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Review

Selected human enteric viruses and viral indicators in drinking-water sources and treated waters: a review on prevalence and monitoring methods

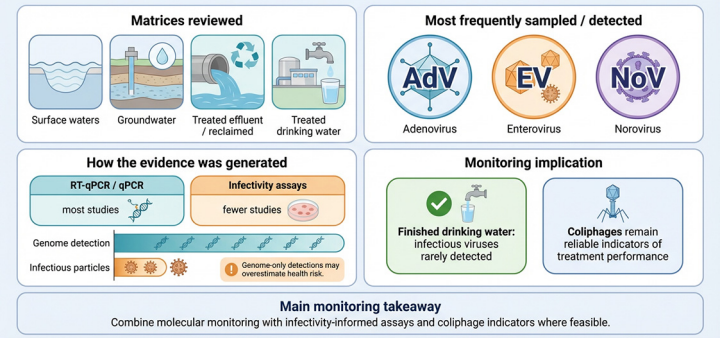
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HIGHLIGHTS

- Selected enteric viruses and indicators reviewed across drinking-water matrices
- Virus occurrence varies strongly by matrix, strain, and analytical method
- Large-volume concentration remains a major bottleneck for low-level detection
- Coliphages support treatment verification; PMMoV/TMV need careful interpretation
- Standardised multi-method monitoring is needed for source and treated waters

GRAPHICAL ABSTRACT



ABSTRACT

Viruses transmitted via contaminated water pose a major public health risk, requiring accurate detection and quantification in diverse water matrices. This review assessed the occurrence of selected human enteric viruses and commonly used viral indicators using 30,085 data points from 3094 sites across surface waters (e.g., lakes, reservoirs, rivers and streams), groundwater, treated effluents and reclaimed waters, and treated drinking water. Viral genomes were primarily quantified using reverse transcription-quantitative polymerase chain reaction ((RT-)qPCR), while infectivity was evaluated in a smaller subset of studies using cell culture approaches (e.g., integrated cell culture-PCR and cytopathic effect-based observations). Culturable coliphages were also reported as indicators of faecal

Abbreviations: Abbreviation, Term; (RT-)qPCR, Reverse Transcription-Quantitative Polymerase Chain Reaction; BSL, Biosafety Level; CDPH, California Department of Public Health; CGMMV, cucumber green mottle mosaic virus; DAL, Double-Layer Agar; ddPCR, Digital droplet Polymerase Chain Reaction; CFR, Code of Federal Regulations; CPE, Cytopathic Effect; DALY, Disability-Adjusted Life Year; DeUF, Dead-End Ultrafiltration; DNA, Deoxyribonucleic Acid; DWTP, Drinking Water Treatment Plant; EMA, Ethidium Monoazide; EU, European Union; FRNAPH, F-Specific RNA Bacteriophages; GC, Genome Copies; GI, Genogroup I (commonly referring to Norovirus GI); GII, Genogroup II (commonly referring to Norovirus GII); IESWTR, Interim Enhanced Surface Water Treatment Rule (USEPA); ISO, International Organization for Standardization; LRV, Log Reduction Value; MNV, Murine Norovirus; MPC, Molecular Process Control; MS2, Male-Specific (F+) RNA Bacteriophage MS2; PEG, Polyethylene Glycol; PFU, Plaque-Forming Units; phi6, Pseudomonas Bacteriophage phi6; PMMoV, Pepper Mild Mottle Virus; PMA, Propidium Monoazide; qPCR, Quantitative Polymerase Chain Reaction; QMRA, Quantitative Microbial Risk Assessment; RNA, Ribonucleic Acid; SAL, Single Agar Layer; SWTR, Surface Water Treatment Rule (USEPA); $TCID_{50}$, 50 % Tissue Culture Infective Dose; TFUF, Tangential Flow Ultrafiltration; TMV, Tobacco Mosaic Virus; UKWIR, UK Water Industry Research; USEPA, United States Environmental Protection Agency; WHO, World Health Organization; WPC, Whole Process Control; WSP, Water Safety Plan.

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contamination and as operational surrogates for treatment performance, but data in treated drinking water remain limited, emphasising the need to understand their strengths and limitations. Regulatory and guidance frameworks are evolving, with increasing emphasis on demonstrating virus log reduction through multi-barrier treatment and on risk-based operational monitoring (including somatic coliphages in some jurisdictions). Low-level virus detection remains constrained by the need to concentrate large sample volumes, particularly for on-site sampling at drinking water treatment works. Across the studies reviewed, reported viral genome concentrations spanned approximately 1.5-7 $\log_{10}$ genome copies/L, and culturable indicator concentrations varied widely across matrices. Recovery of both gene targets and infectious virus varied substantially between methods, indicating methodological challenges that can affect interpretation and risk assessment. Overall, the evidence supports standardised, multi-method monitoring (molecular and, where feasible, infectivity-informed approaches) and improved surveillance designs to better protect public health from viral contamination.

1. Introduction

The vast majority of global viruses exist in water, including the oceans, rivers, and lakes (Suttle, 2007). These aquatic viruses, primarily bacteriophages, play an indispensable role in regulating bacterial populations, thereby maintaining ecological balance and nutrient cycles. A small subset of enteric viruses are pathogenic to humans and can contaminate drinking water sources, posing significant public health risks (Jamal et al., 2020).

Contamination of water sources by enteric pathogens can occur via multiple mechanisms and transmission routes. Sources include sewer leakage, septic systems, human and animal faeces, and treated or untreated wastewater discharges to rivers, lakes, reservoirs and coastal waters that may be used for drinking-water abstraction (Singh et al., 2022). Sewage-impacted waters can pose a public health risk because pathogens can be transmitted via recreational exposure and via shortcomings or failures in water treatment and distribution. Most pathogens of concern in drinking water are associated with human and livestock wastes and are primarily linked to faecal-oral transmission (Xagoraraki et al., 2014).

