HEV infectivity in water samples

Subjects: Virology | Biochemistry & Molecular Biology | Microbiology
Contributor: Maria Caeiro

Hepatitis E virus (HEV) is a non-enveloped single-stranded positive-sense RNA virus, belonging to the Hepeviridae family, resistant to environmental conditions, and transmitted by the consumption of contaminated water. This virus is responsible for both sporadic and epidemic outbreaks, leading to thousands of infections per year in several countries, and is thus considered an emerging disease in Europe and Asia. This study refers to a survey in Portugal during 2019, targeting the detection and eventual quantification of enteric viruses in surface and drinking water sources. Samples positive for HEV RNA were recurrently found by reverse transcription quantitative PCR (RT-qPCR), in both matrices. The infectivity of these samples was evaluated in cultured Vero E6 cells and RNA from putative viruses produced in cell cultures was subjected to RT-qPCR targeting HEV genomic RNA. Our results evidenced the existence of samples positive either for HEV RNA (77.8% in surface water and 66.7% in drinking water) or for infectious HEV (23.0% in surface water and 27.7% in drinking water). These results highlight the need of effective virological control of water for human consumption and activities.

Keywords: drinking water ; hepatitis E virus ; RT-qPCR ; surface water ; Vero E6 cell line ; viral infectivity ; water quality ; infectious water samples

1. Potential Infectivity of the Water Samples

1.1. Effect on Vero E6 Cultures and Production of Putative Viral Particles

Thirty-four concentrated samples from water collected in 2019, between January and August, and covering all sampling sites, were considered for infectivity assays in Vero E6 cultures. Only samples previously identified by RT-qPCR as positive for HEV RNA were selected, except when the related sample (collected on the same date in the associated water matrix) was positive.

Most cultures (19 in 32), each one inoculated with 0.5 mL of a water sample, did not develop cytopathic effects (CPEs) during the incubation period (15 days). Nevertheless, putative viral particles (pVPs) from this first passage were collected (supernatants of "infected" cultures) and used to "infect" new Vero E6 cultures. From these, 17 developed CPEs within 2–6 days post-inoculation; these second passage pVPs in the supernatants from cultures displaying, or not, CPEs, were collected, concentrated (for 6 h at 17,000× g, and 4 ^{o}C) and subjected to RNA extraction and purification.

1.2. RT-qPCR Evaluation of Putative Infectious HEV Produced in Vero E6 Cultures

RNA extracted from pVPs produced as referred to above, was subjected to RT-qPCR, carried out with CeeramTools Hepatitis E kit (bioMérieux; Marcy-l'Etoile, France). HEV infectivity was confirmed by RT-qPCR positive results that met the quality criteria established by the mentioned kit.

From the samples evaluated, 18 were related samples from the river and WTP_R (eight from each) (Table 1), eight from the dam reservoir and WTP_D (four from each) (Table 2), and six were from the sampling point in the distribution network (Table 3). HEV infectivity was confirmed in samples from all matrices (globally 25%): 3/13 (23.0%) from surface water were positive (two from the river and one from the dam reservoir) as well as 5/18 (27.7%) from drinking water (three from WTP_R, one from WTP_D and one from the sampling point in the distribution network) (Table 1, Table 2 and Table 3).

Table 1. Evaluation of related concentrated water samples (river and WTP_R) for the presence of HEV RNA and infectious particles.

Months	HEV RNA (gc/L)		HEV Reduction (%) after Treatment	HEV Infectivity (*)	
	River	WTP_R		River	WTP_R
February	355.5	320.8	9.8	Negative	Negative
February	78.2	49.3	37	Negative	Negative
March	4,029.1	0	100	Negative	Negative
April	7,383.1	2,379.3	67.8	Negative	Negative
May	1,936.5	428	77.9	Negative	Positive
June	1,394.9	126	91	Positive	Positive
July	1,755	22	98.7	Negative	Negative
August	206.5	24.2	88.3	Negative	Negative
August	113.3	0	100	Positive	Positive

Table 2. Evaluation of related concentrated water samples (dam reservoir and WTP_D) for the presence of HEV RNA and infectious particles.

Months	HEV RNA (gc/L)		HEV Reduction (%) after Treatment	HEV Infectivity (*)	
	Dam Reservoir	WTP_D		Dam Reservoir	WTP_D
February	29.1	75.2	NR	Negative	Negative
April	109,687.5	5,617.1	94.9	Negative	Negative
May	2,412	0	100	Negative	Negative
June	0	58.7	NR	Positive	Positive

Table 3. Evaluation of concentrated water samples from a sampling point in the distribution network, for the presence of HEV RNA and infectious particles.

