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Enteroviruses in Water: Epidemiology, Detection and Inactivation

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ABSTRACT

Enteroviruses are small non-enveloped RNA viruses commonly found in environmental water samples. Worldwide analysis of water provides evidence of the broad distribution of enteroviruses. In addition to their medical importance, they are used as markers of faecal contamination of water. This review focuses on water-borne human enteroviruses and their transmission. Methods for detecting enteroviruses in water are also presented as well as current strategies to control these enteric viruses in water.

1 | Introduction

Enteroviruses (EVs) are small non-enveloped RNA viruses, with an icosahedral capsid of around 30 nm in diameter. They belong to the *Picornaviridae* family, and the *Enterovirus* genus comprises 15 species (Genus: Enterovirus | ICTV). Their genome is composed of a positive-sense single-stranded RNA of around 7.4 kb that contains two open reading frames (ORFs) (Guo et al. 2019) flanked by untranslated regions (UTR) at the 5′ and 3′ ends. The main ORF codes for most of the proteins, including four structural proteins (VP1, VP2, VP3 and VP4), and seven non-structural proteins including two proteases, $2A^{pro}$ and $3C^{pro}$ (Ryu 2017). The species *Enterovirus krodeni* appears to have two $2A^{pro}$ sequences (Du et al. 2016), and *Enterovirus geswini* contains an inserted porcine torovirus-like papain-like cysteine protease (PLCP) coding region inserted between 2C and 3A (Tsuchiaka et al. 2018). A shorter ORF (ORF2) encodes a single protein, known as ORF2p, that plays a role in the infection of intestinal cells (Lulla et al. 2019) (Figure 1).

EVs are considered of medical importance (Jubelt and Lipton 2014). Most human EV infections are asymptomatic or induce mild symptoms such as fever, headache, common cold and cutaneous eruptions. However, several serotypes induce more severe outcomes. For example, poliovirus, belonging to the *Enterovirus coxsackiepol* species, is the best-known human pathogen that causes both acute poliomyelitis and post-polio syndrome. To date, two of the three wild poliovirus serotypes (WPV2 and WPV3) have been eradicated thanks to the worldwide polio vaccination programmes (Geiger et al. 2024). EV-A71 and several other EVs are responsible for the hand, foot and mouth disease (Chang et al. 2019). Some coxsackieviruses induce organ inflammation such as myocarditis or meningitis, for instance (Liu et al. 2000; Bouin et al. 2016). Coxsackievirus B (CVB), and especially CVB4, is strongly suspected to play a role in the onset of type-1 diabetes (Hober and Sauter 2010).

The global burden of infectious diseases associated with contaminated water is high. In 2019, it is estimated that 1.4 million deaths could have been prevented by safe drinking water, sanitation and hygiene, representing 2.5% of global deaths from all causes (Wolf et al. 2023). Among the agents responsible for water-related diseases, there are EVs that can be transmitted by the feco-oral route. Globally, EVs infect millions of people, but the socio-economic burden is hard to evaluate, as many infections are not formally attributed to EVs due to a lack of comprehensive EV surveillance systems (Lugo and Krogstad 2016; Xie et al. 2024). EVs therefore belong to the paraphyletic group