Sewage and untreated wastewaters typically contain higher concentrations of enteric viruses than source waters or finished drinking water. Direct contact with raw sewage is relatively uncommon, whereas exposure can occur via recreational use of sewage-impacted surface waters and via abstraction of impacted surface water or groundwater for potable supply. Key contamination sources include sewer leakage, septic discharges and combined sewer overflows, as well as treated and untreated wastewater effluents, particularly during wet-weather events (Guo et al., 2022; Rusiñol et al., 2015). Agricultural runoff and land application of manure or biosolids can also introduce viruses to catchments, and wildlife and livestock may act as reservoirs of zoonotic viruses (Xagoraraki et al., 2014). Hydroclimatic events (snowmelt and heavy rainfall) can trigger spikes in viral loads through runoff and sewer overflows, and projected climate change is expected to increase the frequency and intensity of such events, placing additional strain on water and wastewater infrastructure (Fauvel et al., 2016; Sylvestre et al., 2021; Semenza and Paz, 2021).

Diarrhoeal disease remains a major contributor to global morbidity, and viral agents such as norovirus and rotavirus are leading causes of acute gastroenteritis across age groups (Anderson and Weber, 2004; Bartsch et al., 2020). In the United States, norovirus gastroenteritis has been estimated to impose a substantial annual clinical and economic burden (Bartsch et al., 2020), while in England norovirus-associated hospitalisations contribute to avoidable bed occupancy and opportunity costs (Hassard et al., 2017; Sandmann et al., 2018). Because enteric viruses can persist in the environment and are often present at low concentrations in drinking-water sources, sensitive monitoring approaches are needed to inform risk management (Fong and Lipp, 2005; Haramoto et al., 2018).

This review focuses on selected human enteric viruses and commonly used viral indicators relevant to potable-water safety. Specifically, we (i) synthesise reported occurrence (detection frequencies and concentrations) across key matrices (surface waters, groundwater, treated effluents, reclaimed water and finished drinking water) and (ii) critically evaluate sampling strategies and virus concentration/detection workflows used to monitor low-level viral contamination in source and treated waters. We also discuss how methodological choices (sample volume, concentration approach, process controls, inhibition management and infectivity assessment) influence recovery and interpretation, and we highlight practical priorities for routine monitoring and risk assessment. Accordingly, this review does not attempt to catalogue the broader freshwater virome or metagenomics detections of non-enteric viruses. There has been an increase in studies using qPCR-calibrated metagenomics (Wang et al., 2020), but these studies were outside the predefined scope of this review.

Interpreting viral occurrence data requires explicit consideration of how viruses partition and persist in water, and of what the analytical signal represents. Viruses may occur as free virions, associated with microorganisms, or adsorbed to suspended particles and natural organic matter (Fig. 1). Capsid surface charge and isoelectric point, together with water chemistry (e.g., pH, ionic strength and natural organic matter), influence adsorption, detachment and analytical recovery (Michen and Graule, 2010; Heffron and Mayer, 2021). Virus survival in source waters is shaped by intrinsic viral characteristics and by environmental conditions such as temperature, sunlight/UV exposure, salinity, pH and organic matter (Fong and Lipp, 2005; Boehm et al., 2019). Culture-based methods quantify infectious virus through plaque assays, endpoint dilution or cytopathic effect in permissive host systems, whereas (RT-)qPCR and digital PCR quantify nucleic acids and therefore require careful interpretation in relation to infectivity (Fig. 2).

2. Data search and inclusion criteria

We reviewed studies published from 2000 onwards, with a focus on those published after 2007. Studies were initially identified through literature searches in 2023 (July) and 2024 (September) using database search terms including: virus, bacteriophage, coliphage, drinking water, water supply, groundwater, surface water, raw water, treated water, wastewater, treated effluent, reclaimed water, and quantitative PCR. Terms related to treated effluent and reclaimed water were included because wastewater discharges and water reuse can influence source waters (including via de facto reuse) and because potable reuse schemes increasingly rely on virus log-reduction verification.

(i) contained quantification results for one or more of the 10 human pathogenic viruses (Aichivirus, Adenovirus, Astrovirus, Enterovirus, Hepatitis A virus, Hepatitis E virus, Norovirus, Polyomavirus, Rotavirus and Sapovirus); or one or more of the two surrogate viruses (pepper mild mottle virus (PMMoV), tobacco mosaic virus (TMV)).
(ii) contained quantification results for phages (coliphage (F-specific, somatic), crAssphage).
(iii) were published in English.
(iv) For the prevalence sections only field studies that reported naturally occurring phages were retained; laboratory spiking trials were excluded. However, for the methods and recovery analysis studies using spiked virus/phage were included.

Studies focused exclusively on non-enteric viruses (e.g., SARS-CoV-2, influenza viruses, or rat hepatitis E virus) and metagenomic surveys

without matrix-specific quantitative data were excluded. Such studies broaden understanding of freshwater viromes, but they fall outside the scope of this review. Broader syntheses of coliphage occurrence in raw wastewater, ambient waters and wastewater effluents are already available elsewhere (Nappier et al., 2019; Jones et al., 2022); accordingly, the present review retained a potable-water focused scope and included wastewater-derived matrices only where they inform drinking-water sources, treated waters or potable reuse.

