

Human adenovirus (HAdV), human enterovirus (hEV), and genogroup A rotavirus (GARV) in tap water in southern Brazil

M. Kluge, J. D. Fleck, M. C. Soliman, R. B. Luz, R. B. Fabres, J. Comerlato, J. V. S. Silva, R. Staggemeier, A. D. Vecchia, R. Capalonga, A. B. Oliveira, A. Henzel, C. Rigotto and F. R. Spilki

ABSTRACT

The effects of viral gastroenteritis are more devastating in children than in any other age category. Thus, children exposed to the consumption of low quality water are at an increased risk of infection, especially in regions where sanitation is inadequate. The present study aimed to provide a survey of the occurrence of representative enteric viruses: human adenovirus (HAdV), human enteroviruses (hEV), and genogroup A rotavirus (GARV) in tap water samples collected in public schools located at six municipalities of Rio Grande do Sul, southern Brazil. Seventy-three schools were included in the study and tap water samples were analyzed by conventional PCR for the presence of HAdV, hEV, and GARV genomes. hEV showed the highest detection rate (27.4%), followed by HAdV (23.3%), and GARV (16.4%). New approaches to water monitoring should be considered to promote a better water quality and reduce the risk of waterborne diseases, especially considering drinking water to be served to vulnerable individuals.

Key words | GARV, HAdV, hEV, tap water, virological analysis of water

M. Kluge
J. D. Fleck
M. C. Soliman
R. B. Luz
R. B. Fabres
J. V. S. Silva
R. Staggemeier
A. D. Vecchia
A. Henzel
C. Rigotto
F. R. Spilki (corresponding author)
Laboratório de Microbiologia Molecular (LMM),
Instituto de Ciências da Saúde (ICS),
Universidade Feevale, Novo Hamburgo, RS,
Brazil
E-mail: fernandors@feevale.br

J. Comerlato
Laboratório de Virologia,
Instituto de Ciências Biológicas da Saúde,
Universidade Federal do Rio Grande do Sul,
Porto Alegre, RS,
Brazil

R. Capalonga
A. B. Oliveira
CECANE – UFRGS,
Universidade Federal do Rio Grande do Sul,
Porto Alegre, RS,
Brazil

INTRODUCTION

The transmission of viruses through consumption of contaminated water and food is well recognized. Gastroenteritis associated with water ingestion is commonly reported and is an important health concern that can be caused by a wide array of viruses (Gallimore *et al.* 2004; Fabiana *et al.* 2007; Grant *et al.* 2012). Water quality may be greatly affected by the presence of pathogenic microorganisms derived from fecal pollution. Enteric viruses, which are transmitted by the fecal–oral route, are one of the most important causes of diarrhea in infants and children (Cheng *et al.* 2005; Boschi-Pinto *et al.* 2008; Hamza *et al.* 2011).

The most commonly studied enteric viruses on environmental matrices are human adenovirus (HAdV) and human

enteroviruses (hEV), genogroup A rotaviruses (GARV), norovirus and hepatitis A and E viruses (Leclerc *et al.* 2002; Fong *et al.* 2005; Abdel-Moety *et al.* 2008; Gibson *et al.* 2011). Most of these viruses are non-enveloped, which makes them highly resistant in the environment as well as more resistant than bacteria to decontamination processes used in drinking and wastewater treatments (Sobsey & Meschke 2003; Van Heerden *et al.* 2003; Bofill-Mas *et al.* 2006; Bosch *et al.* 2006).

