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ORIGINAL PAPER

Gastroenteric Viruses Detection in a Drinking Water Distribution-to-Consumption System in a Low-Income Community in Rio de Janeiro

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Abstract

The availability of drinking water is one of the main determinants of quality of life, disease prevention and the promotion of health. Viruses are important agents of waterborne diseases and have been described as important markers of human faecal contamination. This study aimed to investigate viruses' presence as an indicator of drinking water quality in low-income communities in the Manguinhos area, Rio de Janeiro, Brazil. Three hundred and four drinking water samples (2L/each) were collected along the drinking water distribution-to-consumption pathway in households, as well as healthcare and school units. Water samples were collected both directly from the water supply prior to distribution and after storage in tanks and filtration units. Using qPCR, viruses were detected 50 times in 45 water samples (15%), 19 of these being human adenovirus, 17 rotavirus A and 14 norovirus GII. Viral loads recovered ranged from 5E+10 to 8.7E+10^6 genome copies/Liter. Co-detection was observed in five household water samples and there was no difference regarding virus detection across sampling sites. Precarious and inadequate environmental conditions characterized by the lack of local infrastructure regarding basic sanitation and waste collection in the territory, as well as negligent hygiene habits, could explain viral detection in drinking water in regions with a water supply system.

Keywords Gastroenteric viruses · Drinking water · Rio de Janeiro

Introduction

Poor water quality continues to pose a threat to human health, especially in low-income countries, where a considerable part of the population still lives with scarce or inadequate water supplies, associated with poor sanitation conditions. According to the World Health Organization, 785 million individuals lack access to improved drinking water, at least 2 billion lack improved sanitation, and 842,000 people die each year from diarrhoea due to unsafe drinking water, as well as lack of adequate sanitation and hygiene (WHO 2019).

Water quality monitoring is an important strategy for the protection of human health, being an integral part of Health Surveillance Programs worldwide, following recommendations from the World Health Organization (WHO 2011). Despite efforts to improve water quality worldwide, outbreaks of drinking water-related diseases as well as the difficulties in measuring their burden are known (WHO 2011;

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Tam et al. 2012; Gibson 2014). In high-income countries, incidents in the drinking water distribution system, associated with pathogen contamination within the system, have been identified as contributing to endemic gastrointestinal illness (Ercumen et al. 2014; Säve-Söderbergh et al. 2017).

Currently, new enteric pathogens have been suggested as indicators of human faecal contamination, although bacteria parameters have still been used for scrutinized drinking water quality control (Figueras and Borrego 2010; Rachmadi et al. 2016; Rames et al. 2016; Verani et al. 2019). The importance of monitoring waterborne viruses in drinking water, as well as viral detection associated or not with outbreaks of gastrointestinal disease, has been well documented worldwide (Albinana-Gimenez et al. 2009; Fongaro et al. 2013; Kluge et al. 2014; Pons et al. 2015; Gall et al. 2015; Moreira and Bondelind 2017). Among enteric viruses, human adenovirus (HAdV), rotavirus A (RVA) and norovirus are the most commonly reported viruses causing gastrointestinal disease, the last one being of great impact on waterborne diseases worldwide (Gibson 2014; Guzman-Herrador et al. 2015; Moreira and Bondelind 2017).

The present study aimed to assess viral contamination along the drinking water distribution-to-consumption system in low-income communities in Manguinhos, Metropolitan Region of Rio de Janeiro, Brazil. Despite poor sanitation conditions due to informal and disorderly growth, the region has a potable water supply system. The occurrence of waterborne gastroenteritis agents such as HAdV, RVA and norovirus was investigated as an indicator of possible sources of human faecal contamination in water samples obtained directly from the water supply system, as well as after storage in tanks or residential filters.