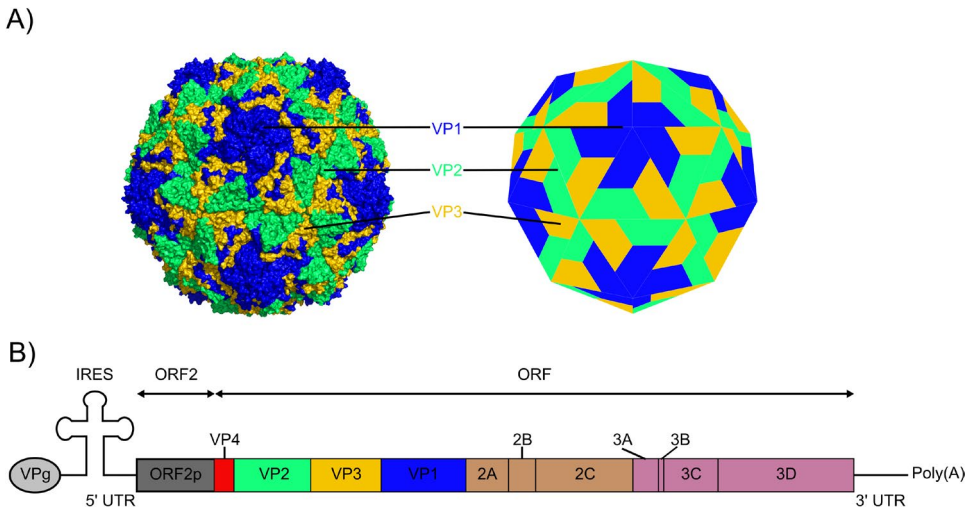

FIGURE 1 | Schematic representation of enteroviruses structure and genome. (A) The icosahedral capsid consists of an arrangement of 60 monomers or protomers each composed of four structural proteins, VP1 in blue, VP2 in green, VP3 in yellow and VP4, which is located on the internal side of the capsid (PDB ID: 6ZMS). (B) The positive-sense single-stranded RNA genome (~7.4 kb) contains two open reading frames (ORF and ORF2) flanked by a 5′-untranslated region (UTR) covalently linked to the viral protein VPg (3B) and by a 3′UTR terminated with a poly-A tail, mimicking cellular mRNA. The IRES structure upstream of the two ORFs recruits the ribosome that transcribes the ORFs. ORF2 encodes an ORF2p protein, which is involved in intestinal infection. The large ORF encodes a polyprotein, which is cleaved by the proteases $2A^{pro}$ and $3C^{pro}$ into eleven proteins.

of enteric viruses, which includes *Caliciviridae* (e.g., Norwalk virus), *Picornaviridae* (e.g., poliovirus type 1, Aichi virus, hepatitis A virus), *Hepeviridae* (e.g., hepatitis E virus), *Reoviridae* (e.g., rotavirus), *Astroviridae* (e.g., Human astrovirus (type 1–8)) and *Adenoviridae* (e.g., adenovirus 40, 41). Enteric viruses are characterised by their ability to induce human diseases through the ingestion of contaminated food or water. Humans are thought to be the only important natural reservoir for human EVs (Knipe and Howley 2013). Children are the main transmitters of EVs, particularly in households located in areas with poorer sanitation or socio-economic conditions (Knipe and Howley 2013). They can be shed in faeces at a high number, from 10^5 to 10^{12} particles per gram (Gerba 2000). They can be found in drinking water sources, recreational waters and shellfish harvest waters (Gerba and Rose 1990; Muscillo et al. 1994; Reynolds et al. 1995; Patti et al. 1996). They are used as a marker of faecal contamination in water (Wong et al. 2012). Therefore, the monitoring and elimination of such viruses are crucial to provide safe water.

This review focuses on water-borne human EVs and their transmission. Methods for detecting EVs in water are also presented as well as current strategies to control these enteric viruses in water.

2 | Epidemiology of Enteroviruses Waterborne Outbreaks

2.1 | Evidence for Waterborne Enteroviruses

From a historical perspective, Joseph Melnick was the first in 1947 to document the spreading of poliovirus strains from sewage, a founding step of environmental virology (Melnick 1947). This has been constantly expanding ever since, and many studies are currently focusing on environmental contamination by EVs. EVs can be found worldwide, in every continent: Europe, North America, South America, Asia or Oceania. In addition, EVs are detected in virtually every water source, as raw or inadequately treated sewage can contaminate marine water, fresh surface water or groundwater, which are reservoirs used for irrigation water, recreational water or drinking water (Rajtar et al. 2008). Monitoring of EV infections can also be carried out in clinical samples such as blood, faeces, cerebro-spinal fluid or saliva.