Data points were extracted and analysed using Excel 2016 (Microsoft, USA) and *graphreader* web interface. Data from articles meeting the inclusion criteria outlined above were categorized into five different water matrices as: *treated effluent* (tertiary treated effluents from wastewater treatment plants), *reclaimed water*, *surface water* (includes waters from lakes, reservoirs, watersheds, catchments, dams, rivers, creeks and streams), *subsurface water* (groundwater and well water), and *treated potable water* (treated drinking water from drinking water treatment plants (DWTPs), the distribution system and point of use supply). Where available, the following information for the abovementioned 12 viruses and 3 phages was extracted matrix-wise from each of the studies into a database (Microsoft Office Excel) (Table S1, S2, Table 1):

(i) The virus and phage type monitored,
(ii) Viral quantification method employed (i.e., gene, infectivity),
(iii) Number of sampling data points (nD), number of positive detects (nD+), number of sampling sites (nS),
(iv) Maximum abundance of viruses and phages including genome copy (GC) and infectivity results (when available),
(v) Sampling location,
(vi) Sampling volume,
(vii) Sample concentration methods (primary concentration method, eluant technique, secondary concentration method, tertiary concentration).

3. Nomenclature

Virus taxonomy and nomenclature follow the International Committee on Taxonomy of Viruses (ICTV) recommendations (accessed December 2024) (ICTV, 2024). Because many studies use common names and epidemiological groupings, we retain those terms (e.g., 'norovirus GI/GII') alongside the corresponding ICTV genus/species where useful. 'CrAssphage' is an informal term for crAss-like bacteriophages; ICTV currently classifies many of these viruses within the order Crassvirales.

- Adenovirus: Human adenoviruses are members of genus Mastadenovirus (family Adenoviridae) and are currently classified into species Human mastadenovirus A-G.
- Astrovirus: Human astroviruses belong to genus Mamastrovirus (family Astroviridae), with multiple species/lineages; clinical literature often refers to 'human astrovirus' or specific genotypes/serotypes.
- Enterovirus: Enteroviruses are members of genus Enterovirus (family Picornaviridae); human-infecting enteroviruses span several species (e.g., Enterovirus A-D) and include polioviruses, coxsackieviruses and echoviruses.
- Norovirus: Noroviruses belong to genus Norovirus (family Caliciviridae). Human-associated strains are commonly reported by genogroup (e.g., GI, GII); ICTV species nomenclature includes Norovirus norwalkense (prototype Norwalk virus).
- Rotavirus: Rotaviruses belong to genus Rotavirus within family Sedoreoviridae (formerly Reoviridae in older literature); the clinically important human group is most often Rotavirus A.
- Sapovirus: Sapoviruses belong to genus Sapovirus (family Caliciviridae). Human-associated strains are commonly grouped into genogroups (e.g., GI, GII, GIV and GV), which are epidemiological/phylogenetic groupings rather than ICTV taxonomic ranks.
- Aichivirus: Human Aichi virus is classified within genus Kobuvirus (family Picornaviridae), species Aichivirus A (formerly referred to as 'Aichi virus').
- Hepatitis A virus: Hepatitis A virus is in family Picornaviridae, genus Hepatovirus, species Hepatovirus A.

Polyomavirus: Human polyomaviruses are in family Polyomaviridae; common human-associated targets in water studies include JC polyomavirus and BK polyomavirus (genus Betapolyomavirus).

- Hepatitis E virus: Human hepatitis E virus is in family Hepeviridae, genus Paslahepevirus, species Paslahepevirus balayani (a taxon that

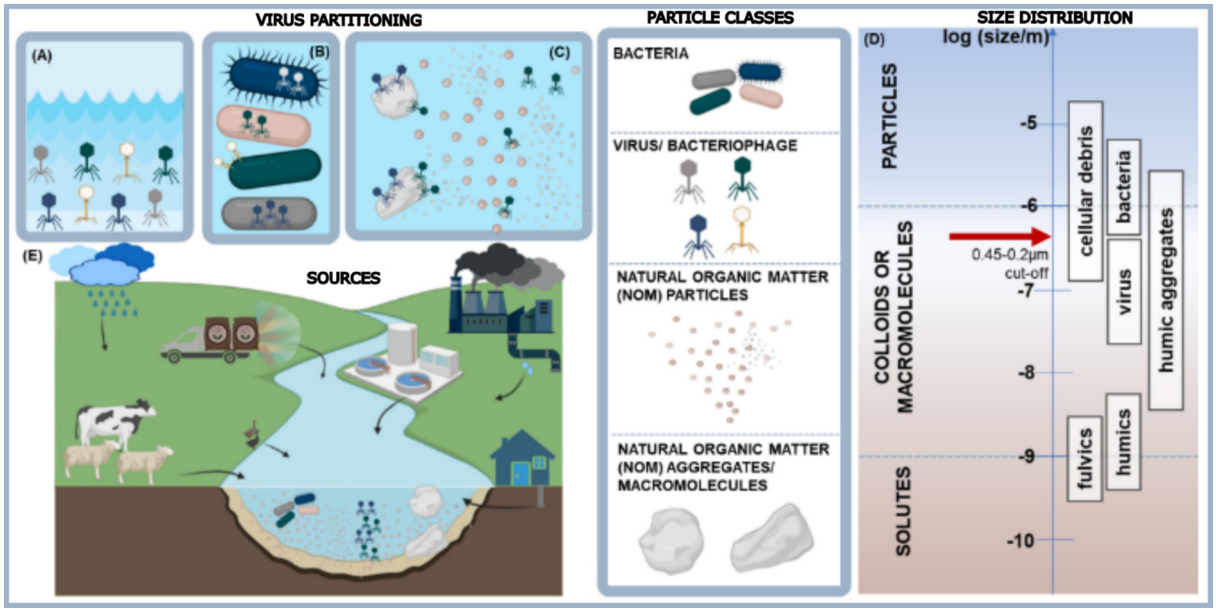


Fig. 1. Partitioning of viruses within a water system: (A) as free-floating, monodispersed particles; (B) associated with microorganisms; or (C) adsorbed to natural organic matter (NOM) aggregates and macromolecules. (D) The size range of these different particles is shown for comparison. (E) depicts how viruses/bacteriophages are discharged into the urban water cycle.

supersedes the older species name Orthohepevirus A in ICTV taxonomy).