Material and Methods

This study is part of a previously approved project by the Committee of Ethics in Research CEP-ENSP nº 408.806 -27/09/13 within the Program "Integrated Territories of Health Care". Participants signed an informed consent form, where water samples were collected. The individual results obtained in this research were returned to the participants and the major findings were presented during meetings in the community.

Studied Area

The Manguinhos territory (i.e. a group of interconnected slums) is a low-income area located on the north side of Rio de Janeiro. It is comprised of 17 adjacent communities, with 38,461 inhabitants living in an area of less than three km^2, with a B (0.899–0.800) and C (0.799–0.700) human development index (IDH) (https://pt.wikipedia.org/wiki/Lista_de_bairros_do_Rio_de_Janeiro_por_IDH). This territory is located in the Canal do Cunha sub-basin, part of the Guanabara Bay basin. Due to this area's disorderly urbanization, its rivers are heavily polluted, grounded and/or covered, being subject to continuous discharge of untreated sewage and solid waste residues. In addition, this area is affected by periodical floods, increasing the transmission risk of several diseases related to inadequate sanitation (Handam et al. 2018).

Drinking Water Sampling

Water samples were collected from October 2012 to December 2014, from residences, schools and healthcare units used by the population of Manguinhos. Two litres of water samples were collected from each tap along the drinking water distribution-to-consumption pathway including: supplied water (S) collected before being distributed to the buildings; tap water (T) collected in the kitchen after storage in tanks; and filtered (F) water, whenever the residents indicated the use of filtration/purification devices before consumption.

Virus Concentration

The adsorption-elution method with a negatively charged membrane was used to concentrate viruses from an initial volume of 2L of water samples, as previously described (Katayama et al. 2002).

Genoma Viral Extraction and Complementary DNA (cDNA)

Nucleic acids were extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, CA, US), according to the manufacturer's instructions. Part of the isolated nucleic acid was transcribed to cDNA using a High Capacity cDNA reverse transcription Kit (Applied Biosystems NY, USA) according to the manufacturer. RNAse/DNAse free water was used as negative control and RVA, HAdV and norovirus positive samples were used as positive controls. Three separate rooms were used for the molecular procedures to avoid sample cross contamination

Virus Detection and Quantification

Noroviruses, HAdV and RVA were detected by quantitative PCR (qPCR) using primers and probes as previously described by Kageyama et al. (2003); Hernroth et al. (2002); Zeng et al. (2008), respectively. All qPCR reactions were carried out in an ABI PRISM 7500® Real-Time System v2.0 (Applied Biosystems, NY, CA) using a TaqMan Universal Master Mix® (Applied Biosystems, NY, CA) according to the manufacturer. Undiluted and tenfold dilutions of the

nucleic acid were analysed in duplicate, and concentrations were estimated as the mean of the data set obtained, correcting for the dilution used. Amplifications were performed in a thermocycler programmed as follows: incubation at 50 °C for 2 min to activate UNG, initial denaturation at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. The standard curve was generated by using tenfold serial dilutions of a plasmid containing the specific DNA fragments of each virus and cloned using the pCR4-TOPO vector (Invitrogen, CA, USA), as previously described (Fumian et al. 2010). For qPCR protocols, positive, negative and non-template controls (NTC) were included. Samples with Ct equal to or less than 38 were considered positive.

Internal Process Control (IPC)

Bacteriophage PP7 was seeded in all water samples as IPC before the concentration step. PP7 was kindly provided by Dr. Verónica Rajal (Salta University, Argentina) and was produced by culture in the host *Pseudomonas aeruginosa* (ATCC 15692) using a previously described protocol (Rajal et al. 2007).

Results

Three hundred-four water samples were collected during the studied period, including 262 from households, 33 from 3 Healthcare Units and 9 from two schools. Often, more than one sample was collected in each building (i.e. healthcare unit, school, household). At least one of the studied viruses was detected in 15% (45/304) of analysed samples, while a co-detection of two viruses was observed in five samples. Fifty viral genomes were obtained, 19 of these being HAdV, 17 RVA and 14 norovirus. Viruses were detected in 76.5% (13/17) of the communities included in this study (Fig. 1; Table 1). The success rate of bacteriophage PP7 recovery was 100%.

