Universidade Feevale

Programa de Pós-Graduação em Qualidade Ambiental

Doutorado em Qualidade Ambiental

VIVIANE GIRARDI

Risco microbiológico e diversidade de adenovírus em águas de recreação do Sul do Brasil

Novo Hamburgo

2019

Universidade Feevale

Programa de Pós-Graduação em Qualidade Ambiental

Doutorado em Qualidade Ambiental

VIVIANE GIRARDI

Risco microbiológico e diversidade de adenovírus em águas de recreação do Sul do Brasil

Tese de doutorado apresentada ao Programa de Pós-Graduação em Qualidade Ambiental como requisito para a obtenção do título de doutor em Qualidade Ambiental.

Orientador: Prof. Dr. Fernando Rosado Spilki

Co-orientadora: Profa. Dra. Suelen O. Paesi

Novo Hamburgo

2019

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)

Girardi, Viviane.
Risco microbiológico e diversidade de adenovírus em águas de recreação do Sul do Brasil / Viviane Girardi. – 2019.
55 f. : il. color. ; 30 cm.

Tese (Doutorado em Qualidade Ambiental) – Universidade Feevale, Novo Hamburgo-RS, 2019.
Inclui bibliografia.
"Orientador: Prof. Dr. Fernando Rosado Spilki ; Co-orientadora: Profa. Dra. Suelen O. Paesi".

1. Adenovírus. 2. Gene da DNA polimerase. 3. *Escherichia coli*. 4. Infecciosidade viral. 5. Risco de infecção. I. Título.

CDU 504.4(816.5)

Bibliotecária responsável: Janice Moser Corrêa – CRB 10/2315

Universidade Feevale

Programa de Pós-Graduação em Qualidade Ambienta

Doutorado em Qualidade Ambiental

VIVIANE GIRARDI

Risco microbiológico e diversidade de adenovírus em águas de recreação do Sul do Brasil

Tese de doutorado aprovada pela banca examinadora em 19 de Fevereiro de 2019, conferindo ao autor o título de doutor em Qualidade Ambiental.

Componentes da Banca Examinadora:

Prof. Dr. Fernando Rosado Spilki (Orientador)
Universidade Feevale

Profa. Dra. Juliane Deise Fleck
Universidade Feevale

Prof. Dr. Cláudio Wageck Canal
Universidade Federal do Rio Grande do Sul

Prof. Dr. André Felipe Streck
Universidade de Caxias do Sul

AGRADECIMENTOS

Gostaria de agradecer às pessoas e instituições que foram importantes na realização deste trabalho:

- à Universidade Feevale e ao Programa de Pós-Graduação em Qualidade Ambiental, pela estrutura física e recursos humanos imprescindíveis;

- à CAPES, CNPq e FAPERGS que financiaram o projeto e fornecem as bolsas de estudo;

- ao meu querido orientador Prof. Dr. Fernando Spilki, um pesquisador ético e profissional que tenho uma admiração imensurável, agradeço por tudo que me ensinou ao longo destes 4 anos, por toda paciência, orientação, pela confiança e principalmente por acreditar na minha capacidade;

-à minha co-orientadora Profa. Dra. Suelen Paesi que me acolheu ao final do mestrado, e mesmo sabendo que eu nunca havia trabalhado na área da Virologia, me incentivou a seguir este caminho. Agradeço a toda ajuda durante a elaboração deste trabalho;

-à Profa. Dra. Vania Schneider e a equipe do ISAM-UCS, por toda ajuda na escolha dos pontos de coleta e na coleta das amostras;

-à Profa. Dra. Kristina Mena que me orientou durante o estágio de doutorado nos Estados Unidos, me ensinou toda a parte de avaliação de risco e também por todo o acolhimento durante o período que morei lá;

-a todos os colegas do Laboratório de Microbiologia Molecular, em especial a Meriane, Juliana, Fernanda, Ana Karolina e Paula. Amizades que conquistei, por estarem sempre dispostas a me ajudar, ouvir minhas lamentações, e sempre me falarem palavras de conforto e incentivo;

- aos membros da banca pela disponibilidade de avaliação deste trabalho e pelas importantes contribuições;

- à minha mãe Fátima e à minha irmã Mônica, por sempre me ensinarem a nunca desistir de nada por mais difícil que seja, por todo o incentivo e apoiarem minhas escolhas;

- ao meu esposo Rafael por toda paciência, por sempre estar ao meu lado, pela compreensão das minhas ausências, por todo o apoio e por acreditar nos meus ideais.

RESUMO

Os vírus entéricos vêm sendo utilizados como marcadores de contaminação fecal em ambientes aquáticos, sendo que os frequentemente detectados são Adenovírus (AdV), Rotavírus (RV) e Enterovírus (EV). Avaliação de risco microbiológico estima a probabilidade de riscos à saúde associados à exposição a agentes patogênicos. O objetivo do presente estudo foi avaliar a diversidade de AdV, a presença de RV e EV e estimar o risco microbiológico de exposição recreacional no Arroio Belo, um corpo hídrico intensamente impactado e utilizado para recreação no município de Caxias do Sul, RS, Brasil. Para avaliar a qualidade da água foram monitorados quatro pontos de amostragem, com coletas mensais durante 14 meses (março de 2015 à abril de 2016). Coliformes totais (CT) e *Escherichia coli* foram avaliados com o uso do kit Colilert®. Amostras concentradas por ultracentrifugação e amostras não concentradas foram avaliadas. A extração de DNA/RNA foi realizada com kit comercial (Biopur®), seguido de cDNA (kit *High Capacity cDNA synthesis* - Applied Biosystems). O material genético extraído foi submetido à reação PCR para avaliar os HAdV dos grupos C e F, AdV de mamíferos (gene DNA polimerase), EV e RV. Os testes de infecciosidade de HAdV-C e F foram realizados por ICC-qPCR (*Integrated cell culture-qPCR*). Para as análises de coliformes, todas as amostras foram positivas. Quanto às análises virais, não foram detectados genomas de EV e de RV. O gene da DNA polimerase esteve presente em 43,6% das amostras concentradas, sendo identificadas as seguintes espécies: HAdV-C, D, E, e F, adenovírus bovino e adenovírus murino. A partir das amostras não concentradas, 23,6% foram positivas, sendo identificadas HAdV-C, D e F. Para HAdV-C a porcentagem de amostras positivas foi de 8,51% (carga viral variando entre 2,41E+06 e 2,28E+07 CG/L) e 21,27% (2,36E+05 – 1,29E+07 CG/L) para amostras não concentradas e concentradas, respectivamente. Para HAdV-F foi de 12,76% (1,54E+06 – 1,84E+07 CG/L) e 48,93% (1,10E+05 – 4,50E+08 CG/L) para amostras não concentradas e concentradas, respectivamente. Para amostras não concentradas, infecciosidade de HAdV-C foi detectada em 37,20% (1º ICC-qPCR) e 25,58% (2º ICC-qPCR). Para HAdV-F, a infectividade foi detectada em 6,97% tanto para o 1º quanto para o 2º ICC-qPCR. Para amostras concentradas, infecciosidade de HAdV-C foi observada em 17,02% (1º ICC-qPCR) e em 8,51% (2º ICC-qPCR). Para HAdV-F, foram detectados vírus infecciosos em 8,51% para o 1º ICC-qPCR e 2º ICC-qPCR. Análises estatísticas mostraram diferença significativa entre os pontos de coleta quando analisado os dados moleculares de HAdV-F, dados de CT e *E. coli*. Testes de correlação mostraram correlação direta entre HAdV-F com *E. coli* e CT. A menor estimativa de risco de infecção foi para *E. coli* (8,58E-05). As concentrações de HAdV-F foram

associadas com as maiores estimativas de risco de infecção (9,99E-01) e para o grupo C, 1,29E-01 a 9,99E-01. Os resultados encontrados mostram a contaminação fecal e apresentam risco para a saúde humana e a qualidade ambiental. Este é o primeiro trabalho na Região Sul do Brasil que apresenta dados de risco de infecção viral.

Palavras-chave: Adenovírus; Gene da DNA polimerase; *Escherichia coli*; Infecciosidade viral; Risco de infecção.

ABSTRACT

Enteric viruses have been used as markers of fecal contamination in aquatic environments, and the most often detected are Adenovirus (AdV), Rotavirus (RV) and Enterovirus (EV). Microbiological risk assessment estimates the likelihood of health risks associated with exposure to pathogens. The goal of the present study was to evaluate the presence and diversity of AdV, the presence of RV and EV and to infer the microbiological risk of recreational exposure in Belo Stream, an intensely impacted water body used for recreation in the city of Caxias do Sul, Brazil. For water quality assessment, four sampling sites were monitored, with monthly collections for 14 months (March / 2015-April / 2016). Total coliforms (TC) and *Escherichia coli* were evaluated by Colilert® kit. The samples were concentrated by ultracentrifugation and unconcentrated samples were also evaluated. DNA / RNA extraction was performed with commercial kit (Biopur®), followed by cDNA using the High Capacity cDNA synthesis kit (Applied Biosystems). The genetic material was submitted to PCR and was evaluated the presence of HAdV of groups C and F, AdV mammalian (DNA polymerase gene), EV and RV. HAdV-C and F infectivity analysis were performed by ICC-qPCR (Integrated cell culture-qPCR). For coliform analyzes, all samples were positive. Regarding viral analyzes, no genomes of EV and VR were detected. The DNA polymerase gene was present in 43.6% of the concentrated samples. The following species were identified: HAdV-C, D, E, and F, bovine adenovirus and murine adenovirus. From the unconcentrated samples, 23.6% were positive, being identified HAdV-C, D and F. For HAdV-C, the percentage of positive samples was 8.51% (viral load ranging between 1.05E + 05 and 1.27E + 06 GC / L) and 21.27% (2.36E + 05 - 1.29E +07 GC / L) for unconcentrated and concentrated samples, respectively. For HAdV-F it was 12.76% (1.54E + 06 - 1.84E + 07 GC / L) and 48.93% (1.10E + 05 - 4.50E + 08 CG / L) for unconcentrated and concentrated samples, respectively. For unconcentrated samples, HAdV-C infectivity was detected in 37.20% (1st ICC-qPCR) and 25.58% (2nd ICC-qPCR). For HAdV-F, infectivity was detected

in 6.97% for both 1st and 2nd ICC-qPCR. For concentrated samples, HAdV-C infectivity was observed in 17.02% (1st ICC-qPCR) and in 8.51% (2nd ICC-qPCR). For HAdV-F, infectious viruses were detected in 8.51% for the 1st ICC-qPCR and the 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. The lowest estimated risk of infection was for *E. coli* (8.58E-05 to 2.17E-03). Concentrations of HAdV-F were associated with the highest estimates of risk of infection (9.99E-01) and for group C, 1.29E-01 to 9.99E-01. The results show fecal contamination and present a risk to human health and environmental quality. This is the first work in the southern region of Brazil that to presents data of risk of infection viral.

Keywords: Adenovirus; DNA polymerase gene; *Escherichia coli*; Viral infectivity; Risk of infection.

SUMÁRIO

1. INTRODUÇÃO GERAL

Os processos de desenvolvimento industrial e urbanização têm causado considerável impacto no meio ambiente, em especial às bacias hidrográficas de regiões urbanas. A transmissão de doenças de veiculação hídrica está associada ao contato com a água por diferentes vias, como durante atividades de recreação aquática (WHO, 2011). Sistemas de notificação em saúde destacam os vírus como a maior fonte de doenças infecciosas e surtos de gastroenterites, especialmente em áreas com saneamento ambiental deficitário (MCMINN et al., 2016). Além disso, a falta ou a ineficácia de sistemas de tratamento de esgoto agravam os níveis de deterioração dos ecossistemas aquáticos (PRADO & MIAGOSTOVICH, 2014).

As gastroenterites são causadas por vírus entéricos, tais como adenovírus (AdV), rotavírus (RV) e enterovírus (EV) transmitidos pelas fezes, podendo ser utilizados como marcadores de contaminação fecal em águas superficiais e subterrâneas (DE OLIVEIRA et al., 2012; RIGOTTO et al., 2010; OGORZALY et al., 2015; STAGGEMEIER et al., 2017). Os AdV são considerados um dos grupos mais abundantes de vírus entéricos presentes na água, podendo causar surtos em frequentadores de águas de recreação contaminadas (SINCLAIR et al., 2009). Estes vírus são estáveis no ambiente aquático, uma vez que possuem resistência, por exemplo, aos agentes químicos, podendo permanecer viáveis ou potencialmente infecciosos durante longo tempo na água. Os AdV são capazes de resistir a condições ambientais diversas e a processos de tratamento de água e esgoto normalmente destinados ao controle bacteriano. Além disso, podem adsorver-se a partículas sólidas do meio aquático, promovendo maior estabilidade (GARCÍA, 2006; YATES et al., 2006; OGORZALY et al., 2015).

A avaliação quantitativa de risco microbiológico (AQRM) é a aplicação de princípios de avaliação de risco para a estimativa das consequências de uma exposição a microrganismos infecciosos, tais como o vírus entérico AdV. O objetivo é trazer a melhor informação disponível para entender a natureza dos efeitos potenciais à saúde humana em virtude de uma exposição microbiana (HAAS et al., 1999).

1.1 BACIAS HIDROGRÁFICAS DE CAXIAS DO SUL

Segundo dados do Instituto Brasileiro de Geografia e Estatística (IBGE, 2018), até o ano de 2017 o município de Caxias do Sul abrangia uma população de 483.377 habitantes, tornando-se assim a segunda maior cidade do Estado do Rio Grande do Sul em número de habitantes. Em relação à economia, mais de 45% é voltada para indústria, especialmente do

setor metalmecânico, uma vez que este município é considerado o segundo pólo metal-mecânico do País (SIMECS, 2013).

O município de Caxias do Sul é divisor de águas das Bacias Hidrográficas do Rio Caí ao sul e do Rio das Antas ao norte do munício. As Bacias Hidrográficas que fazem parte do perímetro urbano de Caxias do Sul compreendem as Bacias dos Arroios Tega, Maestra, Faxinal, Belo, Pinhal e Piaí. O Arroio Belo é um dos tributários da Bacia Hidrográfica do Rio Caí, tem suas nascentes de montante localizadas em áreas urbanizadas e industrializadas do município de Caxias do Sul, as quais contribuem com efluentes de origem doméstica e industrial. Na zona rural, os impactos são resultantes das atividades agrícolas e de criação animal e próximo ao seu exutório é utilizado para lazer e recreação (FEPAM, 2018). Tais condições são representativas daquelas encontradas para as bacias hidrográficas que drenam as maiores cidades da região Sul do Brasil.

1.2 VÍRUS ENTÉRICOS EM ÁGUAS DE RECREAÇÃO

O uso da água para fins de recreação pode ser classificado de acordo com o tipo de contato com a água. O contato primário refere-se a atividades como a natação, surfe, esqui-aquático e mergulho, nos quais há possibilidade de ingestão de água. A contaminação das águas recreacionais pode ser gerada por fontes poluidoras, como efluentes domésticos, agrícolas e industriais, sendo o primeiro o principal fator responsável por enfermidades advindas do contato com a água. Sendo assim, o contato primário com a água impõe condições mais restritivas à qualidade da água, devido ao risco oferecido à saúde humana pela exposição direta e prolongada a organismos patogênicos (BOSCH et al., 2008; SINCLAIR et al., 2009).

O despejo de resíduos humanos e de animais nos recursos hídricos sem um tratamento adequado arrasta grande variedade de patógenos, entre eles bactérias, vírus e protozoários. Destaca-se, dentre estes, os vírus entéricos que são os mais resistentes em ambientes aquáticos (GIBSON 2014; GALL et al., 2015; PREVOST et al., 2016).

Atualmente, a qualidade da água para atividades de recreação no Brasil deve atender aos padrões estabelecidos pela Resolução CONAMA 274, de 29 de novembro de 2000. Segundo esta resolução, as condições de balneabilidade das águas doces são definidas de acordo com os teores de coliformes termotolerantes ou *E. coli*, sendo para esta última aplicável um limite de 800 NMP/100 mL para a média das amostras em 80% das coletas ou o teto de 2.500 NMP/100 mL em uma única coleta para permissão de balneabilidade. No entanto, tal avaliação não demonstra, necessariamente, a origem humana do material fecal,

uma vez que a *E.coli* pode também ser encontrada em fezes de outros animais, bem como não são adequados para indicar a presença de vírus entéricos, tais como os AdV, RV e EV.

1.3 ADENOVÍRUS

Os AdV foram isolados e caracterizados como agentes virais pela primeira vez no ano de 1953 em estudos realizados por Rowe e colaboradores. Este grupo de pesquisadores observou a degeneração de culturas de células de adenoides de crianças, como resultado da replicação do vírus. Os *Mastadenovirus humanos* (HAdV) são vírus não-envelopados, com capsídeo icosaédrico de 60 a 100 nm de diâmetro, genoma de DNA dupla fita e pertencem a família *Adenoviridae* (Figura 1). Esta família é composta por cinco gêneros, entre eles o gênero *Mastadenovirus*, o qual inclui os 85 genótipos de HAdV que são subdivididos em 7 espécies, nomeadas de A à G. Além de HAdV, neste gênero estão incluídos vírus de morcegos, bovinos (BAdVs), caninos, equinos, murinos (MAdVs), ovinos, suínos e símios (SANTOS et al., 2015; ICTV, 2017).

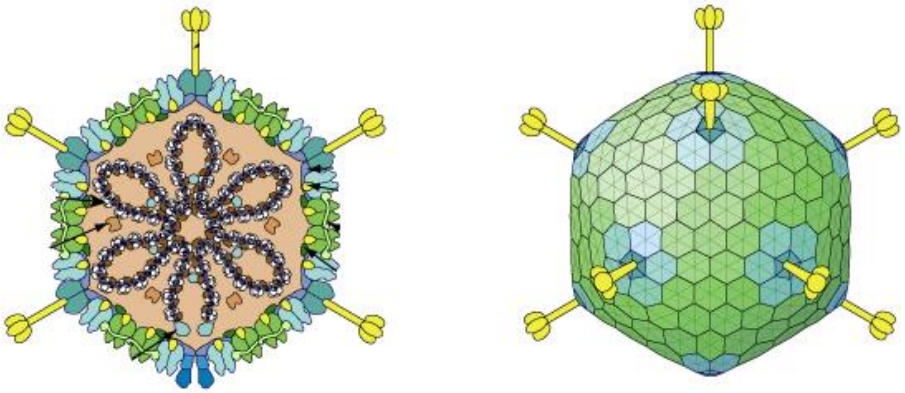


Figura 1: Visão esquemática de partículas de adenovírus (Fonte: http://viralzone.expasy.org).

A transmissão dos AdV pode ocorrer por contato direto ou indireto, através de aerossóis, secreções oculares e respiratórias e pela via fecal-oral, através de água ou alimentos contaminados. As doenças mais comuns associadas aos HAdV são doença respiratória aguda, pneumonia, gastroenterite e conjuntivites. Alguns sorotipos são capazes de estabelecer infecções assintomáticas (SANTOS et al., 2015). Os AdV são associados a numerosos surtos de doenças, particularmente aqueles que envolvem escolas, hospitais e, também, têm sido responsáveis por muitos surtos ligadas a águas de recreação (MENA & GERBA, 2008).

AdV veiculados pela água têm sido detectados durante todos os meses do ano. Além disso, os estudos têm mostrado que sobrevivem mais tempo na águas que os EV e o vírus da

hepatite A, devido provavelmente a estrutura do DNA de fita dupla (MEHNERT et al., 1999; MENA & GERBA, 2008). A presença de AdV vem sendo identificada em águas de recreação dos Estados Unidos (ASLAN et al. 2011, KUNDU et al., 2013; LEE et al. 2014), Europa (WYN-JONES et al., 2011, WYER et al., 2012) e Brasil (MAURER et al., 2015; STAGGEMEIER et al., 2017; DIAS et al., 2018).

1.4 ROTAVÍRUS

Os RV (Figura 2) pertencem ao gênero *Rotavirus* e a família *Reoviridae*, são divididos em 8 espécies (A à H), possuem simetria icosaédrica, são não-envelopados e medem cerca de 100 nm de diâmetro com genoma de RNA fita simples (ICTV, 2015; DA SILVA et al., 2016). A proteína viral VP6 é frequentemente usada para triagem de amostras clínicas e ambientais, uma vez que é altamente conservada entre cepas de RV de diferentes hospedeiros (DE OLIVEIRA et al., 2012). As infecções por RV podem variar de assintomáticas a sintomas como diarreia, vômitos, febre, desidratação e dor abdominal (DA SILVA et al., 2016).

