al., 2018).

HAdV are double-stranded DNA non-enveloped viruses with icosahedral capsid, measuring 60 to 100 nm in diameter and belonging to the *Adenoviridae* family. This family consists of five genera, including the genus *Mastadenovirus*, which includes the 85 genotypes of HAdV subdivided into 7 species (A to G) (Santos and Soares, 2015; ICTV, 2017). Viruses classified in species C primarily affects the respiratory tract (although they may be excreted in the feces), whereas species F members are known to induce disease in the gastrointestinal tract (Ghebremedhin, 2014; Santos and Soares, 2015). In addition to subclinical infections and gastroenteritis, HAdV may be associated with respiratory infections, adenoviral pneumonia, pharyngoconjunctival fever, eye infections, acute hemorrhagic cystitis, and meningoencephalitis (Mena and Gerba, 2008).

EV are single-stranded RNA-positive sense, non-enveloped viruses with capsids ranging 15–30 nm in diameter and belonging to the *Piriconaviridae* family. These viruses may cause a wide spectrum of diseases that may vary from illnesses of short duration to severe disease (Nikonov et al., 2017). RV have a double-stranded RNA genome, belong to the *Reoviridae* family, are non-enveloped viruses, and may be classified by the analysis of the outer capsid proteins VP7 and VP4 (structural protein). (da Silva et al., 2016). The VP6 coding gene is often used for screening clinical and environmental samples since it is highly conserved among RV strains from different hosts (Oliveira et al., 2012). Group A rotaviruses are the most common cause of childhood gastroenteritis worldwide, with transmission occurring via the fecal-oral route (Estes and Greenberg, 2013; da Silva et al., 2016). All AdV, RV and EV are resistant to environmental stressors. Therefore, these viruses have been used as markers of fecal contamination (García, 2006; Sinclair et al., 2009; Rigotto et al., 2010; Oliveira et al., 2012; Staggemeier et al., 2017; Girardi et al., 2018).

In Brazil, the legislation establishing the criteria and limits for appropriateness of a water body to be used for recreation is CONAMA Resolution (*Conselho Nacional do Meio Ambiente*) no 274 from November 29, 2000. Conforming to this resolution, the bathing conditions are defined according to the concentration of thermotolerant coliforms, *Escherichia coli* or *Enterococcus*. However, such an evaluation is not reliable to indicate the presence of enteric viruses, since there is no relationship between coliforms and enteric viruses as indicators of fecal pollution

(Pina et al., 1998; Wyn-Jones et al., 2011; Vecchia et al., 2015; Adefisoye et al., 2016). It should be noted that viral analysis of water bodies is as a recommendation (in the case of outbreaks) for a water supply (Brazil, Ministry of Health, 2011), but not obligatory for drinking or recreational waters. Recently, coliphages (bacteriophages that infect *E. coli*) have also been considered as possible viral indicators of fecal contamination in water (Ravva and Sarreal, 2016; McMinn et al., 2017; Sidhu et al., 2018). United States Environmental Protection Agency (EPA, 2015), has shown interest and has started to consider coliphages as a viral indicator.

Quantitative microbial risk assessment (QMRA) is a method to estimate the probability of health risks associated with exposure to pathogenic microorganisms in the environment (Haas et al., 1999). Instead of conducting an outbreak investigation, QMRA can be performed to predict the possible adverse health outcomes a population may experience following contact with contaminated water (Mena, 2007). QMRA allows for the assessment of a range of pathogens, which is important since estimating health outcomes for one type, such as bacteria, does not necessarily reflect health risks associated with other microorganisms (like viruses). The goal of this study was to evaluate recreational waters in southern Brazil for bacterial and viral contamination, evaluate adenovirus infectivity and estimate associated human health risks from exposure using QMRA. In addition, risk estimates for bacteria will be compared with risks calculated for virus exposure to better characterize these waters for risk management decisions.

2. Materials and methods

2.1. Study area and sampling

Water sampling was performed monthly from May 2015 to April 2016 at four sites along Belo Stream in Caxias do Sul (RS, Brazil, 483,377 inhabitants) in accordance with the Brazilian Association of Technical Standards (ABNT) 9897 (Planning of sampling of liquid effluents and receiving bodies) and ABNT 9898 (Preservation and sampling techniques of liquid effluents and receiving bodies) (ABNT, 1987a, 1987b) (Fig. 1). Sterile bottles were used to collect samples.

Belo Stream has a drainage area of 75.01 km² and its headwaters are located in the northern portion of the basin, which is in the urban perimeter of the city. It has its origin above collection site P1 and continues its route to the Caí River, near the border of the municipalities of Caxias do Sul and Vale Real (5638 inhabitants), incorporating several tributaries during the course. The neighborhood of Desvio Rizzo, which covers most of the stream, has 13,429 inhabitants that contribute to pollution via the release of domestic and industrial effluents. It is worth noting the use of this stream for recreational activities. The stream has an area for bathing and swimming located at collection site P4. Sites P1 to P3 are in the highest region of the stream, while P4 is at the end of the watercourse (Fig. 1).

Forty-seven samples were collected throughout the study: 11 samples from site P1, and 12 samples were taken from P2, P3 and P4 each. A volume of 500 mL of surface water was collected in sterile flasks. These samples were stored at 4 °C until the concentration process.

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