The HAdV is a virus with double-stranded DNA virus genome 28–45 kilobases (kb) long, belonging to genus *Mastadenovirus*, family *Adenoviridae* (ICTV 2009). At least 57 serotypes of HAdV in six subgroups (A–F) have been

described (Buckwalter *et al.* 2012); HAdV serotypes 40/41, included in group F, are the major causes of gastroenteritis in young children and are readily spread by the fecal–oral route. The HAdV can cause respiratory infections, gastroenteritis, conjunctivitis and cystitis in humans (Uhnnoo *et al.* 1984; Lenaerts *et al.* 2008; ICTV 2009). hEVs are positive-sense RNA viruses, with a genome of 7.2–8.5 kb, belonging to *Enterovirus* genus of the *Picornaviridae* family which comprises, among others, polioviruses, coxsackieviruses, and echoviruses. These viruses may replicate in the respiratory tract and the gut and can be transmitted through aerosols and by the respiratory route or via the fecal–oral route (Rodríguez-Lázaro *et al.* 2012). Infections by hEVs are usually asymptomatic or cause mild illness (Pallansch & Roos 2001; ICTV 2009; Lee *et al.* 2009). Rotaviruses (RV) are segmented double-stranded RNA viruses with genome of approximately 18.5 kb. These viruses belong to the *Reoviridae* family, and there are five major groups (A–E); group A RV (GARV) is associated with a large majority of human RV infections and represents the major cause of child mortality (Rodríguez-Lázaro *et al.* 2012) because of severe gastroenteritis in infants (Caprioli *et al.* 1996; Midthun & Kapikian 1996). Approximately 2 million deaths related to diarrhea are reported each year globally; GARV is responsible for almost 440,000 deaths per year among children under 5 years old, and is considered as the most important viral agent in severe cases of gastroenteritis in overcrowded hospitals (WHO 2009).

Given that these non-enveloped viruses are more resistant to decontamination processes used in both drinking and wastewater treatment, the present study aimed to provide a survey of the occurrence of HAdV, hEV, and GARV genomes in tap water samples from schools located at six municipalities of Rio Grande do Sul, southern Brazil.

MATERIALS AND METHODS

Water samples

Seventy-three tap water samples, one for each school, were collected aseptically in 500 mL sterile plastic bottles. The tap water samples were collected from the kitchen faucet of the schools, which used the tap water for the cooking

of meals, preparation of fruit juices and washing of raw vegetables served to children for lunch. The sampling was performed from July to November 2009, in 19 schools from the city of Caxias do Sul (435,564 inhabitants), 16 from Pelotas (328,275 inhabitants), 12 from Santa Maria (261,031 inhabitants), 10 from Passo Fundo (184,826 inhabitants), nine from Bagé (116,764 inhabitants), and seven from Santa Cruz do Sul (118,374 inhabitants) (IBGE 2010). Figure 1 shows the location of the municipalities within the so-called mesoregions of the State of Rio Grande do Sul (geographic economic clusters within this state). All schools received water from conventional water treatment plants and were monitored following the Brazilian guidelines for drinking water quality (Ministry of Health 2011). The conventional water treatment includes the processes of coagulation, flocculation, decantation, filtration, chlorination, and fluoridation. The samples were transported to the laboratory under refrigeration (4 °C), and were kept in this condition until sample concentration.

Sample concentration

Tap water samples were concentrated using an adsorption-elution method with negatively charged membranes (HA, Millipore, USA), based on the method proposed by Katayama *et al.* (2002) with few modifications, as described by Vecchia *et al.* (2012). Briefly, 500 mL of each water sample was mixed with 0.3 g MgCl₂ and pH adjusted to 5.0 with 10% HCl. Subsequently, the resulting mixture was vacuum filtered through a type HA negatively charged sterile membrane (0.45 µm pore size; 47 mm diameter). The membrane was rinsed with 87.5 mL of 0.5 mM H₂SO₄ (pH 3.0), followed by elution of viral particles adsorbed to the membrane with 2.5 mL of 1 mM NaOH (pH 10.5). The filtrate was then neutralized with 12.5 µL of 50 mM H₂SO₄ and 12.5 µL of 100× Tris–EDTA buffer. All procedures were made on biosafety cabinets to avoid sample contamination. The resulting mixture was aliquoted and stored at –80 °C until further processing.