Ninety-six percent of the virus-positive samples (43/45) were from households, where all co-detections were observed. Two noroviruses were obtained from samples collected at two different schools. No viruses were detected in water samples collected at the studied Healthcare Units (Table 2).

Viral occurrence according to the sampling points revealed that 9.3% (10/108) of detection was from water samples collected directly from the distribution system at

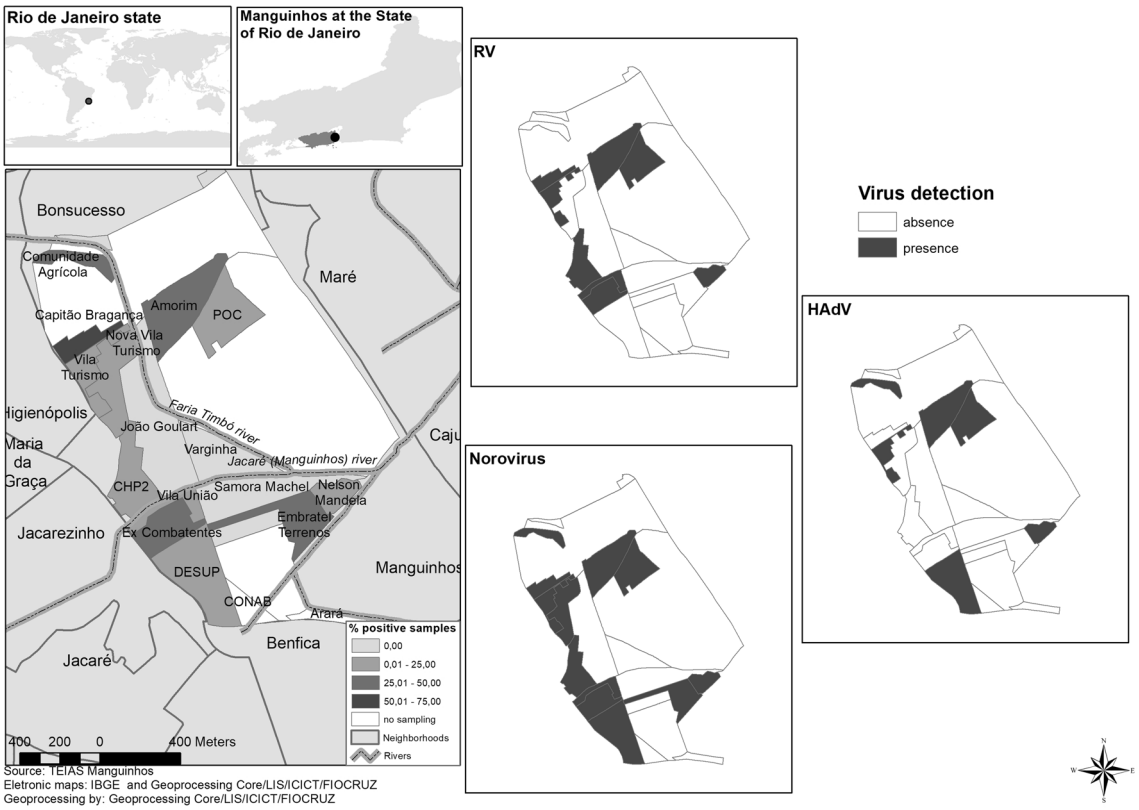

Fig. 1 Distribution of human adenovirus (HAdV), rotavirus A (RVA) and norovirus detection in drinking water according to communities at territory of Manguinhos

Table 1 Human adenovirus (HAdV), rotavirus A (RVA) and norovirus detection according to the communities where the water samples were collected