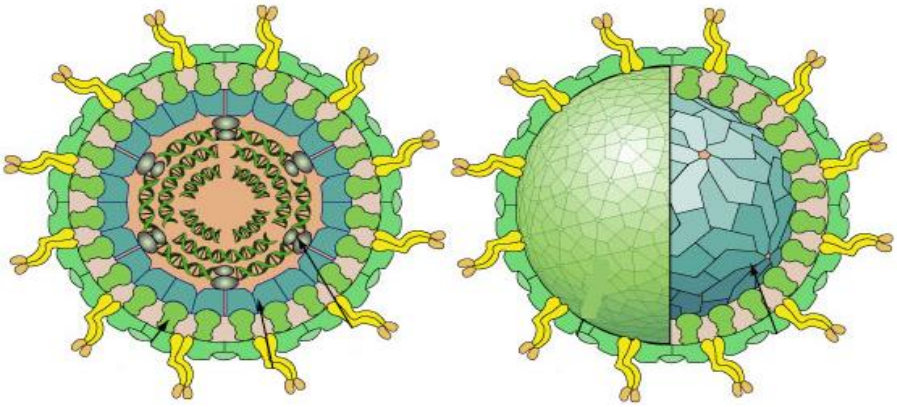


Figura 2: Visão esquemática de partículas de rotavírus (Fonte: http://viralzone.expasy.org).

O RV da espécie A (RV-A) é o principal agente etiológico da doença diarreica em crianças e é reconhecido como um importante contaminante em ambientes aquáticos (PARASHAR et al., 2006; RIGOTTO et al., 2010). Apesar de serem considerados amplamente espécie-específicos, estudos mostram potenciais infecções com caráter zoonótico, ou seja, espécies de RV de animais em humanos e vice-versa (LUCHS & TIMENETSKY 2014a; LUCHS & TIMENETSKY, 2014b; MEDICI et al., 2015). Destaca-se que corpos de água podem apresentar contaminação por RV tanto de humanos quanto de

animais, desempenhando assim um papel fundamental na transmissão desses vírus, causando possíveis infecções zoonóticas (DORÓ et al., 2015).

1.5 ENTEROVÍRUS

Os EV são vírus de RNA de fita simples, não envelopados, com capsídeos variando de 15 a 30 nm de diâmetro e pertencentes à família *Pirconaviridae* (Figura 3). Esses vírus podem se replicar no trato respiratório e no intestino e pode ser transmitido através de aerossóis e pela via respiratória ou pela via fecal-oral. A maioria dos EV causam infecções assintomática. No entanto, também podem causar um amplo espectro de doenças, como gastroenterite, doença respiratória leve, meningite, paralisia flácida aguda, entre outras doenças (PALACIOS e OBERSTE, 2005; RODRÍGUEZ-LÁZARO et al., 2012; NIKONOV et al., 2017).

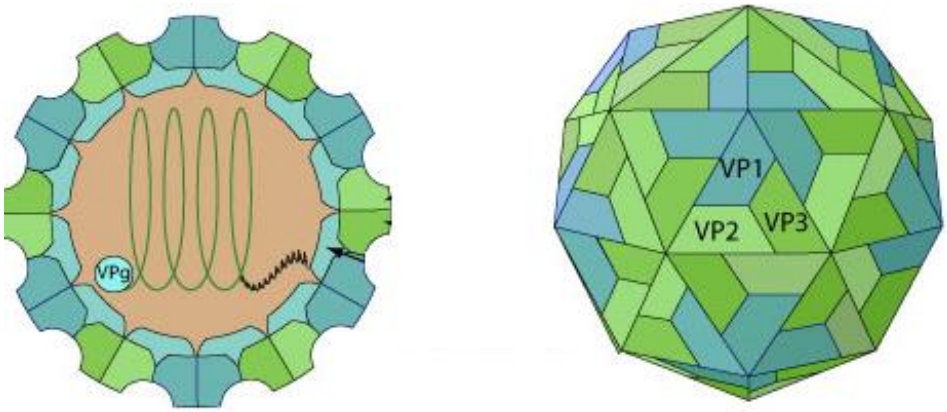


Figura 3: Visão esquemática de partículas de enterovírus (Fonte: http://viralzone.expasy.org).

O risco de infecção por EV através de águas contaminadas é de 10 a 10.000 vezes maior que o de infecção bacteriana, pois possuem baixa dose infecciosa (podendo variar de 1 a 10 unidades virais, representando assim um risco a saúde pública (HAAS et al., 1993). Após o contato com o organismo hospedeiro, os EV se multiplicam no trato gastrointestinal e podem infectar outros órgãos e sistemas extraintestinais (PELLEGRINELLI et al., 2013). A presença de EV em ambientes aquáticos é considerada pela Organização Mundial da Saúde de alta severidade e possível de causar epidemias pelo fato de apresentarem alta infectividade (WHO 2006).

1.6 AVALIAÇÃO QUANTITATIVA DE RISCO MICROBIOLÓGICO

A avaliação de risco é a caracterização qualitativa ou quantitativa e a estimativa de potenciais efeitos adversos à saúde associados à exposição de indivíduos ou populações a

riscos (águas contaminadas por agentes microbianos). A avaliação de risco não é usada isoladamente, mas faz parte do que é conhecido em um contexto mais amplo como análise de risco. A análise de risco inclui avaliação de risco, gerenciamento de risco e comunicação de risco (HAAS et al., 1999):

> Avaliação do risco: inclui o uso da informação científica para descrever a probabilidade e magnitude do dano atribuída a um risco específico. É a caracterização e estimativa de potenciais efeitos à saúde de indivíduos expostos a certos patógenos, por exemplo;
> Gestão do risco: inclui todas as atividades empreendidas para controlar um risco;
> Comunicação do risco: é a troca de informações e opiniões sobre o risco entre as partes envolvidas.

Para a realização completa de uma avaliação quantitativa de risco microbiológico são necessários: identificação do perigo; avaliação da exposição; avaliação da relação dose-resposta e a caracterização do risco (*Microbial Risk Assessment Guideline, 2012*).

A identificação do perigo é a etapa inicial da avaliação do risco microbiano; compreende informações gerais sobre os patógenos e as consequências adversas para a saúde e incorpora uma ampla gama de informações sobre os agentes infecciosos. Um microrganismo que pode infectar e se reproduzir está associado a um ponto final de infecção e pode causar infecções assintomáticas (sem doença) (HAAS et al., 1999; USEPA, 2014).

A avaliação da exposição consiste basicamente em determinar o tamanho e a natureza da população exposta; é a dose do agente patogênico que um indivíduo ingere, inala ou entra em contato. Esse número alimenta os modelos matemáticos de dose-resposta para prever a probabilidade de infecção. Os fatores que são considerados ao avaliar a exposição são: a quantidade (concentração) do patógeno (vírus) na exposição (água de consumo, águas recreacionais, aerossóis), o volume da exposição e a frequência e duração da exposição (HAAS et al., 1999; MENA, 2007).

A avaliação da dose-resposta é uma etapa essencial da avaliação de risco, pois é um elemento quantitativo da estimativa do risco. Estima o risco de uma resposta (por exemplo, infecção, doença ou morte) dada uma dose conhecida de um patógeno, sendo que os valores do risco variam de 0 a 1. Modelos de resposta a dose são funções matemáticas que descrevem a relação dose-resposta para patógenos específicos, rotas de transmissão e hospedeiros. Os

modelos matemáticos exponencial e beta-Poisson são utilizados em estudos de risco envolvendo vírus transmitidos pela água (MENA, 2007; HAAS et al., 2015).

A caracterização do risco é a integração de informações sobre a quantidade de dose recebida com quanto risco está associado para estimar uma probabilidade de risco, a fim de estimar a magnitude do problema de saúde pública. As caracterizações podem variar de simplesmente conectar uma dose em uma função DR para obter um único "ponto estimado" de risco até estimativas mais sofisticadas que consideram a incerteza nos parâmetros adotados no modelo e a variabilidade entre os indivíduos e as subpopulações (SOLLER, 2006; MENA, 2007).

Os estudos desenvolvidos neste doutorado deram origem a publicação de dois artigos que constituem o corpo da presente tese. No primeiro artigo, foi estudada a diversidade de AdV de mamíferos a partir de amostras não concentradas e concentradas pelo método de ultracentrifugação. O segundo artigo aborda a avaliação do risco de infecção tanto por HAdV, quanto por *E. coli.* Além disso, foi estudada a presença de RV e EV e a infecciosidade de HAdV tanto a partir das amostras concentradas, quanto das não concentradas e testes estatísticos para avaliar relação entre coliformes e HAdV e também diferença entre os pontos de coleta.

2. OBJETIVOS

2.1 OBJETIVO GERAL

- Avaliar a diversidade de adenovírus, presença de rotavírus, enterovírus, coliformes totais e *Escherichia coli* ao longo do Arroio Belo e inferir sobre o risco microbiológico de exposição recreacional a essas águas.

2.2 OBJETIVOS ESPECÍFICOS

- Detectar e quantificar os genomas de HAdV dos grupos C e F ao longo dos diferentes pontos de amostragem do Arroio Belo;

- Comparar a presença das diferentes espécies de AdV nos pontos de amostragem tanto da área urbana quanto da área rural;

- Detectar e quantificar os coliformes totais e *E. coli* nas amostras de água;

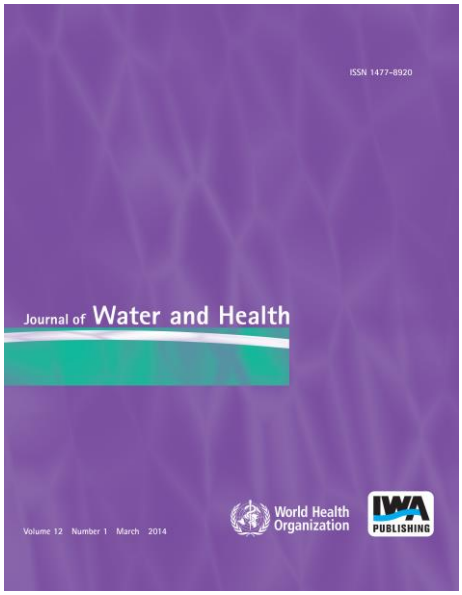
- Avaliar a presença de partículas virais de EV e RV ao longo dos diferentes pontos de amostragem do Arroio Belo;

- Avaliar a infecciosidade viral através de testes em cultivo celular;

- Comparar a presença dos genomas de AdV, bem como a infecciosidade em amostras não concentradas e concentradas;

- Avaliar o risco de infecção em decorrência das práticas recreacionais através de avaliação quantitativa de risco microbiológico.

ARTIGO 1



Assessment of diversity of adenovirus DNA polymerase gene in recreational waters facilitated by ultracentrifugal concentration

Viviane Girardi, Meriane Demoliner, Caroline Rigotto, Vania Elisabete Schneider, Suelen Paesi and Fernando Rosado Spilki

ABSTRACT

Adenoviruses (AdV) are related to respiratory and gastrointestinal diseases in animals and human beings. Their wide genetic diversity in water bodies and their resistance to environmental conditions allow the use of AdV as a reliable marker for detection of fecal contamination. In this work, the diversity of AdV along Belo Stream – in the city of Caxias do Sul, Rio Grande do Sul, Brazil – was evaluated. Samples were compared in both concentrated and unconcentrated forms. The identification of different AdV species was performed by amplifying a partial sequence of the DNA polymerase gene. AdV was detected in 24 out of 55 concentrated samples (43.6%) and the following species were identified: human adenovirus (HAdV) species C (4/55; 7.2%), D (6/55; 10.9%), E (2/55; 3.6%), and F (9/55; 16.3%). AdV related to other mammalian hosts, such as bovine adenovirus (1/55, 1.8%) and murine adenovirus (2/55, 3.6%), have also been identified; 23.6% (13/55) of the unconcentrated samples were positive, and identified as HAdV species C (6/55, 10.9%), D (1/55, 1.8%), and F (6/55, 10.9%). Results obtained evidenced the presence and the great diversity of AdV, mainly of human origin, circulating in Belo Stream. As expected, the concentration step performed helped to detect AdV in more samples.

Key words | adenovirus diversity, DNA polymerase gene, environmental sanitation, nested-PCR, primary contact waters, water resources

Viviane Girardi (corresponding author)
Meriane Demoliner
Caroline Rigotto
Fernando Rosado Spilki
Laboratório de Microbiologia Molecular,
Universidade Feevale,
ERS 239 n° 2755, Novo Hamburgo, RS 93352-000,
Brasil
E-mail: *0178103@feevale.br*

Vania Elisabete Schneider
Instituto de Saneamento Ambiental (ISAM),
Universidade de Caxias do Sul,
Rua Francisco Getúlio Vargas, n° 1130, Caxias do Sul, RS 95070-560,
Brasil

Suelen Paesi
Laboratório de Diagnóstico Molecular, Instituto de Biotecnologia,
Universidade de Caxias do Sul,
Rua Francisco Getúlio Vargas, n° 1130, Caxias do Sul, RS 95070-560,
Brasil

INTRODUCTION

Waterborne diseases, such as gastroenteritis, represent a serious public health problem in developing countries. Several studies in literature confirm that there is a relation between the lack of basic sanitation and the dissemination of viral waterborne diseases, such as the ones caused by adenoviruses (AdV). The poorer the sanitation systems, the greater the impact of these illnesses. In addition, the lack or ineffectiveness of sewage treatment systems aggravates and leads to the deterioration of aquatic ecosystems (Heller *et al.* 2003; Prado & Miagostovich 2014; Spilki 2015).

The use of water for recreation purposes is a common practice in tropical climate countries. The contamination of these water bodies can be generated by several polluting sources, such as domestic, agricultural, and industrial effluents, the first being the main factor responsible for diseases caused by contact with water. During the summer, waterborne disease outbreaks increase due to recreational activities and contact with water contaminated by domestic sewage. Therefore, the quality of the recreational waters should be analyzed more thoroughly, due

doi: 10.2166/wh.2017.144

to the risk offered to human health by direct and prolonged exposure to pathogenic organisms, such as AdV (Bosch *et al.* 2008; Sinclair *et al.* 2009; Lodder *et al.* 2015).

Human adenoviruses (HAdV) are non-enveloped viruses with icosahedral capsid 60–100 nm in diameter and DNA double-stranded genome, and they belong to the family *Adenoviridae*. This family is composed of five genera; among them is the genus *Mastadenovirus*, which includes 57 serotypes of HAdV that are subdivided into seven species that go from A to G. In addition to HAdV, bat, bovine (BAdVs), canine, equine, murine (MAdVs), sheep, swine, simian and viruses from other hosts are included in this genus. HAdVs may have different tropisms, causing a wide variety of diseases that include acute febrile pharyngitis, respiratory infections, acute conjunctivitis, cystitis, gastroenteritis, and systemic infections in immunocompromised patients. HAdV-A, B, C, and E affect the respiratory system, while species D, F and G are more prone to cause gastrointestinal infections (Ghebremedhin 2014; Santos & Soares 2015).

Species classification and AdV serotypes found in aquatic environments help to identify different sources of contamination, as well as to have a better knowledge of their occurrence in water, thus improving the assessment of infectious risk for humans exposed to specific types of HAdV (Kidd *et al.* 1990; García 2006; Robinson *et al.* 2013). AdVs are stable in the aquatic environment and may remain potentially infectious for a long time in water, including diverse environmental conditions and water and sewage treatment processes that are normally intended for bacterial control. Moreover, they can adsorb solid particles of the aquatic environment, promoting greater stability. Due to all these characteristics, AdVs may be used as viral markers for detection of fecal contamination in water (García 2006; Yates *et al.* 2006; Ogorzaly *et al.* 2015).

Belo Stream is one of the tributaries of the Caí River Basin, located in the mountainous region of the state of Rio Grande do Sul (RS), in southern Brazil. Its springs are located in the city of Caxias do Sul, in both urbanized and industrialized areas, which add to domestic and industrial effluents. In the countryside, the impacts are the result of agricultural activities and animal husbandry when the area near to its mouth is used for recreation. Until now, no investigation has been carried out on the presence of enteric viruses in this region.

In this context, the aim of the present work was to compare results obtained from concentrated and unconcentrated samples for the presence and diversity of the mammalian polymerase DNA gene of AdV along a river known to be polluted by human and animal waste.

MATERIAL AND METHODS

Study area and sampling

Sampling was performed from March 2015 to April 2016, monthly, at four points along Belo Stream in Caxias do Sul (RS, Brazil) as shown in Figure 1, 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). Belo Stream drains 21% of the city urban perimeter; it has an area of 75.10 km^2 and a perimeter of 63.11 km.

A total of 55 samples were collected from four different points: 13 samples from P1, and 14 from P2, P3, and P4. A volume of 500 mL of surface water was collected in sterile flasks. These samples were stored at 4 °C until the concentration process took place. A short description of the points is given in Table 1.

Viral concentration

A total of 55 samples containing 500 mL were collected. From these 500 mL, 36 mL were concentrated by ultracentrifugation method, following the protocol: An aliquot was centrifuged at the rate of 41,000 × *g* at 8 °C for 3 h. Thereafter, the precipitate was resuspended in 2 mL of Tris-EDTA buffer (pH 8.0), and vigorously homogenized in vortex for 1 min. The resuspended samples were aliquoted and stored in microtubes at −80 °C until the DNA extraction process. Unconcentrated samples (55) were also evaluated and aliquoted into microtubes and stored at −80 °C for further DNA extraction.

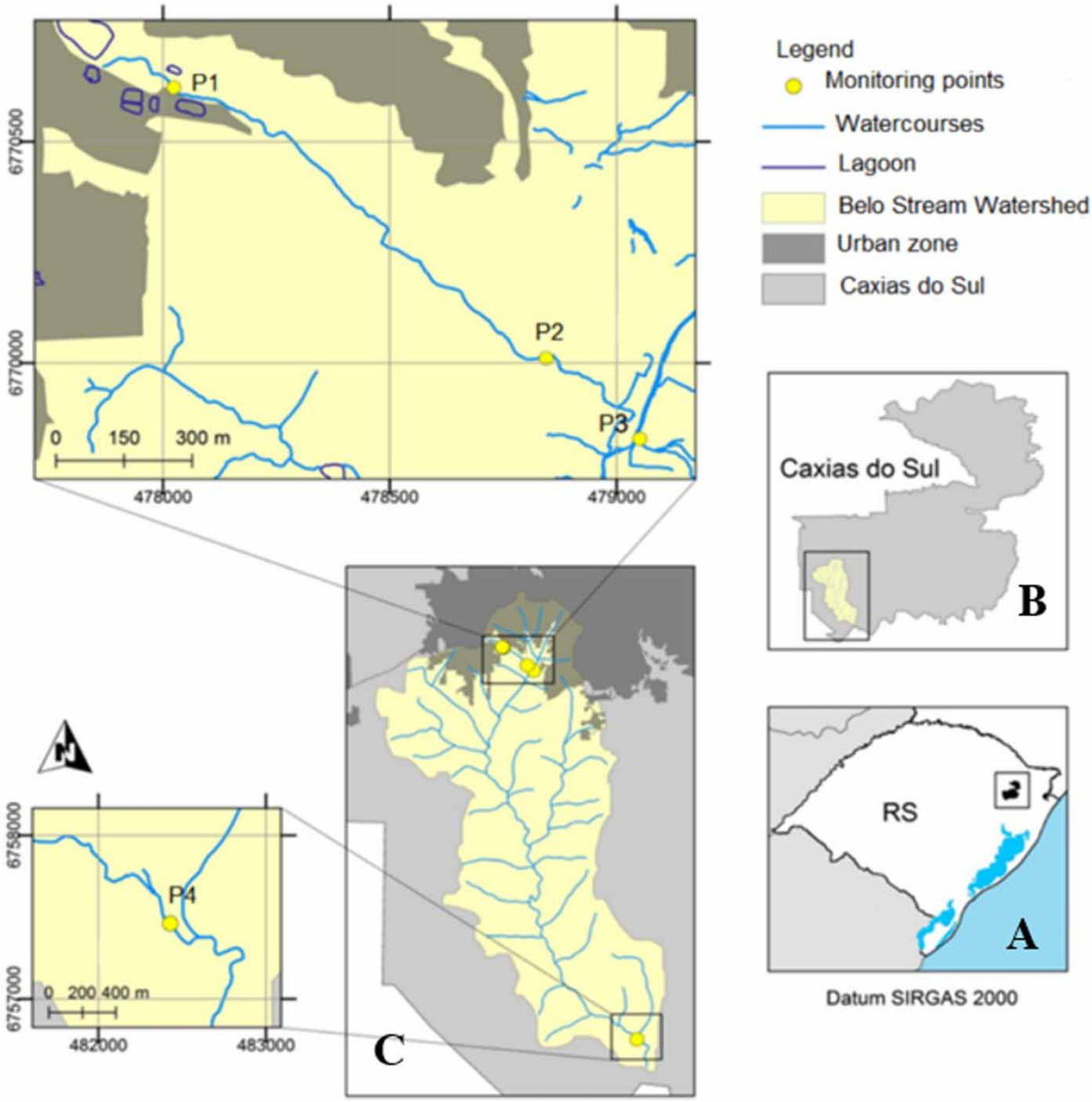


Figure 1 | (a) Map of Rio Grande do Sul – Brazil, in which the location of the city of Caxias do Sul is highlighted. (b) Map of Caxias do Sul, in which the outline and location of Belo Stream are highlighted. (c) Distribution of the sampling points along Belo Stream (elaborated by Geise Macedo dos Santos).