Viral nucleic acid extraction

The viral nucleic acids (DNA of HAdV; and RNA of hEV and GARV) were extracted from 400 µL of the concentrated

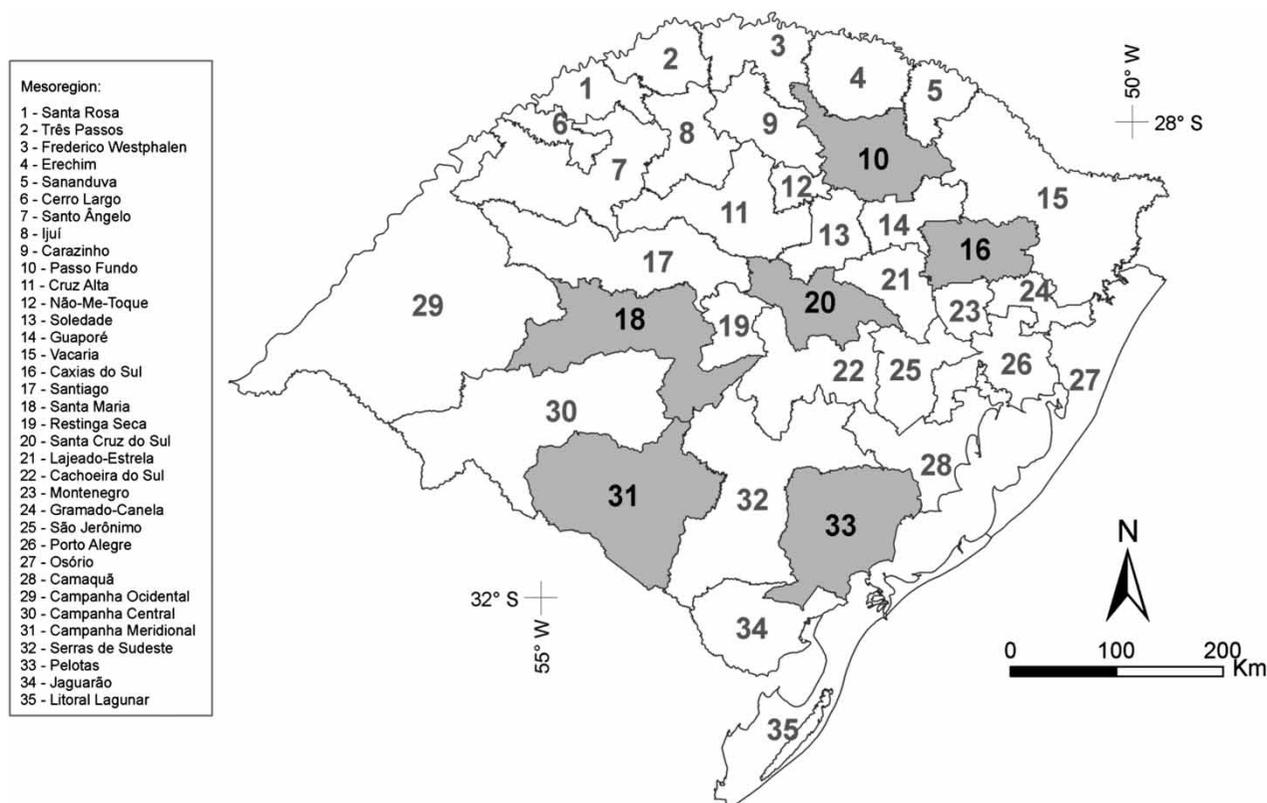


Figure 1 | Mesoregions of the State of Rio Grande do Sul. Each region hachured (in gray) on the map represents one of the municipalities in which sampling was performed.

sample using the RTP[®] DNA/RNA Virus Mini Kit (Invitex, Berlin, Germany) according to the manufacturer's instructions. The obtained viral DNA and RNA were kept at -80°C until further analysis.