Overview of the compiled occurrence dataset: most studies that quantify viruses in drinking-water sources use targeted assays for a limited number of priority pathogens or indicators, because comprehensive characterisation of viral diversity across matrices (including metagenomic approaches) is resource-intensive and not yet routinely feasible for drinking water treatment works (Bibby et al., 2019). Accordingly, this review focuses on a defined set of human enteric viruses and viral indicators commonly used in the potable-water literature, while recognising that other viruses (e.g., respiratory viruses such as SARS-CoV-2) and metagenomic findings may be relevant in specific contexts.

In this study, viral prevalence estimates reported from 3094 distinct sampling sites were quantified using both genome copies (GC) and plaque forming units (PFU). Genome copies refer to the total number of viral DNA or RNA segments detected in the samples, while PFU measure the number of virus particles capable of forming plaques on a host cell layer. The 10 human pathogenic viruses (Aichivirus, Adenovirus, Astrovirus, Enterovirus, Hepatitis A virus, Hepatitis E virus, Norovirus, Polyomavirus, Rotavirus and Sapovirus), the coliphages (total, somatic, F-specific) and three emerging indicators (crAssphage and plant viruses - TMV and PMMoV) were represented by 24,285, 3629 and 2171 data points, obtained from 2307, 327 and 460 sampling sites, respectively. The complete dataset is available: 10.17862/cranfield.rd.20227497.

3.1. Viral sampling, concentration, and elution

Determining viral concentrations in water requires multiple sequential steps because viral abundance is usually low in most drinking-water-relevant matrices. This often entails sampling and capturing viruses from relatively large volumes (or from pre-concentrated solids), followed by elution and secondary concentration (e.g., centrifugation, precipitation or ultrafiltration) prior to nucleic-acid extraction and (RT-)qPCR (Fig. 3; Table 2). To support reliable interpretation, studies should include appropriate quality controls such as field blanks, extraction blanks, whole-process controls, and PCR inhibition checks (e.g., internal amplification controls, matrix spikes, or sample dilution). Whole-process controls help track performance across concentration, elution and extraction steps, whereas extraction/amplification controls isolate later analytical stages; both are important because humic substances, suspended solids and other co-concentrated matrix constituents can suppress enzymatic amplification and bias virus quantification (Pang et al., 2012; Haramoto et al., 2018).

Recovery efficiency depends on the concentration method, water-matrix characteristics (e.g., particulate matter, pH and natural organic matter), virus properties (e.g., size and charge), the choice of lysis/elution chemistry, and the spiked virus concentration (Fig. 1). For efficiency testing, non-pathogenic coliphages (e.g., ΦX174 and MS2) are commonly employed because they can reasonably reflect recovery for other viruses (Fig. 3). However, their baseline prevalence in some water matrices can complicate interpretation of spike-and-recovery experiments. Where endogenous levels are expected, background should be quantified in unspiked aliquots and subtracted from spiked recoveries. Alternative whole-process controls such as mengovirus, murine

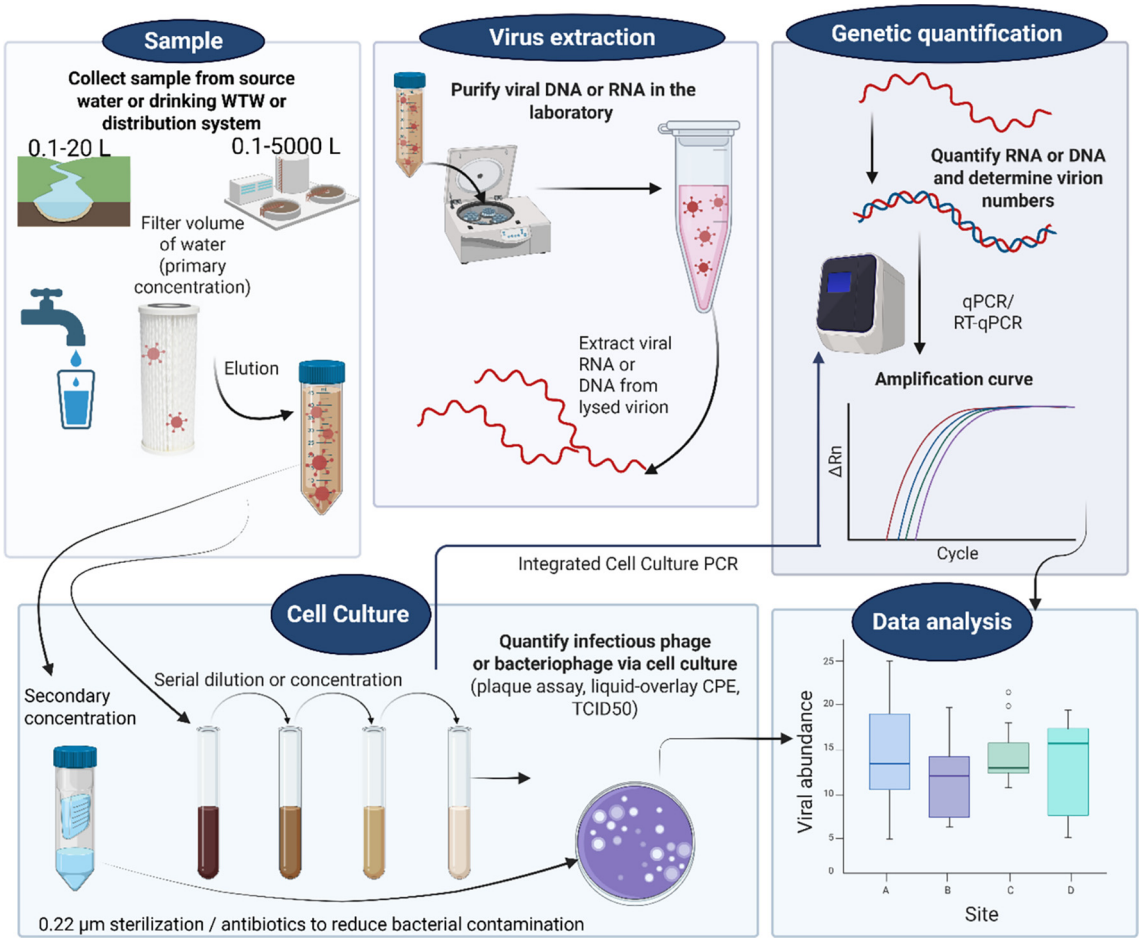


Fig. 2. Measuring viruses in aquatic systems, modified from Ahmed et al. (2020).