Extraction of genetic material

Genetic material from the concentrated and unconcentrated samples was extracted with a BioPur® Kit from an initial volume of 200 μL according to the instructions described by the manufacturer. The final elution was performed in microtubes free of DNAse/RNAse, in which they were stored and maintained at −80 °C until further processing.

Viral detection

To evaluate the presence of different AdV species, a partial sequence of the DNA polymerase (pol) gene was amplified by nested polymerase chain reaction (nested-PCR). Measurements of the reaction were carried out for a final volume of 50 μL, as follows: 1 μL of Pol-F primer (5′-CAGCCKCKGTTRTGYAGGGT-3′), 1 μL of the primer

Table 1 | Name, description and coordinates of collection points along Belo Stream

Point	Description	Coordinates	
		X	Y
P1	It is located in the urban area of the city of Caxias do Sul. It receives effluent from domestic origins. It is upstream of the effluent release of an animal processing industry. It is a sampling point located in a stream that is a tributary of Belo Stream	478,024	6,770,622
P2	It is located in a periurbanized region. It is upstream of the confluence of the stream with Belo Stream and downstream of P1	478,845	6,770,010
P3	It is located downstream of the confluence with the monitored stream (P1 and P2). It receives domestic effluents. Native vegetation and agricultural activities characterize this setting	479,051	6,769,817
P4	It is upstream from the confluence with the Caí River. P4 is characterized by areas of native vegetation and agricultural activities, and it is used for leisure and aquatic recreation because there is a camping area	482,424	6,757,598

Pol-R (5′-GCHACCATYAGCTCCAACTC-3′), both primers at 20 pmoles, 18 μL of DNAse/RNAse free water, 25 μL of GoTaq® Green Master Mix (Promega, USA) and 5 μL of nucleic acid extracted from each sample. After initial incubation at 94 °C for 5 min, 30 cycles of amplification were performed. These consisted of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. Amplification was performed in a Bio-Rad® thermal cycler. The second PCR reaction was performed using the same reagents and quantities from the first one, as well as the same amplification cycles carried out with the products of the first PCR; however, both primers were replaced with Pol-nF (5′-GGGCTCRTTRGTCCAGCA-3′) and Pol-nR (5′-TAYGACATCTGYGGCATGTA-3′) (Li *et al.* 2010).

PCR products were analyzed on 2% agarose gel, 0.5 mg of ethidium bromide/mL was added and the running time was 60 min at 70 V. Molecular sizes of the products were compared with a 100 bp DNA standard (Ludwig brand). The bands stained with ethidium bromide were visualized in UV light; subsequently images were photographed with an Easy Doc 200 UV transilluminator equipment.

Samples testing positive for the DNA polymerase gene by nested-PCR were submitted to DNA sequencing for species identification. Sequencing was carried out by Ludwig Biotec (Sequencing Service) using an automated sequencer (ABI-PRISM 3500 Genetic Analyzer Applied Biosystems). Phylogenetic analysis was performed by comparing the genomic sequences obtained by direct DNA sequencing with other nucleotide fragments available from GenBank, in accordance with the Neighbor-Joining methodology (Saitou & Nei 1987). A phylogenetic tree was elaborated from the calculation of evolutionary distances, using the Kimura-parameter 2 method (Kimura 1980) and operating with Molecular Evolutionary Genetics Analysis software version 5 (MEGA5) (Tamura *et al.* 2011).

RESULTS

Evaluation of the presence of the AdV DNA polymerase gene

The AdV DNA polymerase gene was detected in 43.6% (24/55) of the concentrated samples. On the other hand, 25.4% (14/55) of the unconcentrated samples were positive for AdV. Thus, more positive samples were obtained when submitted to the concentration step in contrast to the unconcentrated ones.

The greatest number of positive samples for both concentrated and unconcentrated samples was from the P3 site (concentrated samples 57.1% (8/14); unconcentrated: 50.0% (7/14)) as seen in Tables 2 and 3. The sites with the lowest positivity were P2 (21.4% (3/14)) and P4 (7.1% (1/14)) (Tables 2 and 3) for concentrated and unconcentrated samples, respectively.

Among the concentrated samples (Figure 2), AdV was found in at least one collection point every month.

Table 2 | Number of positive samples detected by nested-PCR along the collection points of Belo Stream (concentrated samples)

Concentrated samples

AdV species	P1	P2	P3	P4	Total (per species)
HAdV-C	0	2	0	2	7.3% (4/55)
HAdV-D	4	0	2	0	10.9% (6/55)
HAdV-E	0	0	1	1	3.6% (2/55)
HAdV-F	1	1	4	3	16.4% (9/55)
BAdV	1	0	0	0	1.8% (1/55)
MAdV	1	0	1	0	3.6% (2/55)
Total (per collection point)	7/13 (53.8%)	3/14 (21.4%)	8/14 (57.1%)	6/14 (42.8%)	

Table 3 | Number of positive samples detected by nested-PCR along the collection points of Belo Stream (unconcentrated samples)

Unconcentrated samples

AdV species	P1	P2	P3	P4	Total (per species)
HAdV-C	1	3	1	1	10.9% (6/55)
HAdV-D	0	0	1	0	1.8% (1/55)
HAdV-F	2	0	5	0	12.7% (7/55)
Total (per collection point)	3/13 (23.0%)	3/14 (21.4%)	7/14 (50.0%)	1/14 (7.1%)	

Furthermore, October and November of 2015 and February of 2016 were the months which presented the highest number of positive results for AdV (three samples tested positive in each of these months). Based on unconcentrated samples (Figure 3), July, October, and November of 2015 and March of 2016 presented greater positivity, as two positive samples were detected in each of these months.

Diversity of the AdV DNA polymerase gene

Based on the results obtained by nucletotide sequencing and phylogenetic analysis, it was found that the most prevalent species of AdV was F in both unconcentrated and concentrated samples, being found at a rate of 16.4 and 12.7%, respectively. On the other hand, the BAdV species was the one with less predominance in concentrated samples,

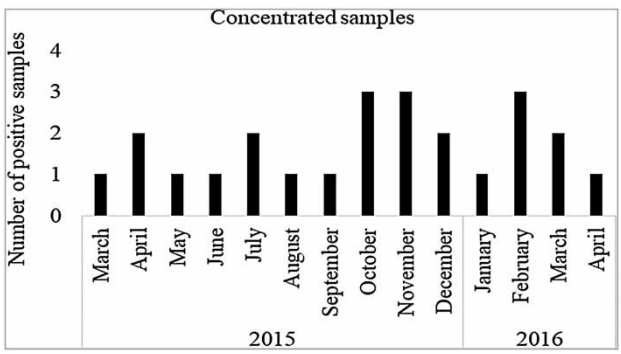


Figure 2 | Total number of positive samples per month of collection along the four sampling points of Belo Stream (concentrated samples).

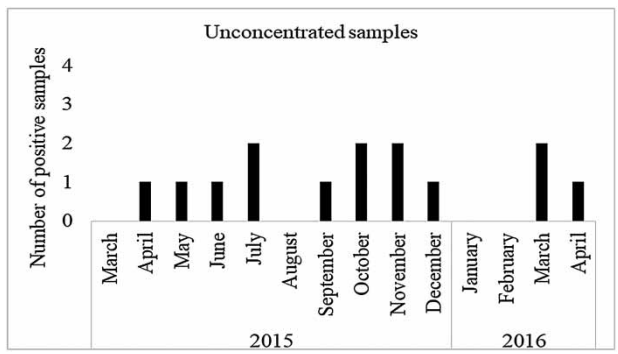


Figure 3 | Total number of positive samples per month of collection along the four sampling points of Belo Stream (unconcentrated samples).

being present in only one sample (1.8%) from P1. In the unconcentrated samples group D was the one less found, being in only 1.8% of the samples. It is important to point out that the HAdV-E, BAdV and MadV species were only identified among the water samples that were submitted to the concentration method (Tables 2 and 3, and Figure 4). Therefore, it may be claimed that the concentration method allows superior results to be obtained, since a great diversity of AdV species was identified, including from other hosts in addition to the human ones.

DISCUSSION

Water resources suffering anthropogenic influence can harbor immense microbiological diversity, as they can be contaminated by different effluent sources. AdV is often found in aquatic ecosystems, since current sewage treatment methods are not fully effective in the removal of viral particles. In the present study, the presence of AdV was detected in 43.6% of the concentrated samples. Taking into consideration this study and the sites investigated, the occurrence of AdV was higher than in other studies. Aw & Gin (2011) evaluated AdV in surface waters in Singapore and reported positivity in 38% of the samples. Kundu *et al.* (2013) found the presence of AdV in 11% of recreational water samples from the USA. However, Maurer *et al.* (2015) found superior results compared with this study. They described the presence of the AdV genome in 77.8% of samples collected in recreational waters in southern Brazil.

Usually, when comparing concentrated with unconcentrated samples, the number of samples found positive is higher after the concentration stage, which allows the finding of a greater diversity of AdV species. This is an important fact to consider when comparing both protocols, since it is not always possible to find the same diversity if samples are not concentrated. Therefore, the concentration method is fundamental to surface water samples, because it allows an increase in the frequency of positive samples. Furthermore, unconcentrated samples contain a number of substances that inhibit or interfere with the viral detection and quantification, due to the receipt of different effluents, such as domestic, industrial, and agricultural (Fumian *et al.* 2010; Silva *et al.* 2011).

HAdV found in recreational waters has been considered to cause gastroenteritis outbreaks and other diseases of the respiratory system due to one of its main characteristics: the resistance to variations in environmental conditions (Sinclair *et al.* 2009). Considering concentrated samples, the total number of positive samples found at P4 (place used for leisure and aquatic recreation) was six (6/14 (42.8%)) (Table 1). It is worth highlighting that among these six positive samples, three belong to group F, a major virus that causes gastroenteritis, and is also considered one of the major etiological agents responsible for infantile gastroenteric infections. Thus, this constitutes a health risk for the population that bathe in these waters, since viral analysis in recreational waters in Brazil is not mandatory (Filho *et al.* 2007; Kundu *et al.* 2013; La Rosa *et al.* 2015). Caxias do Sul (RS, Brazil) is one of the cities that face gastroenteritis problems. Paesi & Magrini (2015) conducted a study on the number of cases of acute diarrheal disease in this municipality from 2004 to 2013. The authors reported that during this period 61,246 cases were recorded, the highest numbers being in low-income neighborhoods. Furthermore, the authors also reported that the area with the second highest register was Desvio Rizzo. In this same area is located P1, which, as previously mentioned (Table 2), was the point with the second highest number of positive samples (7/13 (53.8%)).

In contrast to what is found in the literature, HAdV belonging to the F group was detected more frequently in this study. In the literature, water sample analyses have shown that AdV belonging to group C are in a greater quantity (Bibby & Peccia 2013; Barrios *et al.* 2016; Staggemeier *et al.* 2017). On the other hand, there are studies that have found similar results. Kuo *et al.* (2015), and Wieczorek *et al.* (2015), when analyzing the presence of AdV genome in sewage samples from Taiwan and Poland, respectively, described a higher number of AdV from the F group. Other species of HAdV have also been identified, such as those of groups D and E, viruses that affect the gastrointestinal and respiratory tract, respectively. It is described in literature that HAdV-A, C, and F are more frequently found in water bodies when compared with groups B, D, and E (Kuo *et al.* 2015; Ogorzaly *et al.* 2015; Wieczorek *et al.* 2015). Serotypes 40 and 41 belonging to the F group have been considered one of the most prevalent viruses in

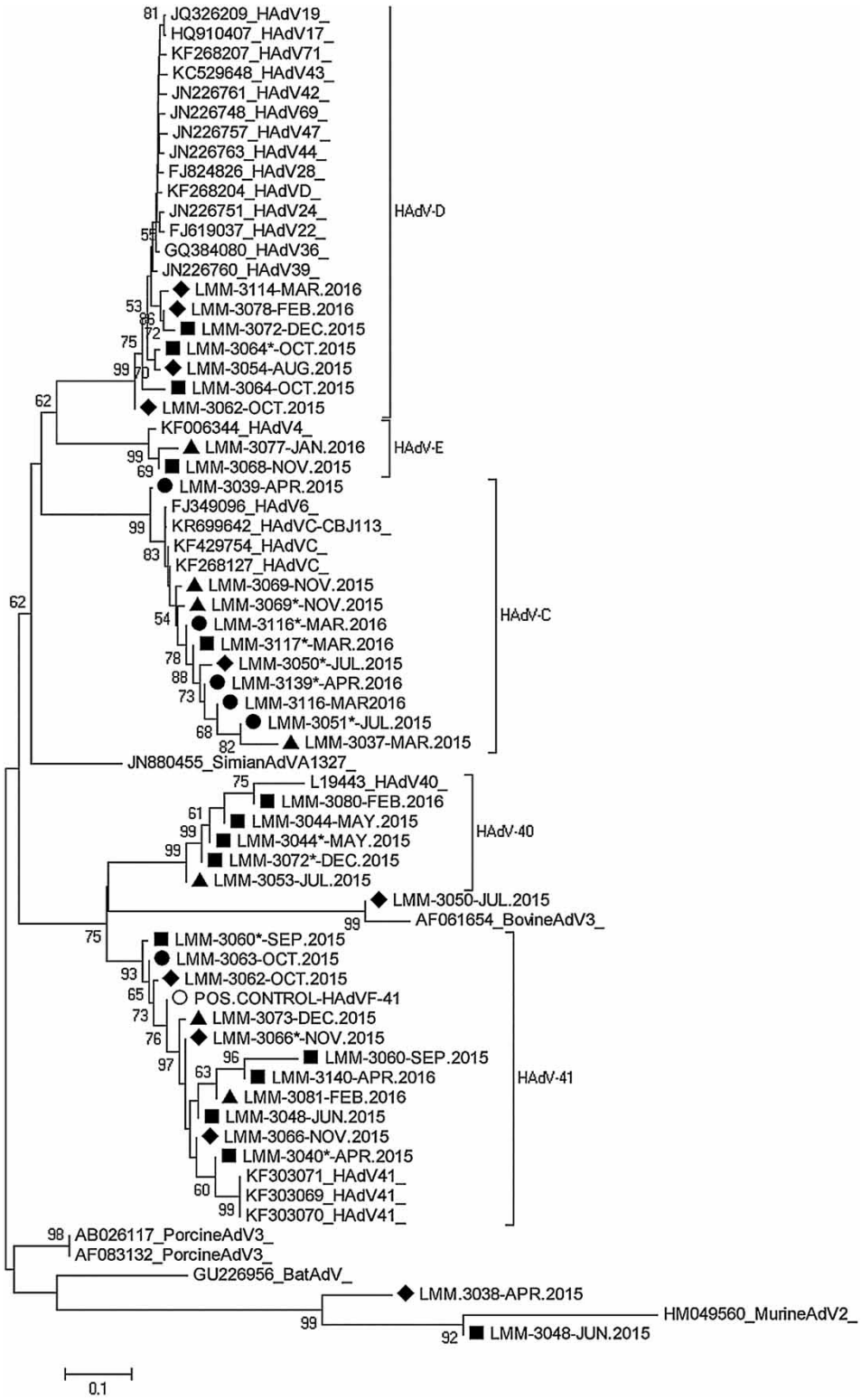


Figure 4 | Phylogenetic analysis showing the identification of six different AdV species (HAdV-C, HAdV-D, HAdV-E, HAdV-F, BAdV and MAdV) from unconcentrated and concentrated samples along four points of Belo Stream. * Unconcentrated samples; ◆ Point 1; ● Point 2; ■ Point 3; ▲Point 4.

acute gastroenteritis in children, and may also cause mortality in immunocompromised individuals. These viruses are released for long periods in feces, urine and respiratory secretions of infected persons. Thus, the high number of positivity found in this species, when compared with others, suggests a high incidence of HAdV-F infections in the population of the region of the present study (Jiang 2006; Filho *et al.* 2007; La Rosa *et al.* 2015).

AdV from other mammalian hosts were also detected, such as MAdV and BAdV. Recently, three types of MAdVs were described, and in this study, type 2 was identified. MAdVs have potential for vector screening in genes and cancer therapy, and may complement vector studies derived from HAdV. Furthermore, MAdVs provide insight into the pathogenesis process of the host, which includes acute and persistent infections (Nguyen *et al.* 1999; Weinberg *et al.* 2005; Klempa *et al.* 2009; Robinson *et al.* 2009; Hemmi *et al.* 2011). In spite of the low detection of BAdV (1.8% (1/55)) in the present study, it is worth noting that this virus can be used as a marker of fecal animal contamination in groundwater and surface waters in rural environment. Due to its structural characteristics, this type of the virus is more stable in the environment and may cause disease in animals. Bovine excrements are often applied to the soil as untreated fertilizer. Because of this, both groundwater and surface water can be contaminated through the dragging of fecal material to water due to the runoff. As a result of this, environmental degradation and the transmission of diseases caused by these pathogens are concerning, since the animals have access to contaminated rivers and streams (Spilki *et al.* 2009; Wong & Xagoraraki 2010).

In the face of the presented results, it is worth mentioning that the great diversity of AdV identified by the nested-PCR technique followed by sequencing represents a result that has not been reported in the literature. Most of the studies on diversity use techniques that are restricted to PCR or quantitative PCR (qPCR) using specific primers for a given species, thus requiring more detailed work (Filho *et al.* 2007; La Rosa *et al.* 2015; Wieczorek *et al.* 2015; Adefisoye *et al.* 2016; Barrios *et al.* 2016).

In general, AdV detection occurred during all the months of the study, and it can be stated that they did not present seasonally, a fact also observed by other studies (Wieczorek *et al.* 2015; Adefisoye *et al.* 2016). Among the months that presented the highest number of positive samples, the month of October 2015 stands out the most. According to INMET (National Meteorological Institute), the months with the highest rainfall in 2015 were September and October. Therefore, a possible reason for the increase in the number of positive samples in October is the increase in precipitation index during this period. Rainfall events can introduce a large quantity of microbial contaminants, which include human enteric viruses, through flow of contaminants and flow of fecal material to the sampling sites (Hata *et al.* 2014; Rodrigues *et al.* 2015).

In this study, the identification of AdV was performed by nested-PCR, which does not necessarily mean the presence of infectious viral particles, since PCR techniques detect both infective and non-infective genomes. However, literature confirms the risk of individuals getting sick when they bathe in water contaminated with AdV (Ogorzaly *et al.* 2010). In order to identify the true presence of infectious particles in water, PCR-integrated cell culture (ICC-PCR) studies should be performed (Rigotto *et al.* 2010).

CONCLUSIONS

The results obtained in the present study are rare in literature regarding diversity, since it is not always possible to find the same amount of species by using the nested-PCR technique. AdV belonging to the F group, an important virus that causes gastroenteritis, was present in most samples (in both unconcentrated and concentrated samples). The concentration step is necessary, for it allows the identification of a greater diversity and quantity of AdV in analyzed samples when compared with unconcentrated ones. However, results obtained from unconcentrated samples should be considered, since three different species of HAdV were found. Because they were detected in all analyzed months, the AdV detected did not present seasonally. The analysis of AdV in these waters reveals mainly human fecal contamination along Belo Stream, which demonstrates the inefficiency or the absence of adequate sewage treatment processes to remove AdV. Considering that part of this stream is used for leisure and recreation, such contamination can put the exposed population at risk.