Polymerase chain reaction (PCR) assays for the detection of viral genomes

In order to achieve amplification of hEV and GARV genomes, a previous step of cDNA synthesis was carried out before amplification, using the High Capacity cDNA Reverse Transcription[™] commercial kit (Applied Biosciences[™], USA), with the aid of random primers and RNase Inhibitor (Applied Biosciences), following the manufacturer's instructions.

Conventional polymerase chain reactions were optimized for the detection of HAdV, hEV, and GARV genomes. Reactions were standardized as follows: (1) HAdV and GARV: 50 μL reaction mixtures consisting of 25 μL of GoTaq[®] Green Master Mix (Promega, USA),

18 μL of nuclease-free water, 1 μL of each primer (20 pmole), and 5 μL of nucleic acid; (2) hEV: 25 μL final volume containing 12.5 μL of 2 \times PCR Master Mix[™] (LGCbio, Brazil), 7.5 μL of nuclease-free water, 1 μL of each primer (20 pmole), and 3 μL of cDNA product. DNase/RNase free water was used as a negative control during all PCR assays. The positive controls used in amplifications were Poliovirus-1 (Sabin strain), kindly provided by Dr Carlos Nozawa; HAdV types 2 and 5, kindly provided by Dr Célia Barardi; and Human-GARV (VP6 I-2 Genotype), isolated from a clinical sample collected from a child with diarrhea at the nearby municipality of Porto Alegre, diagnosed and isolated in our laboratory.

All PCR reactions were performed as described previously (Vecchia *et al.* 2012) using primers designed to anneal in highly conserved regions of each viral genome. The primers used for HAdV amplification targeted the hexon protein gene, VTB2-HAdVCf (5'-GAGACGTACTTCAGCCTGAAT-3') and VTB2-HAdVCr (5'-GATGAACCGCAGCGTCAA-3') (Wolf *et al.* 2010). The primers used in hEV PCR reactions targeted

the 5-UTR region, ENT-F1 (5'-CCTCCGGCCCTGAATG-3') and ENT-R2 (5'-ACACGGACACCCAAAGTAG-3') (Tsai *et al.* 1993; Vecchia *et al.* 2012). Finally, the primers used to amplify GARV genome targeted the VP6 protein gene, ROTAFEEVALE-FW (5'-GATGTCCTGTACTCCTTGT-3') and ROTAFEEVALE-REV (5'-GGTAGATTACCAATTCCTCC-3') (Vecchia *et al.* 2012).

Amplification of the target genomic fragments was performed using a thermal cycler (MultiGene[®]; Labnet International, USA). The PCR conditions were optimized for each virus group and were as follows: (1) HAdV: 98 °C for 7 min, 40 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min; (2) hEV: 98 °C for 5 min, 35 cycles of 94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min; and (3) GARV: 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, i.e., touchdown PCR), 72 °C for 1 min. Then, all reactions were left at 72 °C for 7 min for final elongation and submitted to an infinite cycle at 4 °C.

After the reactions, PCR products were stained with a nontoxic fluorescent dye, SYBR[®] Safe DNA Gel Stain (Invitrogen), analyzed by electrophoresis on 2% (w/v) agarose gel and visualized under ultraviolet (UV) light. The analytical sensitivity of each PCR has been described elsewhere (Vecchia *et al.* 2012). The identity of the amplicons was confirmed by nucleotide sequencing and all the positive samples were confirmed as expected viral targets.

Data analyses

The viral detection rate was compared with the percentage of sewage treatment on each municipality. The secondary data regarding the percentages of sewage treatment were

collected from the Brazilian Sanitation National Information System (SNIS 2010). Correlation coefficients were calculated using the CORREL function on Microsoft Excel 2010[™] spreadsheet software.