Communities	No. positive/No. samples (%)		Virus			Co-detection
			HAdV	RVA	Norovirus	
Parque Amorim	7/19	(36.8)	2	4*	2*	RVA + norovirus (1)
CHP2	5/20	(25.0)	2*	2*	3*	RVA + HAdV (1) HAdV + norovirus (1)
C. Agrícola	9/23	(39.1)	9	0	0	
Desup	2/30	(6.7)	2	0	0	
Embratel prédios	0/4	–	0	0	0	
Embratel terrenos	3/7	(42.9)	0	0	3	
Ex combatentes	2/6	(33.3)	0	1	1	
João Goulart	0/19	–	0	0	0	
Nelson Mandela	5/26	(19.2)	1	4*	2*	RVA + norovirus (2)
POC	2/13	(15.4)	1	1	0	
Samora Machel	0/27	–	0	0	0	
Varginha	0/11	–	0	0	0	
Vila São Pedro	0/10	–	0	0	0	
Capitão Bragança	3/4	(75.0)	0	3	0	
Vila Turismo	4/57	(7.0)	2	1	1	
Nova Vila Turismo	2/20	(10.0)	0	0	2	
Vila União	1/8	(12.5)	0	1	0	
Total	45/304	(14.8)	19*	17*	14*	

*Including strains from co-detection

Table 2 Human adenovirus (HAdV), rotavirus A (RVA) and norovirus GII detection according to local and sampling collection points as supplied water (S), tap water (T) and filtered water (F)

Local	No of positive samples/studied samples	Sampling collection points (*n*)	Number of virus detected			
			HAdV	RVA	Norovirus GII	Total
Units of Health	0/33	S (30)	0	0	0	0
		F (3)	0	0	0	0
Schools		S (7)	0	0	0	0
	2/9	T (2)	0	0	2	2
		S (71)	6	4	0	10
Households	43/262	T (93)	7	4	6	17*
		F (98)	6	9	6	21**
Total	45*/304		19	17	14	50

*3 RVA + norovirus GII

**1 HAdV + norovirus GII and 1 HAdV + RVA

the building entrances, 16.8% (16/95) from kitchen tap water and 18.8% (19/101) from domestic filters. No difference regarding virus detection according to sampling points was observed ($p = 0.073$ and $p = 0.054$, Fisher's exact test). All five samples where viral co-detection was observed came from residences (three from tap water and two from filtered water). Regarding viral concentration, RVA ranged from $8.4E+10^1$ to $8.7E+10^6$ genome copies/Liter (gc/L), while HAdV reached $2.2E+10^3$ gc/L, both detected in the three types of water samples studied. Norovirus concentration ranged from $1.3E+10^2$ to $9.4E+10^5$ gc/L in the tap and filtered water samples (Fig. 2).

Discussion

The occurrence of viral contamination in all water sampling sites throughout the drinking water distribution system revealed this population's vulnerability to this infection route. Here, viral investigation reduced the number of samples defined as safe for consumption from 98 to 85%, due to the detection of at least one virus/sample. Only seven of the 45 virus–positive samples (15.5%) were considered unsatisfactory due to the presence of bacteria and/or physical–chemical parameters analysed (Barrocas