ACKNOWLEDGEMENTS

The authors are grateful for the financial and structural support from University Feevale, the Brazilian Coordination for the Improvement of Higher Level Personnel (CAPES), the Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS), the Brazilian National Council for Scientific Development (CNPq), and the Institute of Environmental Sanitation/ University of Caxias do Sul.

REFERENCES

ABNT – Associação Brasileira de Normas Técnicas 1987a *Planejamento de amostragem de efluentes líquidos e corpos receptores – NBR 9897*. ABNT, Rio de Janeiro.

ABNT – Associação Brasileira de Normas Técnicas 1987b *Preservação e técnicas de amostragem de efluentes líquidos e corpos receptores – NBR 9898*. ABNT, Rio de Janeiro.

Adefisoye, M. A., Nwodo, U. U., Green, E. & Okoh, A. L. 2016 Quantitative PCR detection and characterisation of human adenovirus, rotavirus and hepatitis A virus in discharged effluents of two wastewater treatment facilities in the Eastern Cape, South Africa. *Food Environ. Virol.* **8** (4), 262–274.

Aw, T. G. & Gin, K. H. 2011 Prevalence and genetic diversity of waterborne pathogenic viruses in surface waters of tropical urban catchments. *J. Appl. Microbiol.* **110** (4), 903–914.

Barrios, J. C., Russomando, G. & Espínola, E. E. 2016 Diversidad de adenovirus detectados en niños menores de 5 años hospitalizados por infección respiratoria aguda baja en Paraguay, 2010–2013. *Pediatría (Asunción)* **43** (2), 115–122.

Bibby, K. & Peccia, J. 2013 Prevalence of respiratory adenovirus species B and C in sewage sludge. *Environ. Sci. Process Impac.* **15** (2), 336–338.

Bosch, A., Guix, S., Sano, D. & Pinto, R. M. 2008 New tools for the study and direct surveillance of viral pathogens in water. *Curr. Opin. Biotechnol.* **19** (3), 295–301.

Filho, E. P., Faria, N. R. C., Fialho, A. M., Assis, R. S., Almeida, M. M. S., Rocha, M., Galvão, M., Santos, F. B., Barreto, M. L. & Leite, J. P. G. 2007 Adenoviruses associated with acute gastroenteritis in hospitalized and community children up to 5 years old in Rio de Janeiro and Salvador, Brazil. *J. Med. Microbiol.* **56** (3), 313–319.

Fumian, T. M., Leite, J. P. G., Castello, A. A., Gaggero, A., Caillou, M. S. L. & Miagostovich, M. P. 2010 Detection of rotavirus A in sewage samples using multiplex qPCR and an evaluation of the ultracentrifugation and adsorption-elution methods for virus concentration. *J. Virol. Methods* **170**, 4246.

García, M. E. 2006 Virus en águas de consumo. *Higiene y Sanidad Ambiental* **6**, 173–189.

Ghebremedhin, B. 2014 Human adenovirus: viral pathogen with increasing importance. *Eur. J. Microbiol. Immunol.* **4** (1), 26–33.

Hata, A., Katayama, H., Kojima, K., Sano, S., Kasuga, I., Kitajima, M. & Furumai, H. 2014 Effects of rainfall events on the occurrence and detection efficiency of viruses in river water impacted by combined sewer overflows. *Sci. Total Environ.* **468–469**, 757–763.

Heller, L., Colosimo, E. A. & Antunes, C. M. D. F. 2003 Environmental sanitation conditions and health impact: a case-control study. *Rev. Soc. Bras. Med. Trop.* **36** (1), 41–50.

Hemmi, S., Vidovszky, M. Z., Ruminska, J., Ramelli, S., Decurtins, W., Greber, U. F. & Harrach, B. 2011 Genomic and phylogenetic analyses of murine adenovirus 2. *Virus Res.* **160** (1), 128–135.

Jiang, S. C. 2006 Human adenoviruses in water: occurrence and health implications: a critical review. *Environ. Sci. Technol.* **40** (23), 7132–7140.

Kidd, A. H., Erasmus, M. J. & Tiemessen, C. T. 1990 Fiber sequence heterogeneity in subgroup F adenoviruses. *Virology* **179** (1), 139–150.

Kimura, M. 1980 A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16** (2), 111–120.

Klempa, B., Krüger, D. H., Auste, B., Stanko, M., Krawczyk, A., Nickel, K. F., Überla, K. & Stang, A. 2009 A novel cardiotropic murine adenovirus representing a distinct species of mastadenoviruses. *J. Virol.* **83** (11), 5749–5759.

Kundu, A., Mcbride, G. & Wuertz, S. 2013 Adenovirus-associated health risks for recreational activities in a multi-use coastal watershed based on site-specific quantitative microbial risk assessment. *Water Res.* **47** (16), 6309–6325.

Kuo, H. W., Chen, L. Z. & Shih, M. H. 2015 High prevalence of type 41 and high sequence diversity of partial hexon gene of human adenoviruses in municipal raw sewage and activated sludge. *J Appl. Microbiol.* **119** (4), 1181–1195.

La Rosa, G., Della Libera, S., Petricca, S., Iaconelli, M., Donia, D., Saccucci, P., Cenko, F., Xhelilaj, G. & Divizia, M. 2015 Genetic diversity of human adenovirus in children with acute gastroenteritis, Albania, 2013–2015. *Biomed Res. Int.* **15**, 1–8.

Li, Y., Ge, X., Zhang, H., Zhou, P., Zhu, Y., Zhang, Y., Yuan, J., Wang, L. F. & Shi, Z. 2010 Host range, prevalence, and genetic diversity of adenoviruses in bats. *J. Virol.* **84** (8), 3889–3897.

Lodder, W. J., Schijven, J. F., Rutjes, S. A., de Roda Husman, A. M. & Teunis, P. F. M. 2015 Entero-and parechovirus distributions in surface water and probabilities of exposure to these viruses during water recreation. *Water Res.* **75**, 25–32.

Maurer, C. P., Simonetti, A. B., Staggemeier, R., Rigotto, C., Heinzelmann, L. S. & Spilki, F. R. 2015 Adenovirus, enterovirus and thermotolerant coliforms in recreational waters from Lake Guaíba beaches, Porto Alegre. *J. Water Health* **13** (4), 1123–1129.

Nguyen, T. T., Nery, J. P., Joseph, S., Rocha, C. E., Carney, G. E., Spindler, K. R. & Villarreal, L. P. 1999 Mouse adenovirus (MAV-1) expression in primary human endothelial cells and generation of a full-length infectious plasmid. *Gene Ther.* **6** (7), 1291–1297.

Ogorzaly, L., Bertrand, I., Paris, M., Maul, A. & Gantzer, C. 2010 Occurrence, survival, and persistence of human adenoviruses and F-specific RNA phages in raw groundwater. *Appl. Environ. Microbiol.* **76** (24), 8019–8025.

Ogorzaly, L., Walczak, C., Galloux, M., Etienne, S., Gassiloud, B. & Cauchie, H. M. 2015 Human adenovirus diversity in water samples using a next-generation amplicon sequencing approach. *Food Environ. Virol.* **7** (2), 112–121.

Paesi, S. & Magrini, F. E. 2015 Incidência de doença diarreica aguda em Caxias do Sul, Rio Grande do Sul, Brasil, em uma série histórica de 10 anos. *Sci. Med.* **25** (2), 1–8.

Prado, T. & Miagostovich, M. P. 2014 Environmental virology and sanitation in Brazil: a narrative review. *Cad. Saúde Pública* **30** (7), 1367–1378.

Rigotto, C., Victoria, M., Moresco, V., Kolesnikovas, C. K., Corrêa, A. A., Souza, D. S. M., Miagostovich, M. P., Simões, C. M. O. & Barardi, C. R. M. 2010 Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. *J. Appl. Microbiol.* **109** (6), 1979–1987.

Robinson, M., Li, B., Ge, Y., Ko, D., Yendluri, S., Harding, T., VanRoey, M., Spindler, K. R. & Jooss, K. 2009 Novel immunocompetent murine tumor model for evaluation of conditionally replication-competent (oncolytic) murine adenoviral vectors. *J. Virol.* **83** (8), 3450–3462.

Robinson, C. M., Singh, G., Lee, J. Y., Dehghan, S., Rajaiya, J., Liu, E. B., Yousuf, M. A., Betensky, R. A., Jones, M. S., Dyer, D. W., Seto, D. & Chodosh, J. 2013 Molecular evolution of human adenoviruses. *Sci. Rep.* **3** (1812), 1–7.

Rodrigues, M. T., Henzel, A., Staggemeier, R., De Quevedo, D. M., Rigotto, C., Heinzelmann, L. & Spilki, F. R. 2015 Human adenovirus spread, rainfalls, and the occurrence of gastroenteritis cases in Brazilian basin. *Environ. Monit. Assess.* **187** (11), 1–12.

Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4** (4), 406–425.

Santos, N. S. O. & Soares, C. C. 2015 Viroses entéricas. In: *Virologia Humana* (N. S. O. Santos, M. T. V. Romanos & M. D. Wigg Coord.) Guanabara Koogan, Rio de Janeiro, pp. 209–215.

Silva, H. D., Santos, S. F., Lima, A. P., Silveira-Lacerda, E. P., Anunciação, C. E. & Garcíazapata, M. T. 2011 Correlation analysis of the seasonality of adenovirus gene detection and water quality parameters based on yearly monitoring. *Water Qual. Expo. Health.* **3** (2), 101–107.

Sinclair, R. G., Jones, E. L. & Gerba, C. P. 2009 Viruses in recreational water-borne disease outbreaks: a review. *J. Appl. Microbiol.* **107** (6), 1769–1780.

Spilki, F. R. 2015 Crise hídrica, saúde e parâmetros de qualidade microbiológica da água no Brasil. *Revista USP* **106**, 71–78.

Spilki, F. R., Oliveira, L. K., Dalla Vecchia, A., Comerlato, J., Freza, R. & Silva, J. V. S. 2009 *Detecção e desinfecção de vírus em dejetos de ruminantes.* Revista Conhecimento Online, Novo Hamburgo, v. 1. Available from: http://aplicweb.feevale.br/site/files/documentos/pdf/32677.pdf.

Staggemeier, R., Heck, T. M., Demoliner, M., Ritzel, R. G., Röhnelt, N. M., Girardi, V., Venker, C. A. & Spilki, F. R. 2017 Enteric viruses and adenovirus diversity in waters from 2016 Olympic venues. *Sci. Total Environ.* **586**, 304–312.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011 MEGA5: molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28** (10), 2731–2739.

Weinberg, J. B., Stempfle, G. S., Wilkinson, J. E., Younger, J. G. & Spindler, K. R. 2005 Acute respiratory infection with mouse adenovirus type 1. *Virology* **340** (2), 245–254.

Wieczorek, M., Krzystoszek, A. & Witek, A. 2015 Species-specific identification of human adenoviruses in sewage. *Polish J. Microbiol.* **64** (1), 23–28.

Wong, K. & Xagoraraki, I. 2010 Quantitative PCR assays to survey the bovine adenovirus levels in environmental samples. *J. Appl. Microbiol.* **109** (2), 605–612.

Yates, M. V., Malley, J., Rochelle, P. & Hoffman, R. 2006 Effect of adenovirus resistance on UV disinfection requirements: a report on the state of adenovirus science. *J. Am. Water Works Assoc.* **98** (6), 93–106.

First received 27 June 2017; accepted in revised form 5 October 2017. Available online 3 November 2017

ARTIGO 2

Science of the Total Environment 651 (2019) 298–308

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microbial risk assessment in recreational freshwaters from southern Brazil

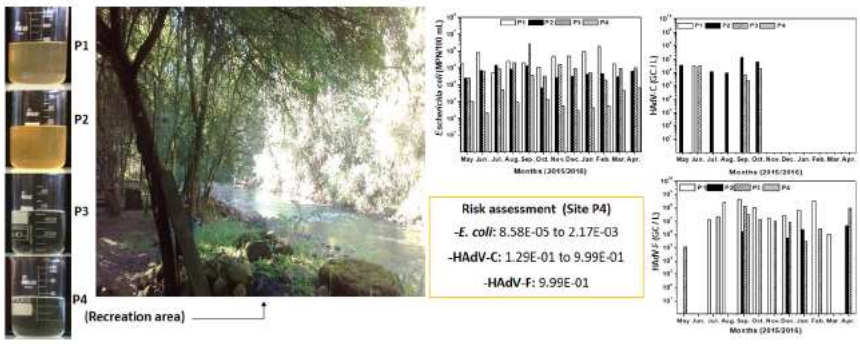
Viviane Girardi [a,*], Kristina D. Mena [b], Suelen M. Albino [c], Meriane Demoliner [a], Juliana S. Gularte [a], Fernanda G. de Souza [d], Caroline Rigotto [a], Daniela M. Quevedo [e], Vania E. Schneider [f], Suelen O. Paesi [g], Patrick M. Tarwater [h], Fernando R. Spilki [a]

[a] Laboratório de Microbiologia Molecular, Universidade Feevale, ERS 239, no 2755, Novo Hamburgo, RS 93352-000, Brazil
[b] School of Public Health, The University of Texas Health Science Center at Houston, El Paso, TX 79902, USA
[c] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Ramiro Barcelos, no 2600, Porto Alegre, RS, Brazil
[d] Laboratório de Vírus, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, no 6627, Belo Horizonte, MG, Brazil
[e] Programa de Pós-Graduação em Qualidade Ambiental, Universidade Feevale, ERS 239, no 2755, Novo Hamburgo, RS 93352-000, Brazil
[f] Instituto de Saneamento Ambiental (ISAM), Universidade de Caxias do Sul, Rua Francisco Getúlio Vargas, no 1130, Caxias do Sul, RS 95070-560, Brazil
[g] Laboratório de Diagnóstico Molecular, Instituto de Biotecnologia, Universidade de Caxias do Sul, Rua Francisco Getúlio Vargas, no 1130, Caxias do Sul, RS 95070-560, Brazil
[h] School of Public Health, Department of Biostatistics, The University of Texas Health Science Center at Houston, El Paso, TX 79902, USA

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



ARTICLE INFO

Article history:
Received 20 July 2018
Received in revised form 12 September 2018
Accepted 13 September 2018
Available online 14 September 2018

Editor: Paola Verlicchi

Keywords:
Adenovirus
Escherichia coli
QMRA
Recreational waters
qPCR
Viral infectivity

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

* Corresponding author.
E-mail address: 0178103@feevale.br (V. Girardi).

https://doi.org/10.1016/j.scitotenv.2018.09.177

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.

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 *Pirconaviridae* 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 awater 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 km^2 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.

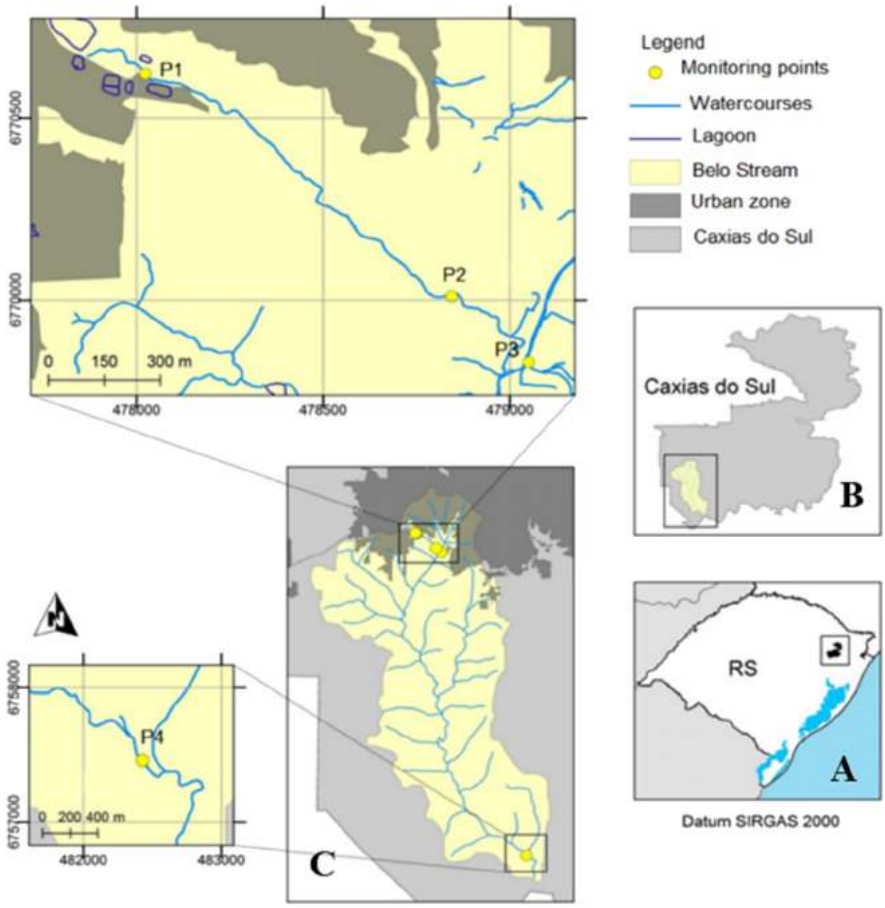


Fig. 1. (A) Map of Rio Grande do Sul – Brazil, in which the location of the city of Caxias do Sul is highlighted. (B) Map of Caxias do Sul, in which the outline and location of Belo Stream is highlighted. (C) Distribution of the sampling points along Belo Stream.

2.2. Coliform analysis

Tests for the detection and quantification of total coliforms (TC) and *E. coli* were performed by the Colilert® substrate enzyme method (Idexx®, USA) according to the instructions described by the manufacturer. All samples were assayed within 24 h after collection. The results were considered positive for total coliforms based on the yellow color indicator and positive for *E. coli* if fluorescing blue following exposure to 300 nm UV light. The test was considered negative in the absence of color and/or fluorescence. Results were expressed as most probable number in 100 mL of water (MPN/100 mL) according to the reference table provided by the manufacturer.

2.3. Viral concentration, DNA and RNA extraction and cDNA synthesis

The samples were concentrated by ultracentrifugation (concentrated samples = CS), in accordance with the Girardi et al. (2018) protocol. Briefly, from each 500-mL sample, 36 mL were concentrated by ultracentrifugation at 41,000 ×*g* at 8 °C for 3 h. Thereafter, the precipitate was resuspended in 2 mL of Tris-EDTA buffer (pH 8.0), and vigorously homogenized in a vortex for 1 min. The resuspended samples were aliquoted and stored in microtubes at −80 °C until the genetic material extraction process. Unconcentrated samples (US) were aliquoted (1.5 mL) into microtubes and stored at −80 °C for infectivity analyses. For the US, the extraction was performed the day after sampling.

Nucleic acids were extracted using a commercial kit (BioPur®, Mobius Life Science, Brazil) from an initial volume of 200 μL according to the manufacturer's instructions. The final elution was performed in microtubes free of DNAse/RNAse and maintained at −80 °C. For the RV and EV analyses, after extraction step the cDNA synthesis was carried out with the commercial High Capacity cDNA synthesis kit (Applied Biosystems, USA) according to the instructions described by the manufacturer.

2.4. Rotavirus and enterovirus detection

The tests for EV and RV detection were performed by polymerase chain reaction (PCR), following the cDNA step. Reaction measurements were carried out for a final volume of 50 μL as follows: 1 μL of primers ENT-F1 5′CCTCCGGCCCCTGAATG3′, ENT-R2 5′ACACGGACACCCAAAG TAG3′ (Tsai et al., 1993; Vecchia et al., 2012), RV-FW 5′GATGTCCTGTA CTCCTTGT3′ and RV-REV 5′ GGTAGATTACCAATTCCTCC3′ (Vecchia et al., 2012) for target gene 5′UTR (EV) and VP6 (RV). All primers with 20 pmol, 18 μL of DNAse/RNAse free water, 25 μL of GoTaq® Green Master Mix (Promega, USA), and 5 μL of nucleic acid were extracted from each sample. Amplification was performed in a Bio-Rad® thermal cycler (USA) with the following conditions: EV - 98 °C for 5 min, 35 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min; and RV - 94 °C for 5 min, 40 cycles of 94 °C for 1 min, 54 °C for 1 min (which was decreased by 0.5 °C at each of the 39 subsequent cycles), and 72 °C for 1 min (Vecchia et al., 2012).

PCR products were analyzed on 2% agarose gel with 0.5 mg of ethidium bromide/mL added, and a running time of 60 min at 70 V. Molecular size of the products was compared to a 100 bp DNA standard (Ludwig brand). The bands stained with ethidium bromide were visualized in UV light; subsequently, images were photographed with an Easy Doc 200 UV transilluminator.