Table 1
Number of samples reported as positive for virus presence, compared with the number of samples tested, by either enumerating genome copies (using PCR), applying infectivity-informed methods, or observing cytopathic effects (CPE). The 'Infectivity-informed methods' column groups integrated cell culture (e.g., ICC/ICC-qPCR) with capsid-integrity or host-binding proxy approaches (e.g., EMA-(RT-)qPCR, SD-CDDP-(RT-)qPCR and PGM-MBs); these should not be interpreted as equivalent to direct culture-based infectivity measurements unless explicitly identified as ICC/ICC-qPCR. The column '% infectivity/GC' represents the percentage of samples estimated as infective or intact relative to the samples that tested positive for viral genome copies. Data are sorted by virus type and matrix type. ΣnD+ denotes the cumulative number of positive detections, while ΣnD indicates the cumulative number of sampling data points. Where 'ID not found' = no robust infectious-dose estimate (e.g., ID50 or HID50) was identified in the cited literature for that virus.

Virus	Matrix	Genome copy (n_D^+/ n_D)	Infectivity-informed methods (nD+/ nD)	CPE (n_D^+/ n_D)	% infectivity ratio (Infectivity: GC)	References
	Treated effluent	10/10	4/10 ICC	10/10*	40-100	(Simmons and Xagoraraki, 2011)
		50/50	10/50 ICC		20	(Qiu et al., 2018)
		17/24	13/24 ICC	9/12	53-76	(Fongaro et al., 2013)
		39/180	1/180 ICC		2.6	(Pang et al., 2019)
		15/20	9/20 ICC		60	(Schijven et al., 2019)
		21/24		15/24	71.4	(Mackowiak et al., 2018)
	Surface waters	15/15	11/15 ICC		73.3	(Sedji et al., 2018)
		15/20	14/20 EMA-(RT)-qPCR		93.3	(Prevost et al., 2016)
		53/68	0/68 ICC		0	(Merrett et al., 2013)
Adenovirus		5/15	8/15 ICC		160	(Dong et al., 2010)
(HID_{50} 6.6 particles)		13/20	9/20 SD-CDDP-(RT-)qPCR		69	(Canh et al., 2021)
		16/16	8/16 ICC		50	(Sylvestre et al., 2021)
		24/964	5/24 ICC	1/24	4-21	(Stokdyk et al., 2020)
	Subsurface	157/1204	40/157 ICC		25.5	(Borchardt et al., 2012)
	water	15/15		0/15	0	(Betancourt et al., 2014)
		0/6	2/6 ICC		positive ICC-PCR but not positive for qPCR	(Sylvestre et al., 2021)
	Treated water					
		8/12	7/12 ICC	4/12	50-87.5	(Fongaro et al., 2013)
		16/49	27/49 EMA-(RT)-qPCR		168	(Prevost et al., 2016)
		2/11	4/11 ICC		200	(Dong et al., 2010)
	Surface waters	4/20	4/20 EMA-(RT)-qPCR		100	(Prevost et al., 2016)
		13/20	4/20 SD-CDDP-(RT-)qPCR		31	(Canh et al., 2021)
Aichi virus (ID not found)	Subsurface water	15/15		0/15	0	(Betancourt et al., 2014)
	Treated water	2/43	0/43 SD-CDDP-(RT-)qPCR		0	(Canh et al., 2021)
		3/49	0/49 EMA-(RT)-qPCR		0	(Prevost et al., 2016)
	Treated effluent	10/23	5/23 ICC		50	(Morsy El-Senousy et al., 2007)
Astrovirus (ID not found)	Surface waters	2/20	2/20 EMA-(RT)-qPCR		100	(Prevost et al., 2016)
		1/12	1/12		100	(Morsy El-Senousy et al., 2007)
	Treated water	1/12	0/12		0	(Morsy El-Senousy et al., 2007)
		46/50	7/50 ICC		15	(Qiu et al., 2018)
	Treated effluent	6/10	0/10	10/10*	0-166	(Simmons and Xagoraraki, 2011)
		26/180	2/180 ICC		7.7	(Pang et al., 2019)
		1/20	0/20 EMA-(RT)-qPCR		0	(Prevost et al., 2016)
	Surface waters	9/20	4/20 SD-CDDP-(RT-)qPCR		44.4	(Canh et al., 2021)
Enterovirus ($0.07\text{-}175 \times 10^6$ PFU)		3/16	1/16		33.3	(Sylvestre et al., 2021)
		5/24		5/24	100	(Mackowiak et al., 2018)
		15/15		0/15	0	(Betancourt et al., 2014)
	Subsurface	9/964	0/9 ICC	0/9	0	(Stokdyk et al., 2020)
	water	109/1204	31/109 ICC		28	(Borchardt et al., 2012)
	Treated water	5/49	0/49 EMA-(RT)-qPCR		0	(Prevost et al., 2016)
Hepatitis E virus ($TCID_{50}$ 160-27,000)	Surface waters	21/27		3/13	23*	(Salvador et al., 2020)
	Treated water	24/36		5/19	26*	(Salvador et al., 2020)
Polyoma virus (ID not found)	Surface waters	14/40	9/40 SD-CDDP-(RT-)qPCR		64	(Canh et al., 2021)
	Treated effluent	14/39	8/39 Capsid integrity (PGM-MBs)		57	(Farkas et al., 2018)
Norovirus GI (HID_{50} 1320-2800 GC) †	Surface waters	16/20	15/20 EMA-(RT)-qPCR		94	(Prevost et al., 2016)
		4/52	1/52 Capsid integrity (PGM-MBs)		25	(Farkas et al., 2018)
		9/20	6/20 SD-CDDP-(RT-)qPCR		66.67	(Canh et al., 2021)
	Treated water	4/49	0/49 EMA-(RT)-qPCR		0	(Prevost et al., 2016)
	Treated effluent	16/39	12/39 Capsid integrity (PGM-MBs)		75	(Farkas et al., 2018)
		5/52	2/52 Capsid integrity (PGM-MBs)		40	(Farkas et al., 2018)
Norovirus GII (HID_{50} 1320-2800 GC) †	Surface waters					
		9/20	4/20 SD-CDDP-(RT-)qPCR		44.44	(Canh et al., 2021)
		11/20	9/20 EMA-(RT)-qPCR		81.2	(Prevost et al., 2016)
		2/24		0/24	0	(Mackowiak et al., 2018)
	Treated water	5/49	0/49 EMA-(RT)-qPCR		0	(Prevost et al., 2016)
	Treated effluent	50/50	4/50		8	(Qiu et al., 2018)