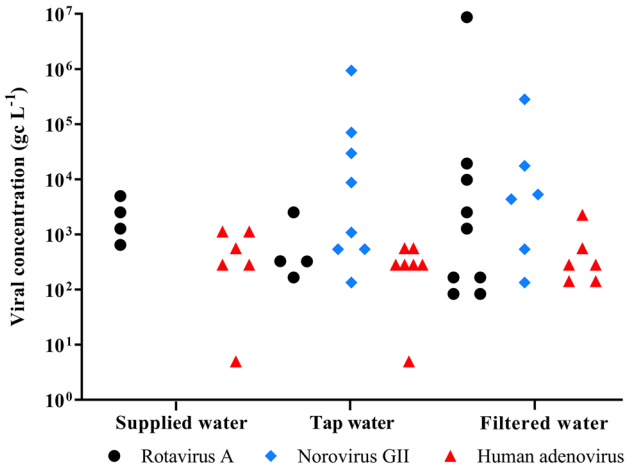

Fig. 2 Viral concentration at the different sampling points at households

P., data not published). The use of viruses as a more sensitive indicator of water quality has been making evident human faecal contamination in water samples classified as suitable for human consumption, according to physical–chemical and microbiological parameters (Payment and Locas 2011; Miagostovich et al. 2014). In agreement, Grassi et al. (2010) also showed a relatively weak relationship between RVA detection and faecal coliforms concentration in different types of environmental water samples.

The occurrence of viruses suggests areas of vulnerability within the territory, although comparative analysis of the viral contamination data from the three sampling sites revealed equally important aspects that should be considered. Viral detection observed in the samples collected directly from the distribution system at the building entrances may suggest that water treatment was insufficient in reducing the source viral load; since viral detection is not routinely used to evaluate final water quality. Lower concentrations of HAdV were found in different treatment steps of a drinking water treatment plant in Barcelona (Albinana-Gimennez et al. 2009). However, the lack of adequate sanitation within the territory, associated with a vulnerable water distribution system with illegal connections to the official system, pipe ruptures and leaks due to the lack of adequate maintenance could explain the observed contamination. According to information obtained with the local population, some opportunities made it possible to associate viral detection with sampling sites where a rupture of the sewage ducts near the water supply system was reported, during the water sampling campaigns. Sudden changes in the physical–chemical characteristics of the water as well as diarrhoea cases in the family unit were observed in those circumstances. The proximity of the drinking water distribution pipelines to areas of direct open sewage disposal represents another system vulnerability in the Manguinhos territory. Additionally, it is important to emphasize that the drainage network in the region has changed, generating river transformations including artificialized rivers (Dias and Cunha 2018). Our data corroborate other studies regarding the quality of water provided in poor areas with inadequate urbanization (e.g. lack of sanitation and garbage collection), showing the vulnerability of these areas' populations and the health risks they were subject to (Remigio et al. 2019; Adams et al. 2016; Snyder et al. 2014; Copeland et al. 2009; Dasgupta 2008).

Cultural and educational aspects of the population should also be considered in order to understand the viral contamination observed, although it is not possible to determine the role of those factors. Water accumulation in tanks as well as the use of domestic filters, often made of clay, due to a poor perception of water quality by the population, is still very common in the country, even in regions that have a continuous water supply, such as in metropolitan Rio de Janeiro. However, manual handling of these filters could be an additional factor for water contamination. Detecting viruses in tap water from home kitchens may pose a higher risk of exposure as it increases the chances of infection by ingestion or food contamination. Foodborne virus outbreaks are associated with manual handling by asymptomatic individuals and/or cross contamination, representing public healthcare and economic burden (Bosch et al. 2018). The relevance of hygiene habits can be evidenced by the absence of virus detection in samples from all Healthcare Units, probably reflecting a greater awareness on behalf of the heath sector professionals, when compared to the regular population.

The viral concentrations detected in this study are sufficient to threaten human health and can be explained by the viruses' ability to resist for prolonged periods in water and solid particles, although the methodological aspect should also be measured (Bosch et al. 2008). Molecular methods for viral detection are unable to distinguish between infectious particles, overestimating the risk of infection (Gironés et al. 2010). Prevost et al. (2016) demonstrated that 55% (27/49) of drinking water samples previously positive for enteric viruses using molecular detection were negative after the association of an intercalating dye pre-treatment method. On the other hand, using the same viral concentration and quantification methodologies, our group demonstrated the infectivity of HAdV when associated to cell culture isolation (Miagostovich et al. 2014). Studies comparing molecular methodologies of viral quantification have shown log-scale differences in the values obtained by methodologies capable of differentiating the detection of nucleic acid and integral viral particles (Heider and Metzner 2014).