EVs can be found in irrigation water, contaminated vegetables and sewage as detected in central Argentina in 37 water samples collected during July–December 2012 (Prez et al. 2018). In China, recreational fresh water of three lakes was tested for the presence of EVs. Out of the 25 sites, 14 were positive for EV detection in all three lakes (Allmann et al. 2013). Samples of surface, ground and sewage water collected in 2004–2005 in Taiwan were positive in 40% of cases (Chen et al. 2008). Sequence analysis reveals relatedness between coxsackievirus A (CVA) strains found in Pakistan and CVA from Greece, Singapore and the USA. Around 19%–28% of drinking water samples were contaminated with EV in three cities of Pakistan (Ahmad et al. 2015). In Karachi, for example, 43% of the 30 samples of tap water contained EVs (Rashid et al. 2021). Climate warming increases the risks of infectious diseases (Curriero et al. 2001; Chen et al. 2012; Tornevi et al. 2013), including EV infections. Heavier rains in the Mediterranean basin are correlated with an elevation of EVs in sediments and in river water as EVs are detected in 50% of samples collected just after rainfall events, compared to 20% of samples collected 15 days or more after the last rainfall (García-Aljaro et al. 2017). The contamination of water sources and shellfish in Africa has been reviewed by Upfold et al. (2021). Eight samples of contaminated streams collected in 2017–2018 in Nigeria revealed a wide diversity of EVs with 93 strains belonging to 45 serotypes identified (Majumdar et al. 2021).

The limitation of these observations is that the causality between enterovirus detection in water and epidemiology is unknown. The presence of enteroviruses in water is probably due to wastewater contamination linked to an ongoing epidemic. The emergence of wastewater-based epidemiology has been proven useful in the monitoring of infectious diseases (Hassard et al. 2023), such as the SARS-CoV-2 pandemic (Cancela et al. 2023), but is also successfully used for enteroviruses monitoring (Asghar et al. 2014; Boehm et al. 2024; Verani et al. 2024). A study monitoring EV-D68 in wastewater and in nasopharyngeal samples found a strong correlation between these two observations (Erster et al. 2022). Furthermore, a study indicates that the presence of avian influenza virus in wastewater was an early indicator of the onset of disease outbreaks (Falender 2025). This underlines the usefulness of water monitoring for enteroviruses.

2.2 | Enteroviruses and Other Markers of Water Pollution

Between June 2016 and June 2017, samples from river water, drinking water and human peripheral blood were collected in three cities in Pakistan, Lahore, Islamabad and Rawalpindi to monitor the level of EVs and hepatitis A viruses with PCR after a concentration step with negatively charged membranes. EVs were found in 28%–40% of river waters and 11%–13% of drinking waters of Lahore and Rawalpindi, while HAV was found in 12%–21% of blood samples. The parallel surveillance of both of these markers may be helpful to monitor waterborne illnesses (Ahmad et al. 2018). A study revealed that the five most common EV serotypes (EV-D68, E11, CVA6, CVB5 and CVA2) showed a decrease in their genetic diversity after the COVID-19 lockdown, probably due to the reduction in their transmission during this period (Forero et al. 2023). EVs have been proven to be good viral indicators of surface water pollution and could report the presence of other enteric viruses since EVs and group A rotavirus were always co-detected in a survey carried out for three years, from June 2013 to October 2015, in Argentina (Masachessi et al. 2021). In the Jinhe River in Tianjin located in China, the analysis of 48 samples over 4 years from 2012 to 2016 found a strong correlation between the presence of total coliforms and EVs (Miao et al. 2018). Overall, these observations may be useful to create correlation matrices in the near future to monitor water quality globally, whatever the technical facilities available.