Months	HEV RNA (gc/L)	HEV Infectivity (*)
January	46.9	Negative
April	8,926.6	Negative
May	1,473.5	Negative
June	133.3	Negative
July	221.4	Positive
August	186.6	Negative

It was possible to determine that 1) most positive samples for HEV infectivity had also tested positive for HEV RNA (exceptions were WTP_R from August and dam reservoir from June) and 2) positive samples for HEV infectivity were frequently found in related samples, i.e., in river/WTP_R and dam reservoir/WTP_D sampled on the same date; one exception was found in river/WTP_R from May (only WTP_R was positive for infectious HEV) (Table 1, Table 2 and Table 3). Moreover, a relationship was not evidenced between the number of RNA copies detected in a water sample and its potential infectivity because, from the 11 samples presenting more than 1000 gc/L, only one (river from June) evidenced infectivity; values of gc/L between 0 and 428 had been found in all the others able to produce infectious HEV in Vero E6 cells.

2. Discussion

This study followed a complex approach to assess the presence of HEV, starting from high volumes of water (EPA Method 1615 of the United States Environmental Protection Agency [1]) and combining, in the same procedure, the possibility to detect viral RNA by RT-qPCR as well as evaluate infectivity. The use of cell cultures overcame the limitation of evaluations based only on RT-qPCR. In fact, RT-qPCR has been increasingly used to detect enteric viruses in water and food samples, with high specificity/sensitivity and the possibility of obtaining results in less than four hours [2][3][4]. However, this methodology does not allow assessing the infectivity associated with the viral genomes detected in the reaction [2][5][6]. Beyond the confirmation of viral genomes, it is crucial in the evaluation of risks to public health, to determine whether they correspond to viral particles with the ability to infect human cells [3][4][7][8]. Despite being expensive and time consuming, relying on cell cultures is the most used standard method for assessing the infectivity of viral particles [2][9][10].

This one-year survey evaluated the presence of HEV in concentrated samples from two bodies of water (a river and a dam reservoir) and from the drinking water sampled on their water treatment plants (WTP_R and WTP_D, respectively) at the end of the treatment process. A mammal cell line (Vero E6) derived from African green monkey (Cercopithecus aethiops) kidney was used, for the first time, to assay the potential infectivity of water samples where HEV RNA had been detected by RT-qPCR. The rationale for the utilization of this cell line was its capability to replicate many different viruses [11][12], also taking into account that HEV has a large host range [13][14]. This approach effectively resulted in the detection of infectious HEV in several samples, by more or less evident induction of CPEs in cultured cells and subsequent confirmation of HEV replication through RT-qPCR to RNA extracted from extracellular putative viral particles. Our results agree with a recent study [15] demonstrating that a wild-derived HEV strain replicated in Vero cells, the cell line from which Vero E6 was derived (46).

HEV RNA was detected in concentrated samples from the two bodies of water and from drinking water (Entry _HEV genomic RNA detection), and in an infectious state in several of these samples.

Although the peak HEV RNA concentration was found in April, both in the river and in the dam reservoir, infectious HEV was only detected in June, when HEV RNA concentrations were high in the river (>1000 gc/L) and zero in the dam reservoir, suggesting inexistence of a direct association between the number of detected RNA copies and potential infectivity.

Out of the 18 concentrated drinking water samples selected for evaluation of HEV infectivity, five were positive. Drinking water from WTP_R presented the highest number of infectious samples (three), followed by WTP_D and the water from the point in the distribution network, both with only one infectious sample. Infectious HEV was detected in samples collected between May and August, after the peak of HEV RNA copies, as also observed in the bodies of water. Once again, no clear relationship was found between infectivity and number of HEV RNA copies detected per liter of sampled water.

Even though most of the results did not evidence contradictory aspects, a few should be discussed. One unexpected result was the infectivity of two samples (one from the dam reservoir and the other from WTP_R) originally identified as negative for HEV RNA. This may be explained by eventual mishaps during original RNA extraction procedures and emphasizes the relevance of evaluating results achieved by independent approaches. The detection of an infectious drinking water sample (WTP_R) was also unexpected, when the sampled water from its source (river) did not show infectivity. This may be explained by the large differences in the water volumes subjected to sampling: 1300 L in WTP_R and 152 L in the river. It should be noted that the positive result of infectivity in WTP_R means that HEV was also present in the river to such an extent that infectivity remained after treatment.

The results showing the presence of infectious HEV in concentrated samples of drinking water evidence the need to further investigate eventual threats to human health. It is worth noting that Vero E6 cultures were inoculated with 0.5 mL of concentrated (40×, in average) drinking water samples, equivalent to approximately 17.5 L of the sampled water (from a total of 1400 L, in average), while healthy individuals drink approximately two liters of water each day [16].

Finally, climate change will certainly increase the frequency of pathogens in water systems worldwide, whether due to the occurrence of floods, sewage contamination or the scarcity of safe drinking water sources [2]. In this context and considering the results obtained in this study, monitoring the presence of HEV and other viruses in water supply and distribution systems is strongly advisable. Similar approaches should be conducted in the future, increasing the sampling effort and implementing the application of the quantitative microbial risk assessment (QMRA) [17].