RESULTS

Overall, hEV were present in the majority of samples analyzed, achieving the highest detection rate (27.4%), followed by HAdV (23.3%) and GARV (16.4%). Results are summarized in Table 1. More than one type of the tested viruses was detected in 10 (13.7%) of the 73 samples analyzed. Two samples showed the simultaneous detection of HAdV, hEV, and GARV and eight samples showed the simultaneous detection of two viral species (four samples with HAdV and hEV genomes, three with HAdV and GARV, and one with hEV and GARV genomes). The three groups of viruses investigated in this study were detected in all cities, except for hEV, which was not found in the Santa Cruz do Sul samples.

DISCUSSION

In the present study, we were able to detect enteric viruses in tap water from public schools located at six cities of Rio Grande do Sul, southern Brazil. Our group recently described the presence of Torque teno virus (TTV) at these same sampling sites in a minor study made in parallel to the present one (Vecchia *et al.* 2013). Several studies have reported the presence of enteric viruses in tap water treated by conventional processes, which usually includes

Table 1 | Frequency of detection of the HAdV, hEV, and GARV viruses for each municipality

| City | Number of samples | HAdV | hEV | GARV |
|-------------------|-------------------|---------------|---------------|---------------|
| Bagé | 9 | 1/9 (11.1%) | 1/9 (11.1%) | 1/9 (11.1%) |
| Caxias do Sul | 19 | 4/19 (21%) | 10/19 (52.6%) | 3/19 (15.8%) |
| Passo Fundo | 10 | 4/10 (40%) | 6/10 (60%) | 3/10 (30%) |
| Pelotas | 16 | 5/16 (31.2%) | 1/16 (6.2%) | 3/16 (18.8%) |
| Santa Cruz do Sul | 7 | 1/7 (14.3%) | 0/7 (0%) | 1/7 (14.3%) |
| Santa Maria | 12 | 2/12 (16.7%) | 2/12 (16.7%) | 1/12 (8.3%) |
| Total | 73 | 17/73 (23.3%) | 20/73 (27.4%) | 12/73 (16.4%) |

coagulation, flocculation, sedimentation, filtration, and chlorination (Ehlers *et al.* 2005; Heerden *et al.* 2005; Ye *et al.* 2012). The presence of GARV in drinking water was previously reported by He *et al.* (2009) in Beijing, China, who found GARV genome in 22.4% (32/143) of tap water samples, while in our study GARV genome was present in 16.4% (12/73) of samples. HAdV and hEVs were detected in drinking water of urban areas in South Korea by ICC-PCR in 39.1% (9/23) and 47.8% (11/23) of the tap water samples, respectively (Lee & Kim 2002), while our results showed a detection rate of 23.3% (17/73) for HAdV and 27.7% (20/73) for hEV. In our previous published results for TTV, only 11.6% of the samples were positive (in a limited number of samples analyzed) (Vecchia *et al.* 2013). In another study conducted by Lee *et al.* (2005), HAdV and hEVs were detected in tap water samples in Seoul, South Korea, using ICC-multiplex-nested-PCR assay. Fifty samples were tested and 58% (29/50) were positive for at least one virus, whereas we detected HAdV and/or hEV in 42.74% (31/73) of the total samples. These tap water samples were treated with conventional flocculation, sedimentation, filtration, and chlorination, which is similar to the treatment applied to water samples analyzed in this study. Nevertheless, it is important to keep in mind that the sample volume analyzed in these studies are different from the volume used in our study, thus making difficult the comparison of the results.