2.3 | Enterovirus Persistence in Water

Non-enveloped viruses such as EVs are particularly resistant in water, which allows faecal-oral transmission (Sattar 2007). For instance, CVB2 can remain infectious in drinking and surface water for more than 70 days at temperatures ranging from 4°C to 20°C, although infectious titers drop from 10^5 TCID50.mL^{-1} to 10^1 TCID50.mL^{-1} (Prevost et al. 2016). EV resistance increases the risks of infection, but also makes EVs a good indicator for water pollution. The nucleic acid decay rates of many pathogens in water, including EVs, depend on the environment. Two different estuarine waters collected in the city of Sydney revealed a faster decay rate of enteric viruses under sunlight exposure. Hence, higher turbidity of surface waters could promote the persistence of EVs nucleic acids (Ahmed et al. 2024). A seasonality exists for the detection of EVs in wastewaters, with a peak in summer in the tempered regions, and therefore, temperature is a strong predictor of the intensity of EV transmission, as the main factor involved in aerial persistence in aerosols seems to be the absolute humidity, which is the quantity of water in the air that is higher in summer (Pons-Salort et al. 2018). In addition, infectious doses of EVs are small. Echovirus type 12 (E12) was added to the drinking water of individuals at 330, 1000, 3300, 10,000, 33,000 and 330,000 PFU and given to 50, 20, 26, 12, 4 and 3 volunteers, respectively (Schiff et al. 1984). The infection was confirmed through intestinal shedding of virus and seroconversion. The analysis of all available data indicated that the risk of being infected is 1% when an individual is exposed to 14 PFU of E12.

The circulation of viruses within a population can also be monitored by their detection in water. It has been shown, for instance, that the detection of SARS-CoV-2 RNA in wastewater could be used for monitoring the evolution of the COVID-19 pandemic (Pasha et al. 2024). In 2022, poliovirus type 2 was found in London's wastewater, calling for greater vigilance and demonstrating the relevance of environmental monitoring (Hill and Pollard 2022; Klapsa et al. 2022; Kasstan-Dabush et al. 2024).

3 | Detection of Enteroviruses in Water

3.1 | Cell Culture

The first method used for EV detection in environmental samples is cell culture. Samples are added to cell culture to assess the presence of a cytopathogenic effect (Faleye et al. 2016). Depending on the cell line, this method can be used to detect a single species or the entire genus. Serotyping can then be performed with known serum samples. In this case, a pool of neutralising sera, each specific to a serotype, is used to neutralise the unknown virus. If the virus is neutralised by a serum, it belongs to the serotype to which the serum is specific (Melnick et al. 1973; Chonmaitree et al. 1988). However, this method is time consuming and not all EVs can be cultivated in vitro, limiting the detection in samples. Nevertheless, cell culture remains the reference method for the detection of EVs in water samples. For instance, there are two French norms for laboratories that aim to detect EVs in water samples, NF EN14486 and NF T90-451. NF EN14486 came into effect in 2006 and relies on cell culture and specifically Buffalo Green Monkey (BGM) cell line. The more recent NF T90-451 (2020) defines a guideline with a concentration step on glass wool, followed by the detection by RT-PCR and/or cell culture.

3.2 | RT-PCR

The reverse transcription-PCR (RT-PCR) has become the golden standard for EV detection because of its high sensitivity, short reaction times and low operational cost (Pallin et al. 1997; Hamza et al. 2011). Depending on the specificity wanted, different regions of the genome can be targeted, the 5′ UTR regions for the genus and VP1, VP2, or VP4 for the species or even the strains (Nix et al. 2006). Multiplex PCR allows the detections of several

strains in parallel. In a multiplex real-time PCR assay, Wang et al. (2018b) succeeded to detect EVs inducing hand, foot and mouth disease, namely EV-A71, CVA16, CVA6 and CVA10 with a high specificity and low limits of detection of 3×10^3, 5×10^2, 4×10^2 and 4×10^3 copies per mL, respectively, in samples comprising cerebrospinal fluid, blood, faeces and throat swabs. The detection of EVs depends on a variety of factors due to the technical constraints to purify and concentrate these viruses. For instance, to perform a PCR, chemicals inhibiting the reaction, such as organic and phenolic compounds, glycogen, fats and organic and phenolic compounds, glycogen, fats and Ca^{2+}, phenolic compounds, humic acids and heavy metals, constituents of bacterial cells, non-target DNA and contaminant (Wilson 1997), must be eliminated (Girones et al. 2010). Among the concentration methods used for EVs, three are commons: electronegative membranes (Katayama et al. 2002), electropositive membranes (Pang et al. 2012) and ultrafiltration (Paul et al. 1991; Olszewski et al. 2005). A meta-analysis studying these three methods concluded that there are no significant differences in virus recovery due to water type, filter type or sample volume (Cashdollar et al. 2013). However, higher recoveries are observed for poliovirus compared to norovirus.