References

1. Fout, G.S.; Brinkman, N.E.; Cashdollar, J.L.; Griffin, M.; McMinn, B.R.; Rhodes, E.R.; Varughese, E.A.; Karim, M.R.; Grimm, A.C.; Spencer, S.K; et al. Method 1615-Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR. *JoVE* **2016**, *107*, 1–13, .
2. Pedro Teixeira; Daniel Salvador; João Brandão; Warish Ahmed; Michael J. Sadowsky; Elisabete Valério; Environmental and Adaptive Changes Necessitate a Paradigm Shift for Indicators of Fecal Contamination. *Microbiology Spectrum* **2020**, *8*, 1–20, 10.1128/microbiolspec.erv-0001-2019.
3. Eiji Haramoto; Masaaki Kitajima; Akihiko Hata; Jason R. Torrey; Yoshifumi Masago; Daisuke Sano; Hiroyuki Katayama; A review on recent progress in the detection methods and prevalence of human enteric viruses in water. *Water Research* **2018**, *135*, 168-186, 10.1016/j.watres.2018.02.004.
4. Roberto A. Rodríguez; Ian L. Pepper; Charles P Gerba; Application of PCR-Based Methods To Assess the Infectivity of Enteric Viruses in Environmental Samples. *Applied and Environmental Microbiology* **2008**, *75*, 297-307, 10.1128/aem.01150-08.
5. Paula A. Baez; Maria Camila Lopez; Alejandra Duque-Jaramillo; Dioselina Pelaez; Francisco Molina; Maria-Cristina Navas; First evidence of the Hepatitis E virus in environmental waters in Colombia. *PLOS ONE* **2017**, *12*, 1–16, 10.1371/journal.pone.0177525.
6. G. Shay Fout; Jennifer L. Cashdollar; Eunice A. Varughese; Sandhya U. Parshionikar; Ann C. Grimm; EPA Method 1615. Measurement of enterovirus and norovirus occurrence in water by culture and RT-qPCR. I. Collection of virus samples. *Journal of Visualized Experiments* **2015**, *107*, 1–13, 10.3791/52067.
7. Leifels, M.; Hamza, I.A.; Krieger, M.; Wilhelm, M.; Mackowiak, M.; Jurzik, L; From Lab to Lake – Evaluation of Current Molecular Methods for the Detection of Infectious Enteric Viruses in Complex Water Matrices in an Urban Area. *PLoS ONE* **2016**, *11*, 1–16, .
8. Ibrahim Ahmed Hamza; Lars Jurzik; Klaus Überla; Michael Wilhelm; Methods to detect infectious human enteric viruses in environmental water samples. *International Journal of Hygiene and Environmental Health* **2011**, *214*, 424-436, 10.1016/j.ijheh.2011.07.014.
9. Wyn-Jones, A.P.; Sellwood, J; A review: Enteric viruses in the aquatic environment. *J. Appl. Microbiol.* **2001**, *91*, 945–962, .

10. Tebelay Dilnessa; Zeleke H; Cell Culture, Cytopathic Effect and Immunofluorescence Diagnosis of Viral Infection. *Journal of Microbiology and Modern Techniques* **2017**, *2*, 1-8, 10.15744/2575-5498.2.102.
11. Nicole C. Ammerman; Magda Beier-Sexton; Abdu F. Azad; Growth and Maintenance of Vero Cell Lines. *Current Protocols in Microbiology* **2008**, , , 10.1002/9780471729259.mca04es11.
12. Format, P.; Properties, C.; Level, B.; Conditions, S. VERO C1008 [Vero 76, Clone E6, Vero E6] (ATCC®CRL-1586TM). Available online: https://www.lgcstandards-atcc.org/Products/All/CRL-1586.aspx?geo_country=pt (accessed on 1 April 2020).
13. Michael A. Purdy; Tim J. Harrison; S. Jameel; X-J. Meng; H. Okamoto; W. H. M. Van Der Poel; Donald B. Smith; ICTV Report Consortium; ICTV Virus Taxonomy Profile: Hepeviridae. *Journal of General Virology* **2017**, *98*, 2645-2646, 10.1099/jgv.0.000940.
14. Alessandra Berto; J R Mesquita; R. Hakze-Van Der Honing; M. S. J. Nascimento; W. H. M. Van Der Poel; Detection and Characterization of Hepatitis E Virus in Domestic Pigs of Different Ages in Portugal. *Zoonoses and Public Health* **2012**, *59*, 477-481, 10.1111/j.1863-2378.2012.01488.x.
15. Juozas Grigas; Evelina Simkute; Martynas Simanavicius; Arnoldas Pautienius; Zaneta Streimikyte-Mockeliune; Dainius Razukevicius; Arunas Stankevicius; Hepatitis E genotype 3 virus isolate from wild boar is capable of replication in non-human primate and swine kidney cells and mouse neuroblastoma cells. *BMC Veterinary Research* **2020**, *16*, 1-11, 10.1186/s12917-020-02315-5.
16. Spero Tsindos; What drove us to drink 2 litres of water a day?. *Australian and New Zealand Journal of Public Health* **2012**, *36*, 205-207, 10.1111/j.1753-6405.2012.00866.x.
17. WHO. Quantitative Microbial Risk Assessment: Application for Water Safety Management. WHO Press: Geneva, Switzerland, 2016; pp. 1–187.