The percentage of treated sewage of each municipality was compared with the viral detection rates, since enteric viruses can be introduced in the environment through untreated or inadequately treated wastewater (Bosch *et al.* 2006; Okoh *et al.* 2010). As mentioned before, the sewage treatment rate of each municipality was taken from Brazilian SNIS, which is maintained by the Brazilian Government and provides data on the current situation of sanitation and wastewater treatment in Brazil. However, these data must be used cautiously since there is a lack of studies and supportive data about how the sewage discharge may impact the water quality in these areas. The municipality of Santa Maria has the highest sewage treatment rate (64.7%), followed by Passo Fundo (21.5%), Pelotas (19.2%), Bagé (16.8%), Santa Cruz do Sul (16.1%), and Caxias do Sul (12.1%) (SNIS 2010). Correlation analysis performed between the percentage of treated sewage and the

detection of viruses showed, as expected, a negative relationship ($r = -0.16$). An additional analysis was performed between the percentage of treated sewage and each virus individually, which showed a negative correlation for HAdV ($r = -0.14$), hEVs ($r = -0.14$), and GARV ($r = -0.41$). Nevertheless, these correlation analyses were based upon a small sample size and an increase in the number of samples is required to better evaluate the relationship between the percentage of treated sewage and the viral detection rates.

CONCLUSIONS

The viral monitoring of drinking water is not mandatory according to Brazilian legislation (Ministry of Health 2011); however, virological analyses of water are important for risk assessment studies in order to reduce the health and economic impacts caused by gastroenteritis outbreaks. This is especially important when it comes to environments frequented by children, which are the most vulnerable population to viral gastroenteritis.

Although viral viability was not assayed in this study, the presence of enteric viruses' genomes in drinking water samples represents a risk to public health and may indicate the inefficiency of conventional water treatment to promote the total disinfection of viral agents. Therefore, these analyses must be taken into consideration for a new monitoring approach to promote a better water quality and reduce the risk of waterborne diseases, especially when it comes to susceptible groups such as children.