RT-PCR can be combined with cell culture to amplify and detect infectious EVs in a method called integrated cell culture (ICC)-PCR (Reynolds et al. 1996, 2001). ICC-RT-PCR-nested PCR method provided increased sensitivity compared to the total culturable virus assay-most-probable-number method the standard of US recommendation (Chapron et al. 2000). However, viral interference when two or more viruses are present in a sample may lead to false negative as one of the virus can overtake the others (Sano et al. 2021). Table 1 provides details on these methods.

PCR quantifies the number of copies of the viral genome. However, it does not mean that the virus is infectious and may lead to false positives (Nuanualsuwan and Cliver 2003). In addition, the number of viruses needed for infection also varies (Haas et al. 1993; Lindesmith et al. 2003). For instance, in 68 concentrated water samples from French rivers, EV genomes were detected in 60 (88%) of them, whereas only 2 samples (3%) contained infectious EV assessed with the inoculation of BGM cell cultures, showing a huge gap between the detection of the genome via PCR and the infectious ability of these viruses (Hot et al. 2003). However, we cannot assert that the BGM cell line is permissive to all EVs, which could explain some of this discrepancy.

To avoid the overestimation of viruses with PCR, a pretreatment with ethidium monoazide and propidium monoazide gives closer results to infectivity assays (Leifels et al. 2016; Prevost et al. 2016). These dyes are able to penetrate damaged or compromised capsids and bind to the viral RNA to prevent its amplification with PCR. Thus, only viral genomes contained in virions that have retained capsid integrity are amplified, but this does not mean that they are infectious either.

3.3 | New Techniques

Commercial kits are available and show high sensitivity (89%–100%) in clinical samples. Nevertheless, the results on environmental matrices demonstrate a reduced sensitivity, as it fell to 16%, 56%, 63% and 91% for the Nanogen, Argen, Ceeram and for the Diagenode kits, respectively (Wurtzer et al. 2014). Therefore, alternative methods are developed to better detect EVs in these samples, mainly water samples, as demonstrated by Wurtzer et al. with the use of new EV degenerated primers (Wurtzer et al. 2014). Whole genome sequencing also allows the detection of all EVs and can provide information on the geographic origin of the virus as phylogenetic relatedness can be estimated; however, this method is costly (Majumdar et al. 2021).

The design of serotype specific probes for reverse line blot following a RT-PCR is able to identify up to the 20 most common serotypes of EVs (Zhou et al. 2009). The development of organoids is also useful for EVs detection. For instance, gastrointestinal organoids can be used for the culture of enteric viruses that are not cultivable in classic cell lines (Kolawole and Wobus 2020). Fourier transform infrared spectroscopy, combined with cellular-based sensing, has also been tested to monitor poliovirus infection in BGM cells (Lee-Montiel et al. 2011). With this method, BGM cells, which are used as a biosensor for poliovirus, are seeded onto crystals made of zinc and selenium. The infection of cells alters the resulting spectrum obtained with infrared spectroscopy. This spectrum is quantitatively related to the content of the cell, in amines or lipids for instance; thus, a Fourier transform is used to estimate the quantity of poliovirus in the system. The detection of poliovirus is faster than classic observations of cell death, with a robust prediction 8 h after infection with water samples.