The efficient viral capability of being transmitted from the environment to suitable hosts should be considered a threat to the exposed population. A non-enteric adenovirus A12 gastroenteritis outbreak as well as a great diversity of

norovirus in children with or without clinical symptoms of diarrhoea was previously reported in the Manguinhos area (Cantelli et al. 2019; Portes et al. 2016). Concerning RVA detection, it is important to emphasize that even after the implementation of the Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) vaccine in the Brazilian National Immunization Program since 2006, which reduced mortality and morbidity rates in the country, RVA are still detected in gastrointestinal diseases as well as in environmental matrices (Carvalho-Costa et al. 2019). High concentrations of RVA were detected in both raw and treated sewage at a water treatment plant that serves the Metropolitan Region of Rio de Janeiro, including the Manguinhos area (Fumian et al. 2013).

In a more integrated concept of One-Health, the virologic findings of this study contribute to assessing the impact of environmental conditions and their interaction with the population, especially from low-income areas globally distributed. A comprehension of exposure pathways is critical for an efficient prevention and minimization of viral outbreaks, especially with an underreported disease such as viral gastroenteritis and considering RVA as a zoonotic virus, naturally transmitted between humans and animals (O'Brien and Xagoraraki 2019).

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Adams, E. A., Boateng, G. O., & Amoyaw, J. A. (2016). Socioeconomic and demographic predictors of potable water and sanitation access in Ghana. *Social Indicators Research, 126*(2), 673–687.

Albinana-Gimenez, N., Miagostovich, M. P., Calgua, B., Huguet, J. M., Matia, L., & Girones, R. (2009). Analysis of adenoviruses and polyomaviruses quantified by qPCR as indicators of water quality in source and drinking-water treatment plants. *Water Research, 43*(7), 2011–2019.

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

Bosch, A., Gkogka, E., Le Guyader, F. S., Loisy-Hamon, F., Lee, A., van Lieshout, L., et al. (2018). Foodborne viruses: Detection, risk assessment, and control options in food processing. *International Journal of Food Microbiology, 285*, 110–128.

Cantelli, C. P., da Silva, M. F. M., Fumian, T. M., da Cunha, D. C., Andrade, J. D. S. R., Malta, F. C., et al. (2019). High genetic diversity of noroviruses in children from a community-based study in Rio de Janeiro, Brazil, 2014–2018. *Archives of Virology, 164*(5), 1427–1432.

Carvalho-Costa, F. A., de Assis, R. M. S., Fialho, A. M., Araújo, I. T., Silva, M. F., Gómez, M. M., et al. (2019). The evolving epidemiology of rotavirus A infection in Brazil a decade after the introduction of universal vaccination with Rotarix®. *BMC Pediatriacs, 19*(1), 42.

Copeland, C., Beers, B., Thompson, M., Pinkerton, R., Barrett, L., Sevilleja, J. E., et al. (2009). Faecal contamination of drinking water in a Brazilian shanty town: Importance of household storage and new human faecal marker testing. *Journal of Water and Health, 7*(2), 324–331.

Dasgupta, R. (2008). Exploring intra-household factors for diarrhoeal diseases: A study in slums of Delhi, India. *Journal of Water and Health, 6*(2), 289–299.

Dias, L. S. M., & Cunha, S. B. (2018). Changes in the fluvial channels of the canal do Cunha subbasin (RJ): Anthropic interventions. *Revista Equador, 6*(2), 23–43.

Ercumen, A., Gruber, J. S., & Colford, J. M., Jr. (2014). Water distribution system deficiencies and gastrointestinal illness: A systematic review and meta-analysis. *Environmental Health Perspectives, 122*(7), 651–660.