2.5. Detection of human mastadenovirus from groups C and F

Detection of HAdV genomes of groups C and F was performed by quantitative real-time polymerase chain reaction (qPCR) in accordance with the manufacturer's instructions and protocols previously standardized in the laboratory (Vecchia et al., 2015). For this purpose, primers were used that partially amplify the hexon capsid protein region according to Wolf et al. (2010): HAdV-C: VTB2 - HAdVCf – 5′ GAGACGTACTTCAGCCTGAAT3′; VTB2 -HAdVCr -5′GATGAACCGCAGCG TCAA3′; HAdV-F: VTB1 - HAdVFf - 5′GCCTGGGGAACAAGTTCAA3′; VTB1 – HAdVFr - 5′GCGTAAAGCGCACTT TGTAAG3′. The 25-μL reaction mix was composed of: 12.5 μL of GoTaq® qPCR Master Mix (Promega, USA), 1 μL of each primer (20 pmoles), 5.5 μL ultrapure water (RNAse/DNAse free water), and 5 μL of the nucleic acid extracted from each sample. Analyses were performed using StepOne (Applied Biosystems, USA) real-time PCR systems. Assays occurred in duplicates in 48-well plates, including negative controls and a standard curve formed by positive controls with serial dilutions and known quantification. Each reaction was composed of a denaturation cycle at 95 °C for 10 min, followed by 45 cycles composed of one step at 95 °C for 20 s, and a combined annealing/extension step at 55 °C for 1 min (sensitivity of 6.2×10^1 GC (Genomic Copies)/5 μL). The fluorescence data were collected during the annealing/extension step. A denaturing curve was used to check the specificity of the amplification products (melting step between 55 and 95 °C, ±1 °C).

2.6. Integrated cell culture-qPCR assay and viral isolation

ICC-qPCR (integrated cell culture-qPCR) method is often performed to analyze viral infectivity using permissive cell cultures. Further characterization of the virus is performed by the detection of nucleic acids by qPCR. For the assay, the A549 cell line (human carcinoma cells) was cultured using a 24-well plate maintained with *E*-MEM Medium (Eagle's Minimum Essential Medium) supplemented with 10% Bovine Fetal Serum, 1% antibiotic (Penicillin-Streptomycin), at 37 °C and 5% CO_2. Cell monolayers were used after they reached at least 80% confluence. After incubation, the US and CS (US from May 2015 were not analyzed) were diluted 1:1 with *E*-MEM, membrane-filtered using 0.22 μM filters, and inoculated in the cell cultures. The inoculum remained in contact with the cells for 1 h under standard conditions and uniform shaking every 15 min. The volume of each well was removed and 1 mL of E-MEM containing 1% antibiotic was added. The plate was incubated for 48 h at 37 °C. After this time, the plate was frozen (−80 °C) and thawed (room temperature) three times in order to lyse the cells and release the viral contents. A 200-μL volume of the final lysate was subjected to DNAse treatment and subsequent DNA extraction, while the remainder of the sample (800 μL) was aliquoted and frozen for future viral passages with the aim of performing viral isolation.

For viral isolation, the conditions were the same as described above, with the difference in incubation time of five days. Plates underwent three cycles of freezing and thawing to produce the inoculum of the next pass. After two passages over five days, a new ICC-qPCR assay was performed with the final lysate, as described in the previous paragraph, totaling four passages of the samples in cells.

The resulting lysate from each well of the first and last ICC-qPCR assay was subjected to DNAse (200 μL) treatment. Treatment with DNAse prior to DNA extraction removes nucleic acids devoid of capsid. The DNAse assay was performed with the RQ1 (RNA-Qualified) RNAse Free DNAse kit (Promega, USA), following a protocol adapted for environmental samples. Briefly, 4 μL of DNAse and 36 μL buffer in the 1:10 ratio were added to 200 μL of sample, and incubated at 37 °C for 30 min. At the end, 240 μL of 30 mM EDTA solution was added, followed by incubation at 75 °C for 10 min to inactivate the enzyme. Samples were then stored at 80 °C for further extraction and qPCR, as described in Sections 2.3 and 2.5, respectively.

2.7. Statistical analysis

Initially Kolmogorov-Smirnov's test was performed to verify if the data obtained (both virological and bacteriological) had normal distribution. Thus, the results obtained showed that the data did not present normal distribution. Based on this result, non-parametric multiple comparisons (Kruskal Wallis) tests were applied, followed by Dunn post-hoc. A hierarchical cluster analysis was performed to confirm the distance/similarity between the collection points identified with the multivariate analysis, considering together all the variables of the study. To investigate the correlation between the virological agents (HAdV-C and HAdV-F) and bacteriological (*E. coli* and total coliforms) Spearman's correlation were used. *P* values < 0.05 were considered as statistically significant. Statistical analyzes were performed using SPSS 24.0 software.

2.8. Quantitative microbial risk assessment

QMRA was applied to estimate health risks associated with exposure to waterborne *E. coli* and HAdV C and F within the recreation area of the stream (site P4). Appropriate dose-response parameters from the peer-reviewed literature were applied (parameters used to estimate the risk of infection are shown in the supplemental file in Tables 1 and 2 for *E. coli* and HAdV, respectively). For HAdVs, infection probabilities (P_{inf}) were calculated using the exponential model for adenovirus provided in Table 1 (Couch et al., 1966; Rose et al., 1996; Haas et al., 1999). For *E. coli*, the P_{inf} was calculated using the beta-Poisson model (Table 1) (DuPont et al., 1971; Haas et al., 1999). Risk calculations were performed using Microsoft Excel 2013.

3. Results

3.1. Evaluation of the presence and quantification of the total coliforms, Escherichia coli, and enteric viruses per site and month of collection

A total of 47 samples were evaluated, all of them positive for coliforms with quantification ranging from 4.87E + 03 to >2.42E + 06 MPN/100 mL for TC (Fig. 2) and 2.00E + 02 and >2.42E + 06 MPN/100 mL for *E. coli* (Fig. 3). In the TC analyses, the values of the arithmetic averages were: 1.34E + 06, 4.12E + 05, 3.76E + 05 and 3.39E + 04 MPN/100 mL, respectively for P1, P2, P3 and P4; and the values of the geometric averages were 8.59E + 05, 2.20E + 05, 1.86E + 05 and 2.30E + 04 MPN/100 mL, respectively for P1, P2, P3 and P4 (Supplemental file -Tables 3, 4, 5 and 6, respectively for P1, P2, P3 and P4). When considering both arithmetic and geometric averages by collection point, site P1 showed higher TC concentrations, while P4 had lower values. It is important to note that some results associated with P1 to P3 were above the detection limit of the method. In order to calculate both averages, the value of <2419.6 was multiplied by the dilution factor of 10^{-2} and 10^{-3}. The months that exceeded the detection limits were P1 [May, August, September, October, December and February (Supplemental file -Table 3)], P2

Table 1
Dose-response models used for quantitative microbial risk assessment in the present study.

Microorganism	Dose-response models	References
Adenovirus	$P(response) = 1 - \exp(rN)$	Couch et al., 1966; Rose et al., 1996 Haas et al., 1999
Escherichia coli	$P(response) = 1-[1 + dose\frac{(2^{\frac{1}{\alpha}}-1)}{N_{50}}^{-\alpha}]$	DuPont et al., 1971; Haas et al., 1999

$P_{response}$ = probability of infection; $r = 0.4172$; N = number of ingested or inhaled microorganisms; $\alpha = 0.155$; $N_{50} = 2.11E + 06$.

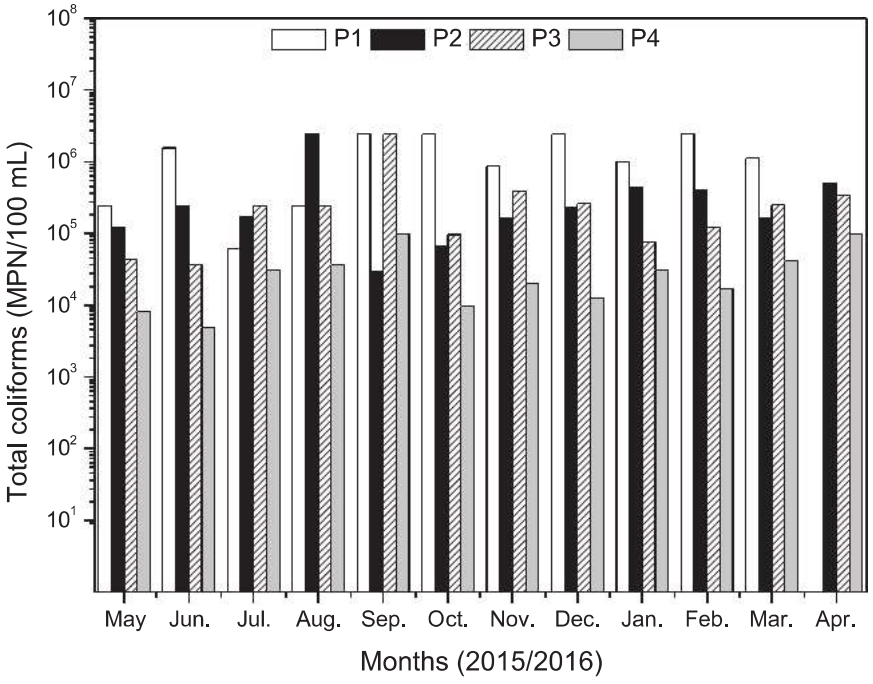


Fig. 2. Total coliform counts expressed in most probable number per 100 mL (MPN/100 mL) per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

[June and August (Supplemental file -Table 4)], and P3 [August and September (Supplemental file -Table 5)].

The *E. coli* results are shown in Fig. 3. The values of the arithmetic averages were: 4.86E + 05, 5.60E + 04, 2.75E + 05 and 4.64E + 03 MPN/100 mL for P1, P2, P3 and P4, respectively, and the values of the geometric averages were 3.02E + 05, 4.25E + 04, 8.68E + 04 and 1.35E + 03 MPN/100 mL for P1, P2, P3 and P4, respectively (Supplemental file -Tables 3, 4, 5 and 6, respectively for P1, P2, P3 and P4). Thus, as previously described for TC, when considering both arithmetic and geometric means, P1 had higher concentrations and P4 showed lower values. In this group of results, P1 and P3 exceeded the detection limits of the method in the months of August and September, respectively. As for the TC analysis, in order to calculate both *E. coli* averages, the value of <2419.6 was multiplied by the dilution factor, which was 10^{-2} and 10^{-3}, respectively, in the months of August (P1) and September (P3) (Supplemental file -Tables 3 and 5, respectively for P1 and P3).

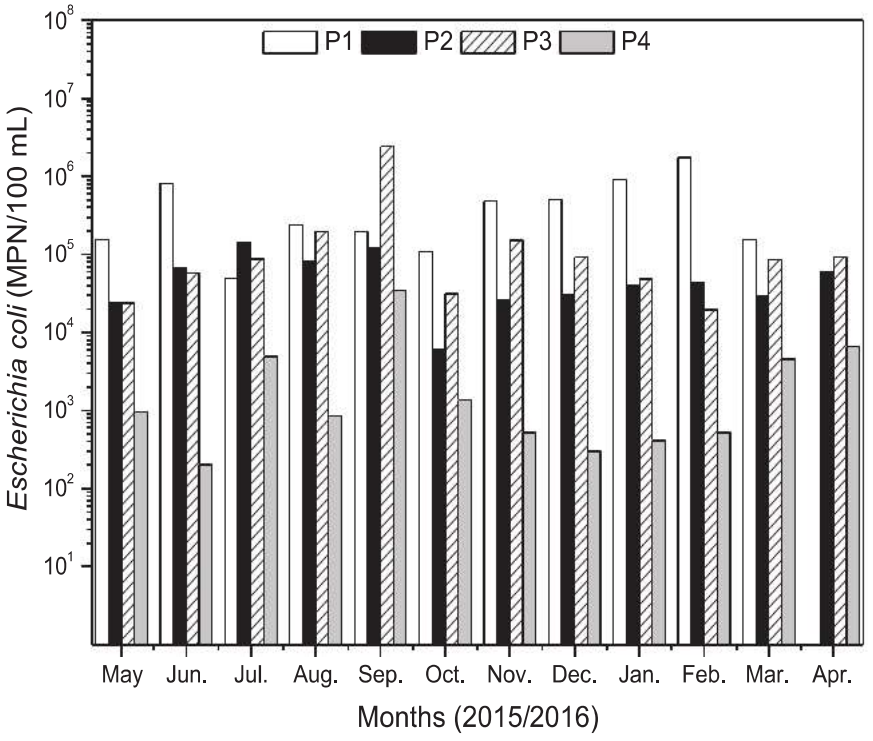


Fig. 3. *Escherichia coli* counts expressed in most probable number per 100 mL (MPN/100 mL) per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

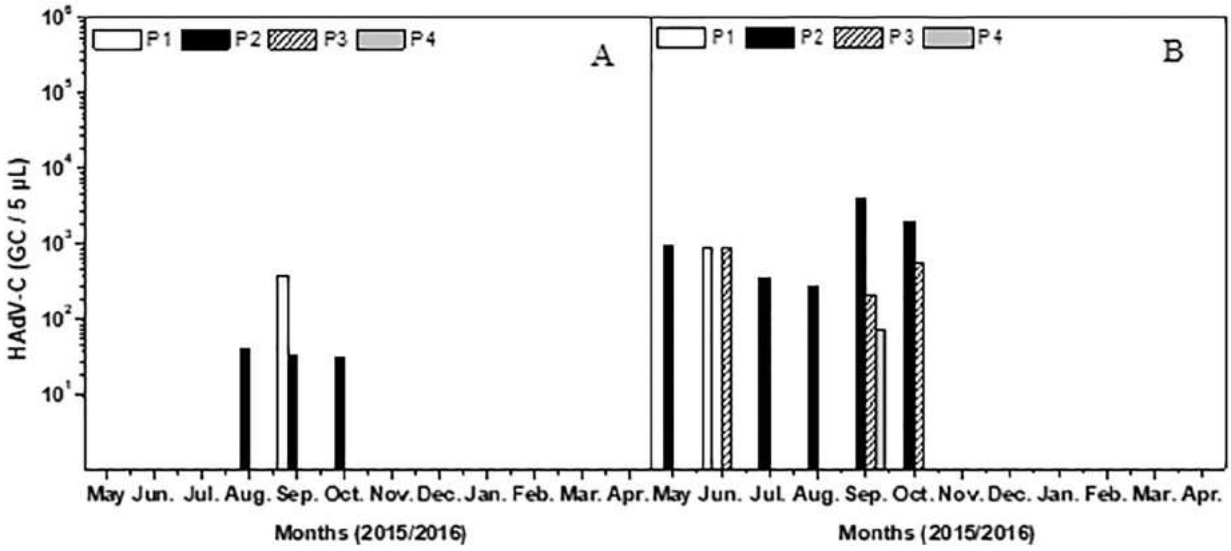


Fig. 4. Genomic copies per liter (GC/L) of *Human mastadenovirus* C (HAdV-C) per month of collection in samples from the Belo Stream, Caxias do Sul, Brazil. A- Unconcentrated samples; B- Concentrated samples.

No positive samples were detected for the presence of RV and EV. The results for HAdV-C are presented in Fig. 4, and show that for both US and CS, site P2 for September had a higher number of positive samples. P1 presented a lower number of positive samples and from November, no viral DNA was detected. For US (Fig. 4A), four positive samples (4/47–8.51%) were detected and at sites P3 and P4, no HAdV-C DNA was detected. At site P1, only one positive sample (2.28E + 07 GC/L - September) was detected; alternatively, three samples were positive at site P2 with arithmetic average of 5.19E + 05 and geometric average of 2.06E + 06 GC/L (Fig. 4A and Supplemental file -Tables 3, 4, 5 and 6, respectively for P1, P2, P3 and P4). For CS (Fig. 4B), HAdV-C DNA in 10 samples (10/47–21.27%) were detected with viral loads ranging from 2.36E + 05 to 1.29E + 07 GC/L (Fig. 4B and Supplemental file -Tables 3, 4, 5 and 6, respectively for P1, P2, P3 and P4). The values of the arithmetic averages were: 2.62E + 05, 2.02E + 06, 4.40E + 05 and 1.96E + 04 GC/L for sites P1, P2, P3 and P4, respectively; and the values of the geometric averages were: 3.00E + 06 and 1.50E + 06 GC/L for P2 and P3, respectively. In comparison when considering the arithmetic average, lower viral load values were observed at site P4. Alternatively, site P2 presented higher values for both arithmetic and geometric mean. However, it should be noted that at sites P1 and P4, HAdV-C DNA was detected in only one sample from each point, corresponding to the months of June and September, respectively (Fig. 4B and Supplemental file -Tables 3, 4, 5 and 6, respectively for P1, P2, P3 and P4).

A greater number of positive samples were detected for HAdV-F in both CS and in US at site P1 (Fig. 5 and Supplemental file -Table 3). At sites P2 and P4, no HAdV-F DNA was detected in the US. As in the HAdV-C results, the month of September had the highest number of positive samples for both CS and US. For the US (Fig. 5A), HAdV-F DNA was detected in six samples (6/47–12.76%), with a viral load ranging from 2.77E + 07 to 3.31E + 08 GC/L. At site P3, only one positive sample was detected (4.58E + 07 GC/L - September). The arithmetic and geometric averages for site P1 were 5.89E + 07 and 9.52E + 07 GC/L, respectively. For CS, 23 samples were positive (23/47–48.93%), with concentrations ranging from 1.10E + 05 to 4.50E + 08 GC/L. The values

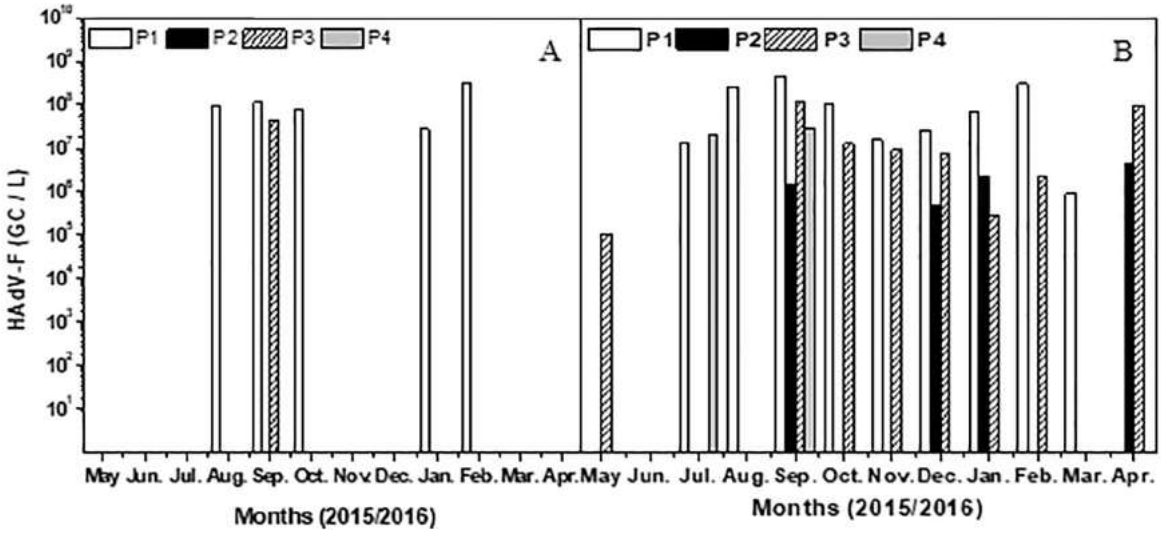


Fig. 5. Genomic copies per liter (GC/L) of *Human mastadenovirus* F (HAdV-F) per month of collection in samples from the Belo Stream, Caxias do Sul, Brazil. A- Unconcentrated samples; B- Concentrated samples.

Table 2
Viral loads measured by ICC-qPCR assays, before and after viral isolation in unconcentrated samples from the Belo Stream, Caxias do Sul, Brazil.