4 | Inactivation of Enteroviruses in Water

4.1 | Common Water Treatments

Sewage is treated to avoid the persistence of infectious agents in water. These treatments include chlorination, UV radiation, ozone addition, or biological treatment. Generally, viruses are more resistant to water treatments, such as UV radiations and chlorination, compared to bacteria (Bitton 2011; Francy et al. 2012; Montazeri et al. 2015); therefore, the assessment of the efficiency of disinfection for EVs is important. Inactivation of infectious viruses in drinking water treatment is usually achieved by chlorination with free chlorine, and monochloramine can be added to minimise disinfectant by-product formation and biofilm growth (Cromeans et al. 2010). Free chlorine, obtained with the dissolution of dichlorine gas (Cl_2) or hypochlorite (ClO^-), is highly reactive, leading to oxidation, addition, or substitution reactions. Reactivity with organic matter is mainly due to oxidation reactions (50%–80% of reactions). Addition of chlorine atoms or substitution of hydrogen atoms with chlorine atoms in organic macromolecules, such as nucleic acids or proteins, can lead to replication failures, conformational changes, or degradation and thus the inactivation of viruses. It has been shown that RNA has a greater affinity than proteins for chlorine assimilation, with the example of the f2 bacteriophage, suggesting a more pronounced effect on RNA degradation (Dennis et al. 1979). However, the quantity of chlorine substituted in viral components does not correlate with viral inactivation, so viral inactivation cannot be merely reduced to the number of hydrogen substitutions with chlorine. Moreover, the degradation

TABLE 1 | Methods commonly used for enterovirus detection in water samples.

	Method		Principle
Concentration	Adsorption/Elution (Shi et al. 2017)	Electronegative membrane	Membrane usually made of a mixture of cellulose nitrate and cellulose acetate, negatively charged. Most viruses are negatively charged, so a preconditioning is often required (Katayama et al. 2002)
		Electropositive membrane	A wide variety of electropositive membrane are commercially available, all positively charged, thus, samples do not require a preconditioning (Sobsey and Jones 1979; Karim et al. 2009)
	Ultrafiltration		Sample flowing through a filter with pore of a given size to retain or let through the components of interests (Clark et al. 1933)
Virus detection and count	Cell culture		Inoculation of the sample to cell lines permissive to enteroviruses (BGM for instance) (Ali et al. 1999; Abd-Elmaksoud et al. 2019)
	Reverse transcription-polymerase chain reaction (RT-PCR)		Molecular biology technique where the genomes are reverse transcripted and then amplified by PCR (Harvala et al. 2018)
	Integrated cell culture-RT-PCR-nested PCR (ICC-RT-PCR-nested PCR)		Association of a first step of amplification through cell culture followed by viral RNA quantification in culture supernatants using RT-PCR and finally by a nested PCR (Chapron et al. 2000)
	Total culturable virus assay-most probable number (TCVA-MPN)		Serial dilutions of the sample are inoculated on BGM cell cultures followed by multiple passages and the infectious virus titre is determined using U.S. Environmental Protection Agency (USEPA) Most Probable Number Calculator (Fout and Cashdollar 2016)
	Reverse line blot RT-PCR (RLB-RT-PCR)		Oligonucleotides that are strain-specific are covalently bound to membranes, and PCR products are hybridised to reveal the content of the sample (Kong and Gilbert 2006; Zhou et al. 2009)
	Fourier transform infrared spectroscopy		The use of the deconvolution of the infrared spectrum obtained via infrared spectroscopy to detect biochemical modifications in cells inoculated with viruses. The spectrum can be quantitatively analysed to assess the viral load (Lee-Montiel et al. 2011)

rate of monomers constituting biopolymers induced by free chlorine has been quantified (Wigginton et al. 2012), and the extrapolation to the whole poliovirus virion based on its composition indicates that free chlorine mainly reacts with proteins, i.e., the capsid. Thus, the formal mechanism by which free chlorine inactivates viruses remains unclear. The assessment of the exposure time required for viral inactivation of human adenovirus, enteroviruses and murine norovirus with free chlorine and monochloramine found that enteroviruses required longer exposure times than human adenoviruses and murine noroviruses with free chlorine to achieve inactivation, and human adenoviruses were the most resistant to the addition of monochloramine (Cromeans et al. 2010). This is confirmed by a meta-analysis on the effect of chlorination on enteric viruses which demonstrated that EVs, and CVB more precisely, are the most resistant to free chlorine while adenoviruses are more resistant to