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REFERENCES

- Abdel-Moety, N. M., Al-Fassi, F. A. & Ali, M. A. 2008 Health aspects of virological water quality: an overview review. *J. Appl. Sci. Res.* **4** (10), 1205–1215.
- Boffill-Mas, S., Albinana-Gimenez, N., Clemente-Casares, P., Hundesa, A., Rodriguez-Manzano, J., Allard, A., Calvo, M. & Girones, R. 2006 Quantification and stability of human adenoviruses and polyomavirus JCPyV in wastewater matrices. *Appl. Environ. Microbiol.* **72** (12), 7894–7896.
- Bosch, A., Pintó, R. M. & Abad, F. X. 2006 Survival and transport of enteric viruses in the environment. In: *Viruses in Foods (Food Microbiology and Food Safety)* (S. M. Goyal, ed.). Springer US, New York, pp. 151–187.
- Boschi-Pinto, C., Velebit, L. & Shibuya, K. 2008 Estimating child mortality due to diarrhoea in developing countries. *Bull. World Health Organ.* **86** (9), 710–717.
- Buckwalter, S. P., Teo, R., Espy, M. J., Sloan, L. M., Smith, T. F. & Pritt, B. S. 2012 Real-time qualitative PCR for 57 human adenovirus types from multiple specimen sources. *J. Clin. Microbiol.* **50** (3), 766–771.
- Caprioli, A., Pezzella, C., Morelli, R., Giammanco, A., Arista, S., Crotti, D., Facchini, M., Guglielmetti, P., Piersimoni, C. & Luzzi, I. 1996 Enteropathogens associated with childhood diarrhea in Italy. *Pediatr. Infect. Dis. J.* **15** (10), 876–883.
- Cheng, A. C., McDonald, J. R. & Thielman, N. M. 2005 Infectious diarrhea in developed and developing countries. *J. Clin. Gastroenterol.* **39** (9), 757–773.
- Ehlers, M. M., Grabow, W. O. & Pavlov, D. N. 2005 Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. *Water Res.* **39** (11), 2253–2258.
- Fabiana, A., Donia, D., Gabrieli, R., Petrinca, A. R., Cenko, F., Bebeci, D., Altan, A. M., Buonomo, E. & Divizia, M. 2007 Influence of enteric viruses on gastroenteritis in Albania: epidemiological and molecular analysis. *J. Med. Virol.* **79** (12), 1844–1849.
- Fong, T. T., Griffin, D. W. & Lipp, E. K. 2005 Molecular assays for targeting human and bovine enteric viruses in coastal waters and their application for library-independent source tracking. *Appl. Environ. Microbiol.* **71** (4), 2070–2078.
- Gallimore, C. I., Cubitt, D. W., Richards, A. F. & Gray, J. J. 2004 Diversity of enteric viruses detected in patients with gastroenteritis in a tertiary referral paediatric hospital. *J. Med. Virol.* **73** (3), 443–449.
- Gibson, K. E., Opryszko, M. C., Schissler, J. T., Guo, Y. & Schwab, K. J. 2011 Evaluation of human enteric viruses in surface water and drinking water resources in Southern Ghana. *Am. J. Trop. Med. Hyg.* **84** (1), 20–29.
- Grant, L., Vinjé, J., Parashar, U., Watt, J., Reid, R., Weatherholtz, R., Santosham, M., Gentsch, J. & O'Brien, K. 2012 Epidemiologic and clinical features of other enteric viruses associated with acute gastroenteritis in American Indian infants. *J. Pediatr.* **161** (1), 110–115.
- Hamza, I. A., Jurzik, L., Überla, K. & Wilhelm, M. 2011 Methods to detect infectious human enteric viruses in environmental water samples. *Int. J. Hyg. Environ. Health.* **214** (6), 424–436.
- He, X. Q., Cheng, L., Zhang, D. Y., Li, W., Xie, X. M. & Ma, M. 2009 First molecular detection of group A rotaviruses in drinking water sources in Beijing, China. *Bull. Environ. Contam. Toxicol.* **83** (1), 120–124.
- Heerden, J., Ehlers, M. M., Vivier, J. C. & Grabow, W. O. 2005 Risk assessment of adenoviruses detected in treated drinking water and recreational water. *J. Appl. Microbiol.* **99** (4), 926–933.
- IBGE – The Brazilian Institute of Geography and Statistics 2010 Available at: <http://www.ibge.gov.br/censo/censobrasil.shtm> (accessed 15 November 2012).
- ICTV 2009 Virus Taxonomy: 2009 Release. Available at: <http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/> (accessed 15 November 2012).
- 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. *Appl. Environ. Microbiol.* **68** (3), 1033–1039.
- Leclerc, H., Schwartzbrod, L. L. & Dei-Cas, E. 2002 Microbial agents associated with waterborne diseases. *Crit. Rev. Microbiol.* **28** (4), 371–409.
- Lee, S. H. & Kim, S. J. 2002 Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. *Water Res.* **36**, 248–256.
- Lee, S. H., Lee, C., Lee, K. W., Cho, H. B. & Kim, S. J. 2005 The simultaneous detection of both enteroviruses and adenoviruses in environmental water samples including tap water with an integrated cell culture-multiplex-nested PCR procedure. *J. Appl. Microbiol.* **98** (5), 1020–1029.
- Lee, T. C., Guo, H. R., Su, H. J., Yang, Y. C., Chang, H. L. & Chen, K. T. 2009 Diseases caused by enterovirus 71 infection. *Pediatr. Infect. Dis. J.* **28** (10), 904–910.
- Lenaerts, L., De Clercq, E. & Naesens, L. 2008 Clinical features and treatment of adenovirus infections. *Rev. Med. Virol.* **8** (6), 357–374.
- Midthun, K. & Kapikian, A. Z. 1996 Rotavirus vaccines: an overview. *Clin. Microbiol. Rev.* **9** (3), 423–434.
- Ministry of Health 2011 PORTARIA, MS N° 2914/2011 do Ministério da Saúde – refers to the procedures for control and monitoring of water quality for human consumption and its potability standards. *Official Gazette of the Federative Republic of Brazil*. Brazilian Ministry of Health, Brasília.
- Okoh, A. I., Sibanda, T. & Gusha, S. S. 2010 In adequately treated wastewater as a source of human enteric viruses in the environment. *Int. J. Environ. Res. Public Health.* **7**, 2620–2637.