(*continued on next page*)

Table 1 (*continued*)

Virus	Matrix	Genome copy (n_D^+/ n_D)	Infectivity-informed methods (nD+/ nD)	CPE (n_D^+/ n_D)	% infectivity ratio (Infectivity: GC)	References
Rotavirus		4/24		3/24	75	(Mackowiak et al., 2018)
(HID_{50} 10PFU)	Surface waters	111/180	0/180 ICC		0	(Pang et al., 2019)
		4/20	4/20 EMA-(RT)-qPCR		100	(Prevost et al., 2016)
		16/16	0/16 ICC-qPCR		0	(Sylvestre et al., 2021)
	Treated water	3/6	0/6 ICC-qPCR		0	(Sylvestre et al., 2021)
	Surface waters	3/20	4/20 EMA-(RT)-qPCR		133	(Prevost et al., 2016)
Sapovirus (1000-2800 GC)						

HID50 – 50 % human infectious dose; TCID50 – 50 % tissue culture infectious dose; * – generic CPE, not confirmed to virus type; CPE – cytopathic effects; ICC/ICC-qPCR – integrated cell culture with qPCR readout where stated; EMA-(RT-)qPCR – ethidium monoazide; SD-CDDP-(RT-)qPCR – sodium deoxycholate cis-dichlorodiamineplatinum; PGM-MBs – porcine gastric mucin-magnetic beads. The 'Infectivity-informed methods' column includes integrated cell culture and capsid-integrity/host-binding proxy methods and is not equivalent to direct culture-based infectivity measurements unless ICC/ICC-qPCR is specified. † Norovirus HID50 estimates vary by genotype, measurement approach (GC vs PFU), and host susceptibility; reported minimum infectious doses may be lower than 1320–2800 GC. Some ratios may use subset denominators when infectivity-informed testing was performed only on a subset and may therefore not be directly comparable.