monochloramine. Noroviruses are more sensitive to both chlorination methods (Rachmadi et al. 2020). Adenoviruses are more resistant to UVs than EVs, probably due to the existence of cellular mechanisms of viral DNA repair in infected cells, as adenoviruses are double-stranded DNA viruses (Prevost et al. 2016). Furthermore, adenoviruses are more resistant to common water treatments such as traditional activated sludge treatment, as well as ozone, than noroviruses, but EVs were not detected in this study (Wang et al. 2018a). Moreover, the final concentration of virus depends on the initial quantity of viruses in influent water of treatment plants. In a one-year study, adenoviruses are more frequently detected in raw and treated wastewater followed by EVs and then noroviruses. In river water, noroviruses and adenoviruses are equally frequently detected while EVs are less frequent (Iaconelli et al. 2017). Adenoviruses and noroviruses are also more numerous in influents than EVs, but only in winter for noroviruses, as a strong seasonality in noroviruses infections is observed (Lodder and de Roda Husman 2005; Katayama et al. 2008).

However, this efficiency of disinfection can vary depending on the type of water (Kahler et al. 2011). Three types of water were studied; the first one was a partially treated source water sample obtained from Cobb County-Marietta Water Authority in Marietta, Georgia, collected from a reservoir/recreational lake and subjected to coagulation and filtration. The second sample was a partially treated source water sample obtained from Washington Aqueduct in Washington D.C. water treatment plants, just prior to chemical disinfection and collected from the Potomac River and subjected to pre-sedimentation, coagulation, flocculation, sedimentation and filtration. The third sample was groundwater obtained from Brunswick-Glynn County Joint Water and Sewer Commission (BGC) in Savannah, Georgia, prior to chlorination. The inactivation of the two EVs tested, namely CVB5 and E11, was significantly different according to water type. However, no consistent trends were observed to indicate that monochloramine disinfection was more or less effective in a particular source water type. EV-A71 exhibited a strong resistance to chlorine dioxide used for disinfection, which has the advantage of not producing toxic by-products. The efficiency of chlorine dioxide is also dependent on temperature and pH (Jin et al. 2013) as is also the case for free chlorine (Cong et al. 2023) and monochloramine (Cromeans et al. 2010). Overall, these elements give insights on how common treatments reduced the viral load in water; however, the efficiency of these treatments varies greatly, even for a given viral strain. For instance, a 3.4-fold variation is observed in the inactivation of CVB5 by free chlorine (Torii et al. 2021) and can go up to a 10-fold variation when comparing CVB strains (Meister et al. 2018).

4.2 | Other Treatment

Bacterial species secrete anti-viral compounds, especially matrix metalloproteases (MMPs) and proteases in general. These compounds can be used to inhibit viral activity (Corre et al. 2022). It was found that incubation of E11 and CVA9 in biologically active water from Lake Geneva induced a 2–4 $\log_{10}$ reduction of infectious titre (Corre et al. 2022). Bacteria present in water were isolated, and it was found that bacterial isolates that produce MMPs induced the most extensive viral decay. In this case, 57 bacterial isolates from five different phyla have MMP activity above 1 $\mu mol.min^{-1}.mL^{-1}$. The addition of MMP inhibitors in water prevented the inactivation of viruses. Thus, MMPs are a potentially effective agent to control EVs in water.

5 | Concluding Remarks

EVs are responsible for infections leading to various outcomes. They are mainly transmitted via the feco-oral route, thus the monitoring and control of EVs in water might be crucial in preventing potential outbreaks. This is why it is necessary, for public health reasons, to monitor outbreaks of EV in water. Several methods exist to monitor EVs in water, including cell culture, which remains the golden standard, but it is time consuming and difficult to identify specific serotypes. Therefore, the use of RT-PCR is increasingly common, although this method cannot discriminate between infectious and noninfectious genomes collected in environmental samples. New experimental techniques, such as organoids, try to overcome these limitations, but more testing is required to assure their robustness.

Author Contributions

Corentin Morvan: writing – original draft, data curation. **Magloire P. Nekoua:** writing – review and editing. **Chaldam J. Mbani:** writing – review and editing. **Cyril Debuysschere:** writing – review and editing. **Enagnon K. Alidjinou:** writing – review and editing. **Didier Hober:** funding acquisition, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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