Figueras, M. J., & Borrego, J. J. (2010). New perspectives in monitoring drinking water microbial quality. *International Journal of Environmental Research Public Health, 7*(12), 4179–4202.

Fongaro, G., Nascimento, M. A., Rigotto, C., Ritterbusch, G., da Silva, A. D., Esteves, P. A., et al. (2013). Evaluation and molecular characterization of human adenovirus in drinking water supplies: Viral integrity and viability assays. *Virology Journal, 28*(10), 166.

Fumian, T. M., Leite, J. P. G., Castello, A. A., Gaggero, A., Caillou, M. S., & 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. *Journal of Virological Methods, 170*(1), 42–46.

Fumian, T. M., Vieira, C. B., Leite, J. P., & Miagostovich, M. P. (2013). Assessment of burden of virus agents in an urban sewage treatment plant in Rio de Janeiro, Brazil. *Journal Water Health, 11*(1), 110–119.

Gall, A. M., Mariñas, B. J., Lu, Y., & Shisler, J. L. (2015). Waterborne viruses: A barrier to safe drinking water. *PLoS Pathogen, 11*(6), e1004867.

Gibson, K. E. (2014). Viral pathogens in water: Occurrence, public health impact, and available control strategies. *Current Opinion in Virology, 4*, 50–57.

Gironés, R., Ferrús, M. A., Alonso, J. L., Rodriguez-Manzano, J., Calgua, B., de Corrêa, A., et al. (2010). Molecular detection of pathogens in water—The pros and cons of molecular techniques. *Water Research, 44*(15), 4325–4339.

Grassi, T., Bagordo, F., Idolo, A., Lugoli, F., Gabutti, G., & De Donno, A. (2010). Rotavirus detection in environmental water samples by tangential flow ultrafiltration and RT-nested PCR. *Environmental Monitoring and Assessment, 164*(1–4), 199–205.

Guzman-Herrador, B., Carlander, A., Ethelberg, S., Freiesleben de Blasio, B., Kuusi, M., Lund, V., et al. (2015). Waterborne outbreaks in the Nordic countries, 1998 to 2012. *Euro Surveillance, 20*(24), 21160.

Handam, N. B., dos Santos, J. A. A., Moraes Neto, A. H. A., Duarte, A. N., Alves, E. B. S., Salles, M. J., et al. (2018). Sanitary quality of the rivers in the Communities of Manguinhos´ Territory, Rio de Janeiro, RJ. *Revista Ambiente & Água, 13*(1), e2125.

Heider, S., & Metzner, C. (2014). Quantitative real-time single particle analysis of virions. *Virology, 462–463*, 199–206.

Hernroth, B. E., Conden-Hansson, A. C., Rehnstam-Holm, A. S., Girones, R., & Allard, A. K. (2002). Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: The first Scandinavian report. *Applied Environmental Microbiology, 68*(9), 4523–4533.

Katayama, H., Shimasaki, A., & Ohgaki, S. (2002). Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. *Applied and Environmental Microbiology, 68*(3), 1033–1039.

Kageyama, T., Kojima, S., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F. B., et al. (2003). Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *Journal of Clinical Microbiology, 41*(4), 1548–1557.

Kluge, M., Fleck, J. D., Soliman, M. C., Luz, R. B., Fabres, R. B., Comerlato, J., et al. (2014). Human adenovirus (HAdV), human enterovirus (HEV), and genogroup A rotavirus (GARV) in tap water in southern Brazil. *Journal of Water and Health, 12*(3), 526–532.

Miagostovich, M. P., Guimarães, F. R., Vieira, C. B., Fumian, T. M., da Gama, N. P., Victoria, M., et al. (2014). Assessment of water quality in a border region between the Atlantic forest and an urbanised area in Rio de Janeiro, Brazil. *Food and Environmental Virology, 6*(2), 110–115.

Moreira, N. A., & Bondelind, M. (2017). Safe drinking water and waterborne outbreaks. *Journal Water and Health, 15*(1), 83–96.