- Pallansch, M. A. & Roos, R. P. 2001 Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: *Fields Virology* (D. M. Knippe & P. M. Howley, eds). Lippincott Williams and Wilkins, Philadelphia, PA, pp. 723–775.
- Rodríguez-Lázaro, D., Cook, N., Ruggeri, F. M., Sellwood, J., Nasser, A., Nascimento, M. S., D'Agostino, M., Santos, R., Saiz, J. C., Rzeżutka, A., Bosch, A., Gironés, R., Carducci, A., Muscillo, M., Kovač, K., Diez-Valcarce, M., Vantarakis, A., von Bonsdorff, C. H., de Roda Husman, A. M., Hernández, M. & van der Poel, W. H. 2012 **Virus hazards from food, water and other contaminated environments**. *FEMS Microbiol. Rev.* **36** (4), 786–814.
- SNIS – Sistema Nacional de Informações sobre Saneamento 2010 Available at: <http://www.snis.gov.br/PaginaCarrega.php?EWRErterterTERTer=29> (accessed 15 November 2012).
- Sobsey, M. D. & Meschke, J. S. 2003 *Virus survival in the environment with special attention to survival in sewage droplets and other environmental media of fecal or respiratory origin*. World Health Organization. Available at: http://www.unc.edu/courses/2008spring/envr/421/001/WHO_VirusSurvivalReport_21Aug2003.pdf (accessed 15 November 2012).
- Tsai, Y. L., Sobsey, M. D., Sangermano, L. R. & Palmer, C. J. 1993 Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. *Appl. Environ. Microbiol.* **59** (10), 3488–3491.
- Uhnoo, I., Wadell, G., Svensson, L. & Johansson, M. E. 1984 Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. *J. Clin. Microbiol.* **20** (3), 365–372.
- Van Heerden, J., Ehlers, M. M., Van Zyl, W. B. & Grabow, W. O. 2003 **Incidence of adenoviruses in raw and treated water**. *Water Res.* **37** (15), 3704–3708.
- Vecchia, A. D., Fleck, J. D., Comerlato, J., Kluge, M., Bergamaschi, B., Da Silva, J. V., Da Luz, R. B., Teixeira, T. F., Garbinatto, G. N., Oliveira, D. V., Zanin, J. G., Van der Sand, S., Frazzon, A. P., Franco, A. C., Roehe, P. M. & Spilki, F. R. 2012 **First description of adenovirus, enterovirus, rotavirus and Torque teno virus on surface water samples collected from the Arroio Dilúvio, Porto Alegre, Brazil**. *Braz. J. Biol.* **72** (2), 323–329.
- Vecchia, A. D., Kluge, M., dos Santos da Silva, J. V., Comerlato, J., Rodrigues, M. T., Fleck, J. D., da Luz, R. B., Teixeira, T. F., Roehe, P. M., Capalonga, R., Oliveira, A. B. & Spilki, F. R. 2013 **Presence of Torque teno virus (TTV) in tap water in public schools from Southern Brazil**. *Food Environ. Virol.* **5** (1), 41–45.
- Wolf, S., Hewitt, J. & Greening, G. E. 2010 **Viral multiplex quantitative PCR assays for tracking sources of fecal contamination**. *Appl. Environ. Microbiol.* **76** (5), 1388–1394.
- World Health Organization 2009 Diarrheal diseases (updated February 2009): Rotavirus. Available at: http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index5.html (accessed 9 July 2012).
- Ye, X. Y., Ming, X., Zhang, Y. L., Xiao, W. Q., Huang, X. N., Cao, Y. G. & Gu, K. D. 2012 **Real-time PCR detection of enteric viruses in source water and treated drinking water in Wuhan, China**. *Curr. Microbiol.* **65** (3), 244–253.

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