Collection sites	1st ICC-qPCR (GC/5 μL)		2nd ICC-qPCR (GC/5 μL)	
	HAdV-C	HAdV-F	HAdV-C	HAdV-F
P1	2/10–20.20% (8.80E + 01–2.66E + 04)	1/10–10.00% (2.13E + 02)	2/10–20.20% (1.08E + 05–2.02E + 06)	ND
P2	5/11–45.45% (1.06E + 02–1.54E + 04)	1/11–9.09% (9.58E + 02)	3/11–27.27% (2.30E + 04–8.63E + 04)	2/11–18.18% (1.10E + 02–7.32E + 02)
P3	4/11–36.36% (2.06E + 02–1.63E + 04)	ND	3/11–27.27% (2.97E + 04–3.10E + 04)	ND
P4	5/11–45.45% (2.39E + 03–1.87E + 04)	1/11–9.09% (1.58E + 06)	3/11–27.27% (3.37E + 04–1.07E + 05)	1/11–9.09% (8.29E + 02)
TOTAL	16/43–37.21%	3/43–6.97%	11/43–25.58%	3/43–6.97%

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected. The provide values represent lower and higher viral loads.

of the arithmetic averages were 1.13E + 08, 7.40E + 05, 2.11E + 07 and 4.11E + 06 GC/L, respectively for sites P1, P2, P3 and P4. The geometric means were 4.81E + 07, 1.68E + 06, 5.42E + 06 and 2.43E + 07 GC/L, respectively for P1, P2, P3 and P4 (Supplemental file -Tables 3, 4, 5 and 6, respectively for P1, P2, P3 and P4).

3.2. Evaluation of infectivity of human mastadenovirus per site of collection

The evaluation for HAdV infectivity of groups C and F were performed for CS collected from May 2015 to April 2016, totaling 47 samples. For the US, analyses were performed for sampling from June 2015 to April 2016, totaling 43 samples. Two viral infectivity tests were performed: a first assay before and another assay after two passages of the samples into cells in an attempt to perform viral isolation. In the results obtained for HAdV-C for US (Table 2), 37.20% (16/43) and 25.58% (11/43) for the 1st and 2nd ICC-qPCR, respectively, were detected as infectious. The viral loads ranged from 8.80E + 01 (P1 - July) to 2.66E + 04 GC/5 μL (P1 - March) for 1st ICC and 2.30E + 04 (P2 - February) to 2.02E + 06 GC/5 μL (P1 – March) for the 2nd ICC. At the 1st ICC-qPCR for sites P2 and P4, a greater quantity of infectious samples (5/11) were detected. Furthermore, in July all samples collected were positive for infectivity. At the 2nd ICC-qPCR in February, March and April, all samples presented viral viability (Supplemental file -Tables 7, 8, 9 and 10, respectively for P1, P2, P3 and P4).

Table 2 shows the results of the HAdV-F infectivity analysis for US. In the 43 samples evaluated, 6.97% (3/43) were determined to be infectious - both for the 1st and 2nd ICC-qPCR. In the 1st ICC-qPCR, the positive samples correspond to sites P1 (March), P2 (June) and P4. At site P4, a sample collected in January showed a higher quantification at 1.58E + 06 GC/5 μL. At the 2nd ICC, at site P2, a greater number of infectious samples (18.18% - 2/11, December and April) were detected with a lower quantification detected (1.10E + 02 GC/5 μL - April) (Supplemental file -Tables 7, 8, 9 and 10, respectively for P1, P2, P3 and P4).

The results of the infectivity analysis for CS are presented in Table 3. For HAdV-C, from 47 samples evaluated, 17.02% (8/47) and 8.51% (4/47) were detected as infectious, for the 1st and 2nd ICC-qPCR, respectively. The highest values were 2.21E + 04 GC/5 μL (P3 - August) and 2.20E + 03 GC/L (P1 - February) for the 1st and 2nd ICC-qPCR, respectively. The lowest values were 1.84E + 02 GC/5 μL (P1 - February) and 2.05E + 02 GC/5 μL (P3 - March) for the 1st and 2nd ICC-qPCR, respectively. In the comparison by collection point - at P2 and P3 - a greater number of infectious samples (25% - 3/12) were observed with the 1st ICC-qPCR (Supplemental file -Tables 11, 12, 13 and 14, respectively for P1, P2, P3 and P4).

For the analysis of CS for the detection of infectivity of HAdV-F (Table 3), 8.51% (4/47) of infectious samples were found in both the 1st and 2nd ICC-qPCR. The highest values were 1.10E + 05 (P3 - August) and 1.20E + 03 GC/5 μL (P3 - December), for the 1st and 2nd ICC-qPCR, respectively. However, the values of lower quantifications were 9.73E + 01 (P2 - December) and 9.27E + 01 GC/5 μL, for 1st and 2nd ICC-qPCR, respectively (Supplemental file -Tables 11, 12, 13 and 14, respectively for P1, P2, P3 and P4).

3.3. Statistical analysis of collection sites

Regarding the results obtained from the molecular analyzes for HAdV-C (Table 4), no significant difference between the sites was observed. The X^2 values were 6.030 and 5.891 for US and CS, respectively, and the *P* values were 0.110 and 0.117 for US and CS, respectively. On the other hand, when HAdV-F, total coliforms and *E. coli* were analyzed (Table 4), a significant difference was observed. Application of the Dunn post-hoc test indicated a significant difference for HAdV-F (US and CS) between site P1 and sites P2, P3 and P4, but P2, P3 and P4 did not differ. Fig. 6 provide cluster analysis, highlighting P1, and grouping P2, P3 and P4 into a cluster of similarity. For total coliforms and *E. coli* only sites P2 and P3 do not differ. Regarding the ICC-qPCR data (Table 5), no significant difference was observed between the sites. Values of X^2 ranged from 0.028 and 3.564, and P values ranged from 0.313 to 0.999.

3.3.1. Statistical correlation analysis between HAdVs with Escherichia coli and total coliforms

Spearman general non-parametric correlation analyzes (grouping the 4 sites) were performed for the molecular results obtained for HAdV-C and HAdV-F with *Escherichia coli* and TC. The analysis showed that there is a significant correlation between HAdV-F (US and CS) (Table 6) with TC and *E. coli*, and the correlation between the variables is direct.

3.4. QMRA for Escherichia coli and HAdV of groups C and F

For the QMRA, health risks associated with the recreational area of the stream (site P4) were estimated (parameters used to estimate the risk of infection are shown in the supplemental file in Tables 1 and 2,

Table 3
Viral loads measured by ICC-qPCR assays, before and after viral isolation in ultracentrifuge-concentrated samples from the Belo Stream, Caxias do Sul, Brazil.

Collection sites	1st ICC-qPCR (GC/5 μL)		2nd ICC-qPCR (GC/5 μL)	
	HAdV-C	HAdV-F	HAdV-C	HAdV-F
P1	1/11–9.09% (1.84E + 02)	ND	1/11–9.09% (2.20E + 03)	1/11–9.09% (9.88E + 02)
P2	3/12–25.00% (3.01E + 02–2.75E + 03)	1/12–8.33% (9.73E + 01)	1/12–8.33% (3.60E + 02)	2/12–16.66% (9.27E + 01–1.03E + 03)
P3	3/12–25.00% (5.03E + 03–2.21E + 04)	2/12–16.66% (2.31E + 03–1.10E + 05)	1/12–8.33% (2.05E + 02)	1/12–8.33% (1.20E + 03)
P4	1/12–8.33% (9.49E + 03)	1/12–8.33% (6.00E + 02)	1/12–8.33% (4.21E + 02)	ND
TOTAL	8/47–17.02%	4/47–8.51%	4/47–8.51%	4/47–8.51%

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected. The provide values represent lower and higher viral loads.

Table 4
Statistical analyzes of the data obtained from the molecular assays in ultracentrifuge-concentrated and unconcentrated samples from the Belo Stream, Caxias do Sul, Brazil.

		Test statistics [a,b]	
		X^2	*P*
US (GC/L)	HAdV-C	6.030	0.110
	HAdV-F	14.311	0.003
CS (GC/L)	HAdV-C	5.891	0.117
	HAdV-F	15.16	0.002
	Total coliforms	26.461	0.000
	Escherichia coli	32.915	0.000

a: Kruskal Wallis Test; b: Grouping Variable: Site; US: unconcentrated samples; CS: concentrated samples GC: genomic copies; X^2:chi-square.

Table 5
Statistical analyzes of the data obtained from the ICC-qPCR assays in ultracentrifuge-concentrated and unconcentrated samples from the Belo Stream, Caxias do Sul, Brazil.

			Test statistics [a,b]	
			X^2	*P*
US (GC/5 μL)	HAdV-C	1st ICC-qPCR	1.737	0.629
		2nd ICC-qPCR	0.083	0.994
	HAdV-F	1st ICC-qPCR	1.083	0.781
		2nd ICC-qPCR	3.564	0.313
CS (GC/5 μL)	HAdV-C	1st ICC-qPCR	2.295	0.513
		2nd ICC-qPCR	0.028	0.999
	HAdV-F	1st ICC-qPCR	2.191	0.534
		2nd ICC-qPCR	2.010	0.570

a: Kruskal Wallis test; b: Grouping variable: site; US: unconcentrated samples; CS: concentrated samples GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; X^2: chi-square.

respectively, for *E. coli* and HAdV). Both daily (one-time exposure) and annual (assuming five days exposure/year) health risks were estimated for HAdV (groups C and F) and *E. coli*. For the HAdV QMRA, values were used that applied a 3-log reduction to the molecular data to address infectivity, since it would be expected that not all of the viruses detected in direct PCR (without passage in a cell line) are capable of initiating infection (Aslan et al., 2011). For viral infectivity values, a 2.0E +05 increase was applied for conversion to GC/L. These health risk predictions assume no level of water treatment has been applied (decimal reduction = 0). When assuming a 1 mL exposure volume, predicted daily and yearly health risks for *E. coli* ranged from 1.27E-05 to 2.17E-03 and 9.53E-05 to 1.08E-02, respectively (Table 7 and Supplemental file –Table 1). For HAdV-C and HAdV-F, both daily and annual risks closely approach 1 (Table 7 and Supplemental file –Table 2).

4. Discussion

In the present study, fecal contamination in the surface waters from Belo Stream (Caxias do Sul - RS - Brazil) was evaluated by detection and quantification of TC, *E. coli*, EV, RV and HAdV of groups C and F. For HAdV, infectivity was also assessed. QMRA was applied to estimate infection risks associated with *E. coli* and HAdV-C and F.

According to the CONAMA Resolution 274/2000, waters considered proper for recreation must not exceed a maximum value of 800 MPN/100 mL for *E. coli* in >80% of the samples collected. Therefore, the recreation area of the studied stream (site P4) is classified as improper for bathing, since only 41.66% of the samples had averages below the maximum allowable value (geometric of 3.67E + 02 and arithmetic of 3.90E + 02 MPN/100 ml). The general values (all samples collected) of the arithmetic mean was 4.64E + 03 MPN/100 mL and the geometric was 1.35E + 03 MPN/100 mL. These results are lower when compared to those performed by Staggemeier et al. (2017), who detected an arithmetic average of 1.10E + 04 MPN/100 mL in recreational water samples from Marina da Glória (RJ-Brazil).

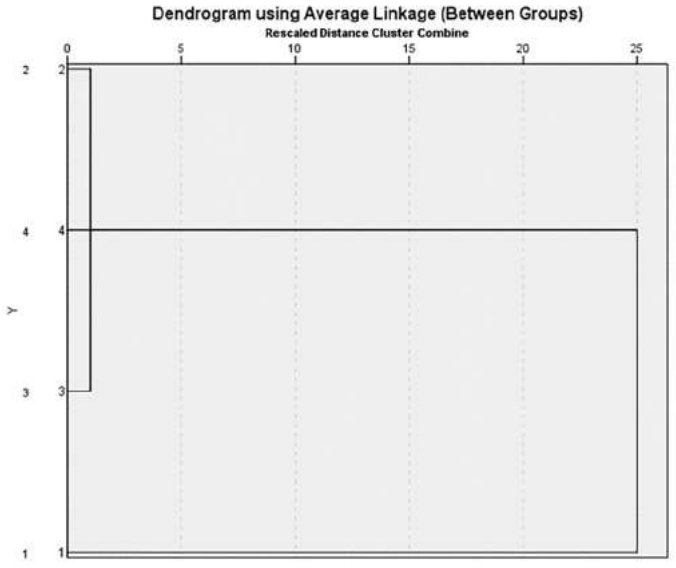


Fig. 6. Hierarchical cluster considering all variables for molecular results obtained for HAdV-F for both ultracentrifuge-concentrated and unconcentrated samples from the Belo Stream, Caxias do Sul, Brazil.

HAdV genome was present in most samples, whereas RV and EV genomes were absent in all samples evaluated. These results are similar to those reported in the literature, in which it is described that in the comparison with AdV in surface waters, the presence of EV and RV viruses is lower (Rigotto et al., 2010; Adefisoye et al., 2016; Ahmad et al., 2016; Staggemeier et al., 2017). In the case of negative results for RV, it may likely be due to the implementation of the vaccine against RV in Brazil that began in 2006, which led to reductions in the number of cases of morbidity and mortality related to diarrhea (Santos et al., 2018).

In the present study, when comparing the results of qPCR obtained for US and CS for both HAdV-C and -F, the amount of positive samples and viral loads were higher when the ultracentrifugation method was used. The use of concentration techniques has an objective to capture all of the target viruses, important tools in the microbiological analysis of water. Methods that generate quantitative data are needed so that estimates of virus concentrations in surface waters are not underestimated (Petterson et al., 2015). Further, viral concentration methods contribute to the reduction of the presence of inhibitors in water samples, which may interfere with PCR analysis (Ahmed et al., 2015).

Table 6
Statistical correlation analyzes between HAdVs with *Escherichia coli* and total coliforms of the data obtained from the molecular assays in ultracentrifuge-concentrated and unconcentrated samples from the Belo Stream, Caxias do Sul, Brazil.

			Correlations	
			Total coliforms	*Escherichia coli*
HAdV-C	US (GC/L)	Correlation coefficient	0.099	0.102
		Significance (bilateral)	0.506	0.495
		N	47	47
	CS (GC/L)	Correlation coefficient	−0.024	0.132
		Significance (bilateral)	0.874	0.378
		N	47	47
HAdV-F	US (GC/L)	Correlation coefficient	0.475**	0.503**
		Significance (bilateral)	0.001	0.000
		N	47	47
	CS (GC/L)	Correlation coefficient	0.494	0.542
		Significance (bilateral)	0.000	0.000
		N	47	47

US: unconcentrated samples; CS: concentrated samples; GC: genomic copies; N: number of samples collected; **The correlation is significant at the 0.01 level (bilateral).

Table 7
Viral and bacterial concentrations, daily and annual risk of infection per month of collection (Adjusted*).

Month	HAdV-F			HAdV-C			*Escherichia coli*		
	GC/L	Daily risk	Annual risk	GC/L	Daily risk	Annual risk	MPN/100 mL	Daily risk	Annual risk
May	ND			ND			9.70E + 02	6.16E-05	3.08E-04
June	ND			5.20E + 08	9.99E-01	9.99E-01	2.00E + 02	1.27E-05	6.36E-05
July	2.02E + 04	9.99E-01	9.99E-01	4.76E + 07	9.99E-01	9.99E-01	4.88E + 03	3.10E-04	1.55E-03
August	ND			ND			8.50E + 02	5.40E-05	2.70E-04
September	2.90E + 04	9.99E-01	9.99E-01	2.35E + 02	1.29E-01	4.99E-01	3.45E + 04	2.17E-03	1.08E-02
October	ND			6.78E + 08	9.99E-01	9.99E-01	1.35E + 03	8.58E-05	4.29E-04
November	ND			ND			5.20E + 02	3.30E-05	1.65E-04
December	ND			ND			3.00E + 02	1.91E-05	9.53E-05
January	1.20E + 08	9.99E-01	9.99E-01	ND			4.10E + 02	2.61E-05	1.30E-04
February	1.66E + 08	9.99E-01	9.99E-01	3.74E + 09	9.99E-01	9.99E-01	5.20E + 02	3.30E-05	1.65E-04
March	ND			8.42E + 07	9.99E-01	9.99E-01	4.55E + 03	2.89E-04	1.44E-03
April	ND			3.18E + 09	9.99E-01	9.99E-01	6.57E + 03	4.17E-04	2.08E-03

* Adjustment: The concentration (GC/L) for HAdV-C and F are the minimum value between molecular values (applying a 3-log reduction) and viral infectivity values (applying a 2.0E +05 increase). ND: not detected.

In the analysis by site of collection, P1, P2 and P3 (sites located in urbanized areas of the city) had a greater number of positive samples. This result is probably explained by the release of domestic sewage at or close to these collection sites. This variation among sampling sites is also observed in other studies, and the detection may vary according to the level of urbanization close to each site (Vecchia et al., 2015). The lower concentrations of TC and *E. coli*, and lower viral detection at site P4, may be associated with the decrease in the number of inhabitants around the site (rural area) and the self-purification capacity of Belo Stream since between sites P3 and P4 there is an altitude of approximately 500 m. Thus, this difference of altitude probably allows for streaming aeration, which improves its self-purification capacity. Dissolved oxygen, biochemical demand, and oxygen chemistry data allow a better affirmation of this hypothesis.

Data provided by the Municipal Health Department of Caxias do Sul show that the number of reports of cases of diarrheal diseases was 5724 and 6819 for the years 2015 and 2016, respectively. In the year 2015, during the period of sampling, the percentage of cases of the disease in the population was 59.38% (3399/5724), with the highest numbers recorded in August (528) and November (529). In the analysis of CS for HAdV-F (qPCR) in September of 2015, collection points had viral DNA and in December three collection points were positive. In 2016 during the sampling months, the percentage of cases of the disease was 34.90% (2380/6819), with the highest number of cases reported in February (683) and April (585).

In this study, opposite to what was obtained in the qPCR analysis, the highest number of samples positive for infectivity was found for HAdV-C, both for CS and US. This may be attributed to the choice of cell line (A549), which is favorable for HAdV-C replication. When comparing these data with those of the literature, Ogorzaly et al. (2013) found 70.6% of the positive samples for HAdV-F in affluent samples using HEK 293 cells. Chapron et al. (2000) showed 37.9% infectivity for enteric adenoviruses using BGMK cells. In relation to the results of HAdV-C, regarding the tests with US, there were more positive samples in the ICC-qPCR assay than in the direct qPCR of the samples. These results may be explained due to both virus replication and withdrawal of inhibitors from the sample during cell passage, improving the sensitivity of PCR (Greening et al., 2002; Rigotto et al., 2005). Alternatively, in CS, the greater detection was in the direct qPCR than in the ICC-qPCR. This may be related to a decrease in the number of viable particles in the ultracentrifugation process, perhaps by disruption of the capsid structure (Fujita et al., 2001; Bettarel et al., 2000).

Statistical analysis of the results regarding to comparison between the collection sites showed that there was a significant difference when considering the molecular data of HAdV-F, data from the analyzes of *E. coli* and TC. Specifically for HAdV-F, the cluster analysis highlights P1 and groups the other collection points. These results may have been probably due to the number of positive samples found, as well as the values of both bacteriological and HAdV-F concentrations that were higher in P1, making this site different from the others. The non-difference found in the other analyzes may have been due to the amount of negative results obtained, and it was not possible to statistically differentiate the points.

Correlation tests showed a direct correlation between HAdV-F with TC and *E. coli*, as opposed to other findings in the literature that found no correlation. Vecchia et al. (2015) when analyzing AdV and total and fecal coliforms in surface waters of southern Brazil, did not find correlation between bacteriological and virological data. The same is reported in studies with water samples from Korea (Lee et al., 2013), South Africa (Adefisoye et al., 2016) and from Germany (Jurzik et al., 2010). The correlation found between these pathogens is probably due to the high contamination rate detected in the Belo Stream, both by coliforms and by HAdV-F. However, these findings do not exclude the need for enteric virus analyzes in water samples, since no correlation was found when considering the HAdV-C data, as well as the majority of the studies (as mentioned previously) do not show correlation between coliforms and enteric viruses.

In the future, other possible viral indicators of fecal contamination of water are coliphages. They have been proposed as indicators in recreational waters due their persistence in the environment and wastewater treatment (Ravva and Sarreal, 2016; McMinn et al., 2017; Sidhu et al., 2018). Benjamin-Chung et al. (2017) conducted a comparative study between coliphages and enterococci, and concluded that there was some evidence that male-specific coliphage had a stronger association with gastrointestinal illness than enterococci in marine waters with human fecal contamination.