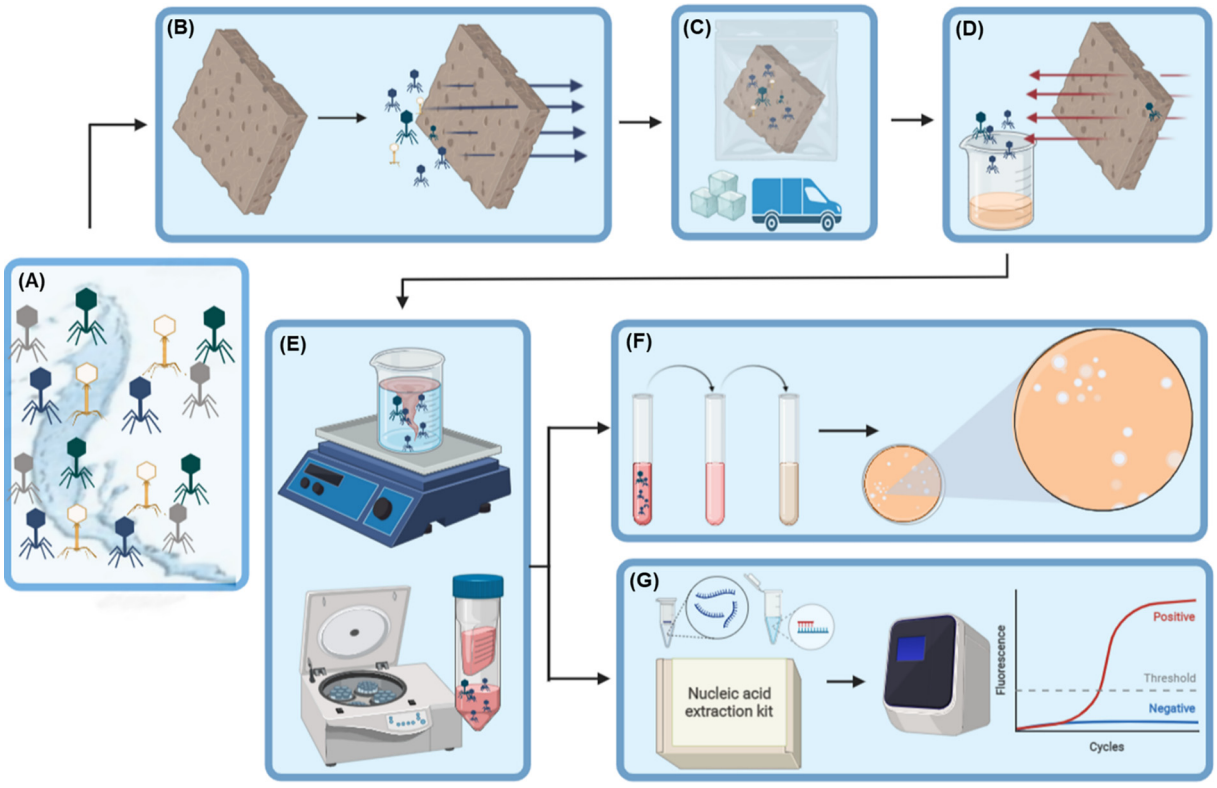


Fig. 3. Steps involved in the concentration and detection of viruses and phages in water matrices: (A) Collection of samples from various water matrices; (B) Primary concentration of viruses and phages, typically accomplished using membranes (through processes like VIRADEL with electronegative filters, or ultrafiltration in either dead-end or tangential flow modes); (C) Safeguarding membrane transport using cold storage (on ice); (D) Elution, or back-washing of viruses and phages that have been retained on the membrane using an elution buffer; (E) Secondary concentration of the eluted solution, which contains the initially retained viruses and phages. The detection of these viruses and phages is done by either (F) evaluating virus or phage infectivity or integrity, or (G) extracting nucleic acids to estimate the quantity of viral or phage gene copies present.

norovirus, *Pseudomonas* bacteriophage phi6, or armoured DNA/RNA surrogates may therefore be preferable in matrices with persistent indigenous phage backgrounds (Coudray et al., 2013).

3.1.1. *Impact of water matrix on virus recovery*

Across the reviewed studies (Table 3), sampling volumes and concentration approaches varied substantially by matrix. For surface waters (rivers, lakes/reservoirs and streams), reported sampling volumes ranged from approximately 10 to 710 L; higher turbidity and particulate loads can reduce effective volumes through filter clogging and can lower recovery, particularly for larger viruses or for viruses strongly associated with particulates. Both adsorption-based membrane methods (electronegative/electropositive filters) and ultrafiltration approaches (e.g., hollow-fibre or dead-end ultrafiltration) have been applied, and performance is influenced by water chemistry and the choice of eluants, including proteinaceous eluants (e.g., beef extract, glycine) and surfactant-based formulations (e.g., Tween-80 or sodium polyphosphate) (Hill et al., 2005; Hill et al., 2007; Rhodes et al., 2016).

For groundwater samples, electropositive filtration was commonly used, and eluants such as beef extract with surfactants (e.g., Tween-80) and salts (e.g., NaCl) were frequently reported. Small volumes (often around 0.5 L) were used in several method-evaluation studies because of lower turbidity and fewer interfering substances; however, these were typically controlled recovery experiments using spiked surrogates and

Table 2
Advantages and disadvantages of commonly used primary concentration methods for viral concentration in drinking water. The methods recommended for onsite viral concentration of large volumes of drinking water are denoted by an asterisk (*). TFUF – Tangential Flow UltraFiltration, DeUF – Dead-End UltraFiltration, PCR – Polymerase Chain Reaction, PEG – PolyEthylene Glycol, DEAE-silica – DiEthylAminoEthyl-silica.

Concentration principle	Concentration method	Advantages	Drawbacks
Ultrafiltration	TFUF*	Useful for large volumes	May require filter conditioning
	DEUF*	Can be automated Useful for large volumes	
Adsorption-Elution	Electropositive filters*	Useful for large volumes Field deployable Virocap/NanoCeram® Recommended by USEPA Method 1615 Can be automated Reduces amount of PCR inhibitors	Turbid samples may clog filters Expensive Multiple steps lead to viral loss
	Electronegative filters	Direct extraction can be done directly from membrane Reduces amount of PCR inhibitors	Turbid samples may clog filters Requires conditioning Multiple steps lead to viral loss Not feasible for large volume samples
Centrifugation-based	All	Good recoveries	Expensive equipment needed
		Useful for turbid samples	Not feasible for large volume samples
	Ultracentrifugation	No preconditioning One step method	Not feasible for large volume samples
Precipitation/Flocculation	All	Useful for turbid samples	Not feasible for large volume samples
		Low cost	May require preconditioning/centrifugation
	PEG/Dextran	Recommended for poliovirus surveillance by WHO	Concentrates enzymatic inhibitors (PCR inhibitors)
	Skimmed Milk Flocculation	Field deployable (Virwatest) One-step method Up to 40 L samples	Time consuming
Others	Sorption chromatographic columns (DEAE silica gel, monolithic chromatography)	Good recoveries for small volumes	Expensive equipment and consumables

should not be interpreted as typical field surveillance volumes. For routine pathogen surveillance in groundwater or treated water, larger volumes and/or replicate sampling may be required because virus concentrations are often very low. Reporting the sampled volume, effective processed volume and overall concentration factor is therefore essential for comparability and for interpreting non-detects (Cashdollar and Wymer, 2013; Ikner et al., 2012).

In addition to matrix-driven method selection, sampling design (where, when and how much) strongly influences detection. Most studies relied on grab sampling at raw-water intakes, downstream of wastewater discharges, or within distribution networks; fewer used time- or flow-weighted composite samples, even though composites better integrate temporal variability and characterise average exposure rather than instantaneous concentration. Event-based sampling during rainfall-runoff and combined sewer overflow events, and seasonally resolved sampling, can improve characterisation of peak viral loads and treatment challenges (Fauvel et al., 2016; Sylvestre et al., 2021). Where feasible, larger sample volumes and replicate samples increase detection probability at low concentrations, but practical limits arise from filter clogging and processing capacity; transparent reporting of sampling context, hydraulics, antecedent weather and other meteorological conditions improves interpretation of occurrence and persistence data.