O'Brien, E., & Xagoraraki, I. (2019). A water-focused one-health approach for early detection and prevention of viral outbreaks. *One Health, 20*(7), 100094.

Payment, P., & Locas, A. (2011). Pathogens in water: Value and limits of correlation with microbial indicators. *Ground Water, 49*, 4–11.

Pons, W., Young, I., Truong, J., Jones-Bitton, A., McEwen, S., Pintar, K., et al. (2015). A systematic review of waterborne disease outbreaks associated with small non-community drinking water systems in Canada and the United States. *PLoS ONE, 10*(10), e0141646.

Portes, S. A., Volotão, E. M., Rocha, M. S., Rebelo, M. C., Xavier, M. P., Assis, R. M., et al. (2016). A non-enteric adenovirus A12 gastroenteritis outbreak in Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz, 111*(6), 403–406.

Prevost, I. B., Goulet, M., Lucas, F. S., Joyeux, M., Moulin, L., & Wurtzer, S. (2016). Viral persistence in surface and drinking water: Suitability of PCR pre-treatment with intercalating dyes. *Water Research, 91*(15), 68–76.

Rajal, V. B., McSwain, B. S., Thompson, D. E., Leutenegger, C. M., Kildare, B. J., & Wuertz, S. (2007). Validation of hollow fiber ultrafiltration and real-time PCR using bacteriophage PP7 as surrogate for the quantification of viruses from water samples. *Water Research, 41*(7), 1411–1422.

Rachmadi, A. T., Torrey, J. R., & Kitajima, M. (2016). Human polyomavirus: Advantages and limitations as a human-specific viral markerin aquatic environments. *Water Research, 15*(105), 456–469.

Rames, E., Roiko, A., Stratton, H., & Macdonald, J. (2016). Technical aspects of using human adenovirus as a viral water quality indicator. *Water Research, 1*(96), 308–326.

Remigio, R. V., Rabello, R. S., Zulaika, G., Carvalho, M. S., Barrocas, P. R. G., & Lovasi, G. S. (2019). Household-level drinking water quality, access, and management practices within an informal community: A case study at Rio das Pedras, Rio de Janeiro. *Journal of Water, Sanitation and Hygiene for Development, 9*(1), 80–89.

Säve-Söderbergh, M., Bylund, J., Malm, A., Simonsson, M., & Toljander, J. (2017). Gastrointestinal illness linked to incidents in drinking water distribution networks in Sweden. *Water Research, 1*(122), 503–511.

Snyder, R. E., Jaimes, G., Riley, L. W., Faerstein, E., & Corburn, J. (2014). A comparison of social and spatial determinants of health between formal and informal settlements in a large metropolitan setting in Brazil. *Journal of Urban Health, 91*(3), 432–445.

Tam, C., Rodrigues, C. L., Viviani, C. L., Dodds, J. P., Evans, M. R., Hunter, P. R., et al. (2012). Longitudinal study of infectious intestinal disease in the UK (IID2 study): Incidence in the community and presenting to general practice. *Gut, 61*(1), 69–77.

Verani, M., Federigi, I., Donzelli, G., Cioni, L., & Carducci, A. (2019). Human adenoviruses as waterborne index pathogens and their use for Quantitative Microbial Risk Assessment. *Science of the Total Environment, 651*(1), 1469–1475.

WHO. (2011). Chapter 2. A conceptual framework for implementing the Guidelines. In *WHO guidelines for drinking-water quality* (4th ed.). https://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf.

WHO. (2019). World Health Organization—Drinking Water. Retrieved November 4, 2019, from Disponível em: https://www.who.int/news-room/fact-sheets/detail/drinking-water.

Zeng, S. Q., Halkosalo, A., Salminen, M., Szakal, E. D., Puustinen, L., & Vesikari, T. (2008). One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. *Journal of Virological Methods, 153*, 238–240.