Applying QMRA to address human health risks associated with exposure to contaminated waters in Brazil is still scarce, and the existing studies do not specifically evaluate risks of HAdV infection. Vieira (2015) evaluated the risk of infection of group A RV in waters of the Rio Negro basin, Manaus, Amazonas-Brazil. Infection risk results showed averages ranging from 0.3954 to 0.868 associated with exposure to recreational activities or hand-mouth contact. QMRA indices obtained in this present study for HAdV are high when compared with studies performed in other countries. Vergara et al. (2016) conducted illness probability studies for HAdV and obtained values of 0.0028 and 0.0048 for first contact of recreational waters of Singapore. Chigor et al. (2014) estimated daily risk of infection of 0.0073 per HAdV in waters of the Buffalo River in South Africa. If it is assumed the water from Belo Stream (or a source water body with similar microbial concentrations) would be used as a drinking water source, the yearly risk estimates would not meet USEPA's risk recommendation that

annual microbial risks of infection not exceed 1E-04, even at a low exposure volume of 1 mL.

5. Conclusion

In conclusion, the absence of efficient treatment for domestic sewage intensifies the deterioration of the water quality of Belo Stream. The site used for recreation by the population presents human infection risks for both HAdV and *E. coli*. This study demonstrates the importance of proper watershed management of surface waters where humans are in contact. Furthermore, our findings reinforce the concept that QMRA based on *E. coli* as a water quality indicator may not reflect the threat of health risks posed by the presence of viruses in recreational freshwaters.

Acknowledgements

The authors are grateful for the financial and structural support from University Feevale, the Brazilian Coordination for the Improvement of Higher Level Personnel (CAPES), the Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS), the Brazilian National Council for Scientific Development (CNPq), the Institute of Environmental Sanitation/University of Caxias do Sul and the Municipal Health Department of Caxias do Sul, for kindly sending the data of diarrheal diseases of the municipality.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.177.

References

Abia, A.L.K., et al., 2017. Microbial remobilization on riverbed sediment disturbance in experimental flumes and a human-impacted river: implication for water resource management and public health in developing sub-saharan African countries. Int. J. Environ. Res. Public Health 14 (306), 1–18.
ABNT – Associação Brasileira de Normas Técnicas, 1987a. Planejamento de amostragem de efluentes líquidos e corpos receptores – NBR 9897. ABNT, Rio de Janeiro.
ABNT – Associação Brasileira de Normas Técnicas, 1987b. Preservação e técnicas de amostragem de efluentes líquidos e corpos receptores – NBR 9898. ABNT, Rio de Janeiro.
Adefisoye, M.A., et al., 2016. Quantitative PCR detection and characterization of human adenovirus, rotavirus and hepatitis A virus in discharged effluents of two wastewater treatment facilities in the Eastern Cape, South Africa. Food Environ. Virol. 8 (4), 262–274.
Ahmad, T., et al., 2016. Prevalence of rotavirus, adenovirus, hepatitis A virus and enterovirus in water samples collected from different region of Peshawar, Pakistan. Ann. Agric. Environ. Med. 23 (4), 576–580.
Ahmed, W., et al., 2015. Comparison of concentrations methods for quantitative detection of sewage-associated viral markers in environmental waters. Appl. Environ. Microbiol. 81 (6), 2042–2049.
Aslan, A., et al., 2011. Occurrence of adenovirus and other enteric viruses in limited-contact freshwater recreational areas and bathing waters. J. Appl. Microbiol. 111, 1250–1261.
Benjamin-Chung, J., et al., 2017. Coliphages and gastrointestinal illness in recreational waters. Epidemiology 28 (5), 644–652.
Bettarel, Y., et al., 2000. A comparison of methods for counting viruses in aquatic systems. Appl. Environ. Microbiol. 66 (6), 2283–2289.
Biscaro, V., et al., 2018. Detection and molecular characterization of enteric viruses in children with acute gastroenteritis in northern Italy. Infect. Genet. Evol. 60, 35–41.
Chapron, C.D., Ballester, N.A., Fontaine, J.H., Frades, C.N, Margolin, A.B., 2000. Detection of astroviruses, enteroviruses, and adenovirus types 40 and 41 in surface waters collected and evaluated by the information collection rule and an integrated cell culture-nested PCR procedure. Appl. Environ. Microbiol. 66 (6), 2520–2525.
Chigor, V.N., et al., 2014. Assessment of the risks for human health of adenoviruses, hepatitis a virus, rotaviruses and enteroviruses in the Buffalo River and three source water dams in the Eastern Cape. Food Environ. Virol. 6, 87–98.
CONSELHO NACIONAL DO MEIO AMBIENTE (CONAMA), 274/2000. Comissão Nacional do Meio Ambiente. Resolução 274, 2000. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento. http://www.mma.gov.br/port/conama/res/res00/res27400.html.
Couch, R.B., et al., 1966. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. Bacteriol. Rev. 30 (3), 517–529.
da Silva, M., et al., 2016. Rotavirus and astroviruses. In: Rose, J.B., Jiménez-Cisneros, B. (Eds.), Global Water Pathogens Project. UNESCO, E. Lansing, MI http://www.waterpathogens.org (J.S Meschke, and R. Girones (eds) Part 3 Viruses) http://www.waterpathogens.org/book/rotavirus Michigan State University.
Dupont, H.L., et al., 1971. Pathogenesis of *Escherichia coli* diarrhea. NEJM 285, 1–9.
EPA-United States Environmental Protection Agency, 2015. Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality. No. 820-R-15-098. Washington, DC.
Estes, M., Greenberg, H., 2013. Rotaviruses. In: Knipe, D.M., Howley, P.M., Cohen, J.I., Griffin, D.E., Lamb, R.A., Martin, M.A., et al. (Eds.), Fields Virology, 6th ed. Wolters Kluwer business/Lippincott Williams and Wilkins, Philadelphia.
Fujita, N., et al., 2001. Paraformaldehyde protects of hepatitis C virus particles during ultracentrifugation. J. Med. Virol. 63 (2), 108–116.
Fumian, T.M., et al., 2018. Enteric viruses dissemination in a private reserve of natural heritage. Lett. Appl. Microbiol. 66 (4), 313–320.
García, M.E., 2006. Virus en águas de consumo. Higiene y Sanidad Ambiental. 6, pp. 173–189.
Ghebremedhin, B., 2014. Human adenovirus: viral pathogen with increasing importance. Eur. J. Microbiol. Immunol. 1, 26–33.
Girardi, V., et al., 2018. Assessment of diversity of adenovirus DNA polymerase gene in recreational waters facilitated by ultracentrifugal concentration. J. Water Health 16 (1), 102–111.
Greening, G.E., et al., 2002. Evaluation of integrated cell culture-PCR (C-PCR) for virological analysis of environmental samples. J. Appl. Microbiol. 93 (5), 745–750.
Gu, X., et al., 2018. Geospatial distribution of viromes in tropical freshwater ecosystems. Water Res. 137, 220–232.
Haas, C.N., et al., 1999. Quantitative Microbial Risk Assessment. John Wiley & Sons, New York.
ICTV_International commitee on taxonomy of viruses, 2017. Adenovírus. (Availale in). http://www.ictvonline.org/>.
Jurzik, L., et al., 2010. Chemical and microbiological parameters as possible indicators for human enteric viruses in surface water. Int. J. Hyg. Environ. Health 213, 210–216.
Klove, B., et al., 2017. Overview of groundwater sources and water-supply systems, and associated microbial pollution, in Finland, Norway and Iceland. Hydrogeol. J. 25 (4), 1033–1044.
Lee, G.C., et al., 2013. A 5-year survey (2007–2011) of enteric viruses in Korean aquatic environments and the use of coliforms as viral indicators. Microbiol. Immunol. 57, 46–53.
Mackowiak, M., et al., 2018. Distribution of *Escherichia coli*, coliphages and enteric viruses in water, epilithic biofilms and sediments of an urban river in Germany. Sci. Total Environ. 626, 650–659.
McMinn, B.R., et al., 2017. Concentration and quantification of somatic and F+ coliphages from recreational waters. J. Virol. Methods 249, 58–65.
Mena, K.D., 2007. Waterborne viruses: assessing the risks. Human Viruses in Water, Cap. 8, pp. 163–175.
Mena, K.D., Gerba, C.P., 2008. Waterborne adenovírus. Rev. Environ. Contam. Toxicol. 198, 133–167.
Nikonov, O.S., et al., 2017. Enteroviruses: classification, diseases they cause, and approaches to development of antiviral drugs. Biochem. Mosc. 82 (13), 1615–1631.
Ogorzaly, L., et al., 2013. Two-day detection of infectious enteric and non-enteric adenoviruses by improved ICC-qPCR. Appl. Microbiol. Biotechnol. 97 (9), 4159–4166.
Oliveira, L.K., et al., 2012. Enteric viruses in water samples from Brazilian dairy farms. Agric. Water Manag. 111, 34–39.
Ordinance No. 2,914, of December 12, 2011.. Ministry of Health - Brazil. http://bvsms.saude.gov.br/bvs/saudelegis/gm/2011/prt2914_12_12_2011.html.
Petterson, S., et al., 2015. Variability in the recovery of a virus concentration procedure in water: implications for QMRA. Water Res. 87, 79–86.
Pina, S., et al., 1998. Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. Appl. Environ. Microbiol. 64 (9), 3376–3382.
Rajendran, V., et al., 2018. Quality assessment of pollution indicators in marine water at critical locations of the Gulf of Mannar Biosphere Reserve, Tuticorin. Mar. Pollut. Bull. 126, 236–240.
Ravva, S.V., Sarreal, C.Z., 2016. Persistence of F-specific RNA coliphages in surface waters from a produce production region along the central coast of California. PLoS One 11 (1), 1–13.
Rigotto, C., et al., 2005. Detection of adenoviruses in shellfish by means of conventional-PCR, nested-PCR, and integrated cell culture PCR (ICC/PCR). Water Res. 39 (2), 297–304.
Rigotto, C., et al., 2010. Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. J. Appl. Microbiol. 109 (6), 1979–1987.
Rose, J.B., et al., 1996. Risk Assessment for Microbial Contaminants in Water. Report for the AWWA Research Foundation. AWWA Research Foundation and AWWA.
Santos, N.S.O., Soares, C.C., 2015. Viroses entéricas. In: Santos, N.S.O., Romanos, M.T.V., Wigg, M.D. (Eds.), Virologia humana. Guanabara Koogan, Rio de Janeiro, pp. 209–215.
Santos, V.S., et al., 2018. Rotavirus genotypes circulating in Brazil before and after the national rotavirus vaccine program. Pediatr. Infect. Dis. J. 37 (3), 63–65.
Sidhu, et al., 2018. Comparative enteric viruses and coliphage removal during wastewater treatment processes in a sub-tropical environment. Sci. Total Environ. 616-617, 669–677.
Sinclair, R.G., et al., 2009. Viruses in recreational water-borne disease outbreaks: a review. J. Appl. Microbiol. 107, 1769–1780.

Spilki, F.R., et al., 2016. Enhancing Water Management Capacity in a Changing World: The Challenge of Increasing Global Access to Water and Sanitation. Universidade Feevale, Novo Hamburgo https://books.google.com.br/books?id=d5qxDAAAQBAJ&pg=PA13&lpg=PA13&dq=Enhancing+water+management+capacity+in+a+changging+world:+the+challenge+of+increasing+global+access+to+water+andsanitation&source=bl&ots=usZtZXaune&sig=ExKPHvA0QzCbS7a81_iFc8Lqzls&hl=pt-BR&sa=X&ved=0ahUKEwjwtarhuJ7bAhUBf5AKHaz0BR8Q6AEILzAB.

Staggemeier, R., et al., 2017. Enteric viruses and adenovirus diversity in waters from 2016 Olympic venues. Sci. Total Environ. 586, 304–312.

Tsai, Y.L., et al., 1993. Simple method of concentrating enteroviruses and hepatitis A virus fromsewage and ocean water for rapid detection by reversetranscriptase polymerase chain reaction. Appl. Environ. Microbiol. 59, 3488–3491.

Vecchia, A.D., et al., 2012. First description of adenovirus, enterovirus, rotavirus and torque teno virus on water samples collected from the Arroio Dilúvio, Porto Alegre, Brazil. Braz. J. Biol. 72, 323–329.

Vecchia, A.D., et al., 2015. Surface water quality in the Sinos River basin, in southern Brazil: tracking microbiological contamination and correlation with physicochemical parameters. Environ. Sci. Pollut. Res. 22 (13), 9899–9911.

Vergara, G.G.R.V., et al., 2016. Risk assessment of noroviruses and human adenoviruses in recreational surface waters. Water Res. 103, 276–282.

Vieira, C.B., 2015. Rastreamento microbiológico de fontes de contaminação humana e animal por marcadores virais e avaliação de risco de infecções por vírus gastroentéricos na bacia do Rio Negro, Manaus. Amazonas (PhD Dissertation). https://www.arca.fiocruz.br/handle/icict/14070.

Wolf, S., et al., 2010. Viral multiplex quantitative PCR assays for tracking sources of fecal contamination. Appl. Environ. Microbiol. 76, 1388–1394.

Wyn-Jones, A.P., et al., 2011. Surveillance of adenoviruses and noroviruses in European recreational waters. Water Res. 45 (3), 1025–1038.

Zhu, H., et al., 2018. Monitoring of Poyang lake water for sewage contamination using human enteric viruses as an indicator. Virol. J. 15 (3), 1–9.

MATERIAL SUPLEMENTAR (ARTIGO 2)

Table 1

Escherichia coli concentration, parameters used to estimate the risk of infection (beta-Poisson model) and daily and annual risk calculated per month of collection.

Month	C (MPN/L)	R	I	DR	V (L)	d	N_{50}	α	Days/year	Daily Risk	Annual Risk
May	9.70E+03	1	1	0	0.001	9.7	2.11E+06	0.155	5	6.16E-05	3.08E-04
June	2.00E+03	1	1	0	0.001	2	2.11E+06	0.155	5	1.27E-05	6.36E-05
July	4.88E+04	1	1	0	0.001	48.8	2.11E+06	0.155	5	3.10E-04	1.55E-03
August	8.50E+03	1	1	0	0.001	8.5	2.11E+06	0.155	5	5.40E-05	2.70E-04
September	3.45E+05	1	1	0	0.001	344.8	2.11E+06	0.155	5	2.17E-03	1.08E-02
October	1.35E+04	1	1	0	0.001	13.5	2.11E+06	0.155	5	8.58E-05	4.29E-04
November	5.20E+03	1	1	0	0.001	5.2	2.11E+06	0.155	5	3.30E-05	1.65E-04
December	3.00E+03	1	1	0	0.001	3	2.11E+06	0.155	5	1.91E-05	9.53E-05
January	4.10E+03	1	1	0	0.001	4.1	2.11E+06	0.155	5	2.61E-05	1.30E-04
February	5.20E+03	1	1	0	0.001	5.2	2.11E+06	0.155	5	3.30E-05	1.65E-04
March	4.55E+04	1	1	0	0.001	45.5	2.11E+06	0.155	5	2.89E-04	1.44E-03
April	6.57E+04	1	1	0	0.001	65.7	2.11E+06	0.155	5	4.17E-04	2.08E-03

C= *Escherichia coli* concentration (MPN/L); R= Recovery (assumption that all microorganisms were recovered); I= Infectivity (assumption that all microorganisms are infectious); DR= Decimal Reduction; V= Exposure volume (L); d=dose; $d=C*(1/R)*I*(10^{(-DR)})*V$; $N_{50}=2.11E+06$; $\alpha=0.155$; Daily Risk (beta-Poisson model) = $1- [1+dose\ (2^{1/\alpha}-1)/N_{50}]^{-\alpha}$

Table 2

Human mastadenovirus concentration, parameters used to estimate the risk of infection (exponential model) and daily and annual risk calculated per month of collection.

Month	C (GC/L)	r	I	V (L)	R	DR	Days/year	N	Daily Risk	Annual Risk
July	2.02E+04[a]	0.4172	1	0.001	0.71	0	5	28.45070423	9.99E-01	9.99E-01
September	2.90E+04[a]	0.4172	1	0.001	0.71	0	5	40.84507042	9.99E-01	9.99E-01
January	1.20E+08[a]	0.4172	1	0.001	0.71	0	5	169014.0845	9.99E-01	9.99E-01
February	1.66E+08[a]	0.4172	1	0.001	0.71	0	5	233802.8169	9.99E-01	9.99E-01
June	5.20E+08[b]	0.4172	1	0.001	0.71	0	5	732394.3662	9.99E-01	9.99E-01
July	4.76E+07[b]	0.4172	1	0.001	0.71	0	5	67042.25352	9.99E-01	9.99E-01
September	2.35E+02[b]	0.4172	1	0.001	0.71	0	5	0.330985915	1.29E-01	4.99E-01
October	6.78E+08[b]	0.4172	1	0.001	0.71	0	5	954929.5775	9.99E-01	9.99E-01
February	3.74E+09[b]	0.4172	1	0.001	0.71	0	5	5267605.634	9.99E-01	9.99E-01
March	8.42E+07[b]	0.4172	1	0.001	0.71	0	5	118591.5493	9.99E-01	9.99E-01
April	3.18E+09[b]	0.4172	1	0.001	0.71	0	5	4478873.239	9.99E-01	9.99E-01

C= HAdV concentration; a= HAdV-F concentration; b= HAdV-C concentration; I= Infectivity; V= Exposure volume (L); R=Recovery efficiency of the ultracentrifugation method ; DR= Decimal Reduction; r= Dose-response (Rose et al., 1996); N (Dose)= $C*(1/R)*I*V*(10^{-DR})$; Daily Risk (exponential model)= 1-exp(-rN); Annual Risk= $1-(1-\text{Daily Risk})^{\text{Days/year}}$

Table 3

Viral and bacterial concentrations from site P1, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C		HAdV-F		Coliforms (MPN/100 mL)	
	US (GC/L)	CS (GC/L)	US (GC/L)	CS (GC/L)	Total Coliforms	*E. coli*
May	ND	ND	ND	ND	>2.42E+05	1.55E+05
June	ND	2.88E+06	ND	ND	1.55E+06	8.16E+05
July	ND	ND	ND	1.29E+07	6.13E+04	4.88E+04
August	ND	ND	9.96E+07	2.58E+08	>2.42E+05	>2.42E+05
September	2.28E+07	ND	1.13E+08	4.50E+08	>2.42E+06	1.99E+05
October	ND	ND	7.56E+07	1.09E+08	>2.42E+06	1.08E+05
November	ND	ND	ND	1.60E+07	8.70E+05	4.79E+05
December	ND	ND	ND	2.66E+07	>2.42E+06	5.00E+05
January	ND	ND	2.77E+07	6.99E+07	1.01E+06	9.14E+05
February	ND	ND	3.31E+08	3.00E+08	>2.42E+06	1.73E+06
March	ND	ND	ND	9.46E+05	1.12E+06	1.57E+05
April	NE	NE	NE	NE	NE	NE
Total	9.09% (1/11)	9.09% (1/11)	45.45% (5/11)	81.81% (9/11)	100%	100%
Arithmetic Average	2.07E+06	2.62E+05	5.89E+07	1.13E+08	1.34E+06	4.86E+05
Geometric Average	-	-	9.52E+07	4.81E+07	8.59E+05	3.02E+05

GC: genomic copies; MPN: most probable number; CS: concentrated samples; US: unconcentrated samples; ND: not detected; NE: not evaluated.

Table 4

Viral and bacterial concentrations from site P2, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C		HAdV-F		Coliforms (MPN/100 mL)	
	US (GC/ L)	CS (GC/ L)	US (GC/ L)	CS (GC/ L)	Total Coliforms	*E. coli*
May	ND	3.08E+06	ND	ND	1.20E+05	2.40E+04
June	ND	ND	ND	ND	>2.42E+05	6.87E+04
July	ND	1.14E+06	ND	ND	1.73E+05	1.41E+05
August	2.41E+06	8.59E+05	ND	ND	>2.42E+06	8.16E+04
September	1.93E+06	1.29E+07	ND	1.51E+06	2.99E+04	1.20E+05
October	1.89E+06	6.29E+06	ND	ND	6.49E+04	6.12E+03
November	ND	ND	ND	ND	1.59E+05	2.59E+04
December	ND	ND	ND	5.16E+05	2.31E+05	3.09E+04
January	ND	ND	ND	2.20E+06	4.36E+05	4.08E+04
February	ND	ND	ND	ND	4.11E+05	4.31E+04
March	ND	ND	ND	ND	1.61E+05	2.92E+04
April	ND	ND	ND	4.66E+06	5.01E+05	6.02E+04
Total	25.00% (3/12)	41.66% (5/12)	-	33.33% (4/12)	100%	100%
Arithmetic Average	5.19E+05	2.02E+06	-	7.40E+05	4.12E+05	5.60E+04
Geometric Average	2.06E+06	3.00E+06	-	1.68E+06	2.20E+05	4.25E+04

GC: genomic copies; MPN: most probable number; CS: concentrated samples; US: unconcentrated samples; ND: not detected.

Table 5

Viral and bacterial concentrations from site P3, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C		HAdV-F		Coliforms (MPN/100 mL)	
	US (GC/ L)	CS (GC/ L)	US (GC/ L)	CS (GC/ L)	Total Coliforms	*E. coli*
May	ND	ND	ND	1.10E+05	4.35E+04	2.38E+04
June	ND	2.82E+06	ND	ND	3.65E+04	5.72E+04
July	ND	ND	ND	ND	2.42E+05	8.66E+04
August	ND	ND	ND	ND	>2.42E+05	1.99E+05
September	ND	6.63E+05	4.58E+07	1.26E+08	>2.42E+06	>2.42E+06
October	ND	1.79E+06	ND	1.27E+07	9.61E+04	3.13E+04
November	ND	ND	ND	9.29E+06	3.88E+05	1.51E+05
December	ND	ND	ND	7.43E+06	2.59E+05	9.09E+04
January	ND	ND	ND	2.85E+05	7.68E+04	4.79E+04
February	ND	ND	ND	2.29E+06	1.21E+05	1.95E+04
March	ND	ND	ND	ND	2.48E+05	8.57E+04
April	ND	ND	ND	9.56E+07	3.44E+05	9.34E+04
Total	-	25.00% (3/12)	8.33% (1/12)	66.66% (8/12)	100%	100%
Arithmetic Average	-	4.40E+05	3.82E+06	2.11E+07	3.76E+05	2.75E+05
Geometric Average	-	1.50E+06	-	5.42E+06	1.86E+05	8.68E+04

GC: genomic copies; MPN: most probable number; CS: concentrated samples; US: unconcentrated samples; ND: not detected.

Table 6

Viral and bacterial concentrations from site P4, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C		HAdV-F		Coliforms (MPN/100 mL)	
	US (GC/ L)	CS (GC/ L)	US (GC/ L)	CS (GC/ L)	Total Coliforms	*E. coli*
May	ND	ND	ND	ND	8.13E+03	9.70E+02
June	ND	ND	ND	ND	4.87E+03	2.00E+02
July	ND	ND	ND	2.02E+07	3.08E+04	4.88E+03
August	ND	ND	ND	ND	3.65E+04	8.50E+02
September	ND	2.36E+05	ND	2.91E+07	9.80E+04	3.45E+04
October	ND	ND	ND	ND	9.59E+03	1.35E+03
November	ND	ND	ND	ND	1.97E+04	5.20E+02
December	ND	ND	ND	ND	1.27E+04	3.00E+02
January	ND	ND	ND	ND	3.04E+04	4.10E+02
February	ND	ND	ND	ND	1.72E+04	5.20E+02
March	ND	ND	ND	ND	4.11E+04	4.55E+03
April	ND	ND	ND	ND	9.80E+04	6.57E+03
Total	-	8.33% (1/12)	-	16.66% (2/12)	100%	100%
Arithmetic Average	-	1.96E+04	-	4.11E+06	3.39E+04	4.64E+03
Geometric Average	-	-	-	2.43E+07	2.30E+04	1.35E+03

GC: genomic copies; MPN: most probable number; CS: concentrated samples; US: unconcentrated samples; ND: not detected.

Table 7

Viral loads measured by ICC-qPCR assays, before and after viral isolation in unconcentrated samples from site P1, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/ 5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC-qPCR	2nd ICC-qPCR
May	NE	NE	NE	NE
June	ND	ND	ND	ND
July	8.80E+01	ND	ND	ND
August	ND	ND	ND	ND
September	ND	ND	ND	ND
October	ND	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	ND	ND
January	ND	ND	ND	ND
February	ND	1.08E+05	ND	ND
March	2.66E+04	2.02E+06	2.13E+02	ND
April	NE	NE	NE	NE
Total	20.00% (2/10)	20.00% (2/10)	10.00% (1/10)	0

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected; NE: not evaluated.

Table 8

Viral loads measured by ICC-qPCR assays, before and after viral isolation in unconcentrated samples from site P2, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/ 5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC- qPCR	2nd ICC-qPCR
May	NE	NE	NE	NE
June	5.12E+03	ND	9.58E+02	ND
July	1.06E+02	ND	ND	ND
August	ND	ND	ND	ND
September	ND	ND	ND	ND
October	3.70E+02	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	ND	7.23E+02
January	3.22E+02	ND	ND	ND
February	ND	2.30E+04	ND	ND
March	1.54E+04	8.63E+04	ND	ND
April	ND	3.73E+04	ND	1.10E+02
Total	45.45% (5/11)	27.27% (3/11)	9.09% (1/11)	18.18% (2/11)

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected; NE: not evaluated.

Table 9

Viral loads measured by ICC-qPCR assays, before and after viral isolation in unconcentrated samples from site P3, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/ 5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC-qPCR	2nd ICC-qPCR
May	NE	NE	NE	NE
June	2.06E+02	ND	ND	ND
July	8.01E+03	ND	ND	ND
August	ND	ND	ND	ND
September	ND	ND	ND	ND
October	ND	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	ND	ND
January	ND	ND	ND	ND
February	1.53E+04	2.98E+04	ND	ND
March	1.63E+04	3.10E+04	ND	ND
April	ND	2.97E+04	ND	ND
Total	36.36% (4/11)	27.27% (3/11)	0	0

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected; NE: not evaluated.

Table 10

Viral loads measured by ICC-qPCR assays, before and after viral isolation in unconcentrated samples from site P4, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/ 5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC-qPCR	2nd ICC-qPCR
May	NE	NE	NE	NE
June	2.61E+03	ND	ND	ND
July	2.39E+03	ND	ND	ND
August	ND	ND	ND	ND
September	ND	ND	ND	ND
October	3.40E+03	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	ND	ND
January	ND	ND	1.58E+06	ND
February	1.87E+04	3.37E+04	ND	8.29E+02
March	ND	4.05E+04	ND	ND
April	1.59E+04	1.07E+05	ND	ND
Total	45.45% (5/11)	27.27% (3/11)	9.09% (1/11)	9.09% (1/11)

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected; NE: not evaluated.

Table 11

Viral loads measured by ICC-qPCR assays, before and after viral isolation in ultracentrifuge-concentrated samples from site P1, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/ 5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC-qPCR	2nd ICC-qPCR
May	ND	ND	ND	ND
June	ND	ND	ND	9.88E+02
July	ND	ND	ND	ND
August	ND	ND	ND	ND
September	ND	ND	ND	ND
October	ND	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	ND	ND
January	ND	ND	ND	ND
February	1.84E+02	2.20E+03	ND	ND
March	ND	ND	ND	ND
April	NE	NE	NE	NE
Total	9.09% (1/11)	9.09% (1/11)	0	9.09% (1/11)

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected; NE: not evaluated.

Table 12

Viral loads measured by ICC-qPCR assays, before and after viral isolation in ultracentrifuge-concentrated samples from site P2, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/ 5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC-qPCR	2nd ICC-qPCR
May	ND	ND	ND	ND
June	ND	ND	ND	1.03E+03
July	ND	ND	ND	ND
August	ND	ND	ND	ND
September	ND	ND	ND	ND
October	ND	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	9.73E+01	ND
January	ND	ND	ND	ND
February	3.01E+02	ND	ND	9.27E+01
March	2.61E+03	ND	ND	ND
April	2.75E+03	3.60E+02	ND	ND
Total	25.00% (3/12)	8.33% (1/12)	8.33% (1/12)	16.66% (2/12)

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected.

Table 13

Viral loads measured by ICC-qPCR assays, before and after viral isolation in ultracentrifuge-concentrated samples from site P3, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/ 5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC-qPCR	2nd ICC-qPCR
May	ND	ND	ND	ND
June	ND	ND	ND	ND
July	5.03E+03	ND	ND	ND
August	2.21E+04	ND	1.10E+05	ND
September	ND	ND	ND	ND
October	ND	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	ND	1.20E+03
January	ND	ND	2.31E+03	ND
February	ND	ND	ND	ND
March	9.06E+03	2.05E+02	ND	ND
April	ND	ND	ND	ND
Total	25.00% (3/12)	8.33% (1/12)	16.66% (2/12)	8.33% (1/12)

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected.

Table 14

Viral loads measured by ICC-qPCR assays, before and after viral isolation in ultracentrifuge-concentrated samples from site P4, per month of collection in samples collected from the Belo Stream, Caxias do Sul, Brazil.

Month	HAdV-C (GC/5 µL)		HAdV-F (GC/ 5 µL)	
	1st ICC-qPCR	2nd ICC-qPCR	1st ICC-qPCR	2nd ICC-qPCR
May	ND	ND	ND	ND
June	ND	ND	ND	ND
July	9.49E+03	ND	ND	ND
August	ND	ND	ND	ND
September	ND	ND	ND	ND
October	ND	ND	ND	ND
November	ND	ND	ND	ND
December	ND	ND	ND	ND
January	ND	ND	6.00E+02	ND
February	ND	ND	ND	ND
March	ND	4.21E+02	ND	ND
April	ND	ND	ND	ND
Total	8.33% (1/12)	8.33% (1/12)	8.33% (1/12)	0

GC: genomic copies; ICC-qPCR: integrated cell culture qPCR; ND: not detected.

5. CONSIDERAÇÕES FINAIS

As análises de AdV, coliformes totais e *Escherichia coli* ao longo do Arroio Belo revelam a contaminação fecal, demonstrando a ineficiência ou a ausência de processos de tratamentos de esgoto adequados. Além disso, evidenciam a deterioração da qualidade da água do arroio. Os resultados de diversidade encontrados no presente estudo são raros na literatura, demonstrando que o processo de concentração de amostras de água são fundamentais para obtenção de resultados superiores, tanto em termos de diversidade quanto em frequência de amostras positivas. Os HAdV do grupo F estiveram presentes na maioria das amostras em ambas as metodologias de análises (Nested – PCR e qPCR), destacando-se que os HAdV deste grupo são um importante vírus causador de gastroenterite.

Os pontos de amostragem localizados no perímetro urbano do município apresentaram maior contaminação, tanto viral quanto bacteriana, mostrando assim a influência antrópica negativa sobre o Arroio. A ausência de partículas virais de RV pode estar associada a implementação de vacinas no Brasil levando a redução de casos de morbidade e mortalidade relacionadas a diarreia. Os resultados superiores de infecciosidade foram obtidos para HAdV do grupo C, para ambas amostras concentradas e não concentradas, provavelmente devido a linhagem celular utilizada que é permissiva a replicação deste grupo viral.

O local usado para recreação pela população apresenta risco de infecção tanto por HAdV quanto por E. coli. Este estudo demonstra a importância do manejo adequado das bacias hidrográficas das águas superficiais onde os seres humanos estão em contato. Mais do que isso, as descobertas deste trabalho reforçam o conceito de que a avaliação de risco baseada somente em E. coli como indicador de qualidade da água pode não refletir os riscos à saúde associados à presença de vírus em águas para fins recreativos.

Frente aos resultados expostos neste trabalho, com objetivo de prevenir surtos em decorrência de infecções, principalmente virais, e impactos ao meio ambiente, há a necessidade da implantação de programas de monitoramento e remoção viral de corpos hídricos. Além disso, destaca-se que profissionais com qualificações em análises virais e o acesso dos órgãos competentes às metodologias investigativas são essenciais nos programas de monitoramento. Estas medidas poderão nortear ações públicas de gerenciamento ambiental e saúde pública, de forma preventiva que requerem investimento de menor valor econômico, socialmente justa e ambientalmente corretas.

6. REFERÊNCIAS

ASLAN, A., et al. Occurrence of adenovirus and other enteric viruses in limited-contact freshwater recreational areas and bathing waters. **Journal of Applied Microbiology**, v.111, p.1250-1261, 2011.

BOSCH, A., et al. New tools for the study and direct surveillance of viral pathogens in water. **Current Opinion Biotechnology**. v. 19(3), p. 295-301, 2008.

CONAMA - CONSELHO NACIONAL DO MEIO AMBIENTE - 274/2000. Comissão Nacional do Meio Ambiente. Resolução 274 (2000). Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento. Disponível em: http://www.mma.gov.br/port/conama/res/res00/res27400.html. Acesso em 22 de agosto de 2018.

DA SILVA, M., et al. Rotavirus and Astroviruses. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogens Project. http://www.waterpathogens.org (J.S Meschke, and R. Girones (eds) Part 3 Viruses) http://www.waterpathogens.org/book/rotavirus Michigan State University, E. Lansing, MI, UNESCO, 2016.

DE OLIVEIRA, L.K., et al. Enteric viruses in water samples from Brazilian dairy farms. **Agricultural Water Management.** v.111, p.34-39, 2012.

DIAS, J. et al. Detection and quantification of human adenovirus (HAdV), JC polyomavirus (JCPyV) and hepatitis A virus (HAV) in recreational waters of Niterói, Rio de Janeiro, Brazil. **Marine Pollution Bulletin**. v.133, p. 240-245, 2018.

DÓRÓ, R. et al. Zoonotic transmission of rotavirus: surveillance and control. **Expert Review of Anti-infective Therapy.** v. 13(11), p. 1337–1350, 2015.

FEPAM – FUNDAÇÃO ESTADUAL DE PROTEÇÃO AMBIENTAL HENRIQUE LUIZ ROESSLER. Qualidade das águas da bacia hidrográfica do Rio Caí. Disponível em: http://www.fepam.rs.gov.br/qualidade/qualidade_cai/cai.asp. Acesso em: 22 de agosto de 2018.

GALL, A. M. et al. Waterborne Viruses: A Barrier to Safe Drinking Water. **PLOS Pathogens.** v. 11(6), p.1-7, 2015.

GARCÍA, M.E. Virus en águas de consumo. **Higiene y Sanidad Ambiental**, v.6, p.173-189, 2006.

GIBSON, K. E. Viral pathogens in water: occurrence, public health impact, and available control strategies. **Current Opinion in Virology**. v.4, p. 68-76, 2014

HAAS, C. N., et al. Quantitative Microbial Risk Assessment. New York: John Wiley & Sons, 1999

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍTICA. População residente no município de Caxias do Sul no ano de 2017. Disponível em: https://cidades.ibge.gov.br/brasil/rs/caxias-do-sul/panorama. Acesso em: 22 de agosto de 2018.

ICTV_INTERNATIONAL COMMITEE ON TAXONOMY OF VIRUSES. **Adenovírus**. Disponível em: <http://www.ictvonline.org/>.

ICTV_INTERNATIONAL COMMITEE ON TAXONOMY OF VIRUSES. Reoviridae. Disponível em: https://talk.ictvonline.org/ictv-reports/ictv_9th_report/dsrna-viruses-2011/w/dsrna_viruses/188/reoviridae

KUNDU, A., et al. Adenovirus-associated health risks for recreational activities in a multi-use coastal watershed based on site-specific quantitative microbial risk assessment. **Water Research**, v.47, p.6309 – 6325, 2013.

LEE, C.S., et al. Occurrence of human enteric viruses at freshwater beaches during swimming season and its link to water inflow. **Science of the Total Environment**, v.472, p.757 – 766, 2014.

LUCHS, A., TIMENETSKY, M. C. S. T. Unexpected detection of bovine G10 rotavirus in a Brazilian child with diarrhea. **Journal of Clinical Virology**, v. 59, p.74– 76, 2014a.

LUCHS, A., TIMENETSKY, M. C. S. T. G8P[6] rotaviruses isolated from Amerindian children in Mato Grosso do Sul, Brazil, during 2009: close relationship of the G and P genes with those of bovine and bat strains. **Journal of General Virology**, v. 95, p. 627–641, 2014b.

MAURER, C. P., et al. Adenovirus, enterovirus and thermotolerant coliforms in recreational waters from Lake Guaíba beaches, Porto Alegre. **Journal of Water and Health**. v.13 (4), p.1123-9, 2015.

MCMINN, B. R., KORAJKIC, A., & GRIMM, A. C. Optimization and evaluation of a method to detect adenoviruses in river water. **Journal of Virological Methods**, v. 231, p. 8-13, 2016.

MEDICI, M. C., et al. Genetic diversity in three bovine-like human G8P[14] and G10P[14] rotaviruses suggests independent interspecies transmission events. **Journal of General Virology**, v. 96, p.1161–1168, 2015.

MEHNERT, D.U., et al. Occurrence of human enteric viruses in sewage and surface waters in the city of São Paulo. **Virus Reviews and Research**, 4: 27, 1999.

MENA, K. D. Waterborne Viruses: Assessing the Risks. In: Bosch A. Human Viruses in Water. 1.ed. Elsevier; p.163-75, 2007.

MENA, K.D., GERBA, C.P. Waterborne Adenovirus. Reviews of Environmental Contamination and Toxicology, v.198, p.133-167, 2008.

MICROBIAL RISK ASSESSMENT GUIDELINE, Pathogenic Microorganisms with focus on food and water, (*USDA*), (*United States Department of Agriculture* United States Environmental Protection Agency (EPA), 2012.

NIKONOV, O. S., et al. Enteroviruses: classification, diseases they cause, and approaches to development of antiviral drugs. Biochemistry (Moscow), v. (82) 13, p.1615-1631, 2017.

OGORZALY, L. et al. Human adenovirus diversity in water samples Using a next-generation amplicon sequencing approach. **Food and Environmental Virology**, p.1-10, 2015.

PARASHAR, U. D., et al. Rotavirus and severe childhood diarrhea. **Emerging Infectious Disease**, v.12, p. 304–306, 2006.

PRADO, T., MIAGOSTOVICH, M. P. Environmental virology and sanitation in Brazil: a narrative review. **Cad. Saúde Pública**, Rio de Janeiro, v. 30 (7),p. 1367-1378, 2014.

PREVOST, M. et al. Viral persistence in surface and drinking water: Suitability of PCR pre-treatment with intercalating dyes. **Water Research**. v.91, p. 68-76, 2016

RIGOTTO, C., et al. Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. **Journal of Applied Microbiology**. v.109 (6), p. 1979-1987, 2010.

RODRÍGUEZ-LÁZARO, D. et al., Vírus hazard from food, water and other contaminated environments. **FEMS Microbiology Reviews**. v.34 (4), p.786-814, 2012.

SANTOS, N. S. O. e SOARES, C. C. Viroses entéricas. In: Santos, N. S. O., Romanos, M. T. V., Wigg, M. D. (Coord.), 2015. Virologia humana. Rio de Janeiro, ed. Guanabara Koogan, pp 209 – 215, 2015.

SIMECS - SINDICATO DAS INDÚSTRIAS METALÚRGICAS, MECÂNICAS E DE MATERIAL ELÉTRICO DE CAXIAS DO SUL. Perfil Socioeconômico de Caxias do Sul, 2013. Disponível em: http://www.simecs.com.br. Acesso em: 24 de agosto de 2018.

SINCLAIR, R.G., JONES, E.L., GERBA, C.P. Viruses in recreational water-borne disease outbreaks: a review. **Journal of Applied Microbiology**, v.107, p.1769-1780, 2009.

SOLLER, J. A. Use of microbial risk assessment to inform the national estimate of acute gastrointestinal illness attributable to microbes in drinking water. **Journal of Water and Health**, v. 4, n. SUPPL. 2, p. 165–186, 2006.

STAGGEMEIER, R., et al. Enteric viruses and adenovirus diversity in waters from 2016 Olympic venues. **Science of the Total Environment**. v.586, p.304-312, 2017.

USEPA. Microbiological Risk Assessment (MRA) Tools , Methods , and Approaches for Water Media. n. December, p. 184, 2014.

WHO- World Health Organization. Guidelines for Drinking-water Quality. 4 ed. 2011. Disponível em: http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf. Acesso em: 22 de agosto de 2018.

WYER, M. D., et al. Relationships between human adenoviruses and faecal indicator organisms in European recreational waters. **Water Research**, v.46, p.4130 – 4141, 2012.

WYN-JONES, A. P., et al. Surveillance of adenoviruses and noroviruses in European recreational waters. **Water Research**, v.45 (3), p.1025-1038, 2011.

YATES, M.V. et al. Effect of adenovirus resistance on UV disinfection requirements: A report on the state of adenovirus science. **Journal American Water Works Association**. v.98, p.93-106, 2006.