3.2. Coliphages as viral indicators in aquatic systems

Various phage groups are used to assess water quality, with somatic and F-specific coliphages being the most common viral indicators. Total coliphages (somatic + F-specific) are also widely used, and phages infecting Bacteroides spp. can be valuable for detecting human-specific contamination. International guideline and regulatory frameworks increasingly recognise coliphages as practical viral indicators to support water safety plans (WSPs) and to verify disinfection and physical removal efficacy for viruses (WHO, 2017b; Goodwin et al., 2015; Gunnarsdottir et al., 2020). For example, North Carolina regulations for Type 2 reclaimed water specify coliphage limits of 5 PFU/100 mL (monthly mean) and 25 PFU/100 mL (daily maximum) (NC OAH, 2011), while the EU Drinking Water Directive includes somatic coliphages as a risk-based operational parameter in certain circumstances (EU Drinking Water Directive, 2020).

Comparing coliphage measurement methods, ISO and USEPA have protocols tailored to different matrices and analytical volumes. ISO 10705-1 (F-specific RNA coliphage) and ISO 10705-2 (somatic coliphage) use a double-layer agar (DAL) plaque assay, typically inoculating 1 mL aliquots; in practice these methods can also be applied to concentrates derived from much larger sample volumes (ISO, 1995; 2000). USEPA Method 1601 provides a qualitative two-step enrichment approach for total coliphage, and Method 1602 provides a single-agar-layer (SAL) enumeration approach for total coliphage, commonly applied to 100 mL groundwater samples (USEPA, 2001a, 2001b). USEPA Method 1642 applies dead-end hollow-fibre ultrafiltration to 1 L recreational water samples (fresh and marine) prior to SAL enumeration, whereas Method 1643 is a SAL enumeration method for untreated secondary wastewater (USEPA, 2018a, 2018b). In general, larger analytical volumes can improve sensitivity for low-concentration waters, but method selection should consider matrix effects, practicality and the availability of validated concentration steps. Commercial rapid formats have also been developed to simplify culture-based coliphage detection (e.g., Bluephage for somatic coliphages) (Muniesa et al., 2018).

Multiple guidelines exist to ensure sufficient log reduction values (LRVs) for pathogenic viruses across drinking water treatment steps, and viral indicators such as coliphages are often used to verify performance. In the US, the Surface Water Treatment Rule (SWTR) requires at least 4-log (99.99 %) removal/inactivation of viruses and 3-log (99.9 %) removal/inactivation of *Giardia lamblia* for systems using surface water or groundwater under the direct influence of surface water (SWTR, 1989). The Interim Enhanced SWTR added strengthened requirements for Cryptosporidium, including a minimum 2-log (99 %) removal requirement for systems that provide filtration (IESWTR, 1998). California's indirect potable reuse regulations specify cumulative treatment targets of 12/10/10 log reductions for viruses, *Cryptosporidium* and *Giardia*, respectively, from raw wastewater to potable output (CDPH, 2014; Olivieri et al., 2020), and pathogen log-reduction targets for direct potable reuse are also being refined (Gerrity et al., 2023). In Canada, guidance recommends at least 4-log removal and/or inactivation of enteric viruses in drinking water systems (Health Canada, 2019).

Table 3
Recovery of phages and viruses (including cucumber green mottle mosaic virus (CGMMV), murine norovirus (MNV), and mengovirus (MengoV)) utilized as whole process controls (WPC) in various water matrices, as reported in the selected studies. The legend displays the sampling volume, viral concentration (primary and/or secondary), and elution methods employed. Onsite concentration of samples is indicated by an asterisk (*). PEG refers to polyethylene glycol, NaPP stands for sodium polyphosphate, and PBS denotes phosphate-buffered saline.

SAMPLING VOLUME (L)	MATRIX	PRIMARY CONCENTRATION METHOD	SECONDARY CONCENTRATION METHOD	ELUANT	WPC PHAGE RECOVERY (%)	WPC VIRUS RECOVERY (%)
14	Surface water	Electronegative filtration	none	acid rinse-alkaline elution	MS2≥10 %	CGMMV≥10 %
20				none	'Not reported'	MNV 1-100 %
5-6			Flocculation-filtration	Lysis buffer		MNV 0.005-1.8 %
5-6				Lysis buffer		MNV 7.4-68 %
5-6				Lysis buffer		MNV 9-54 %
5-6			Electronegative filtration	acid rinse-alkaline elution		MNV 9-54 %
150-500*	Treated potable water		none	acid rinse-alkaline elution	MS2≥10 %	CGMMV≥10 %
710*	Surface water	Electropositive filtration	Flocculation-filtration	Beef extract		MengoV <5 %
0.5*	Groundwater		none	Beef extract, Tween-80, NaCl	Somatic 81-97 %	
0.5*			none	Beef extract, Tween-80, NaCl	F specific 8-36 %	
0.5	Treated potable water		none	none	'Not reported'	MNV 8.7-30.8%
10	Treated effluent	Ultrafiltration	PEG precipitation	Beef extract	'Not reported'	MengoV 10-100%
10				Beef extract		MengoV 13-81 %
10	Reclaimed water		PEG precipitation	Tween 80, NaPP, Antifoam Y	PRD1 86-139 %	
10				Tween 80, NaPP, Antifoam Y	SP phage (Genogroup IV) 90-127 %	
10			Celite precipitation	Tween 80, NaPP, Antifoam Y	PP7 phage 7.8-112 %	
10	Surface water		PEG precipitation	Beef extract	'Not reported'	MengoV 10-100%
10				Beef extract		MengoV 19-100%
10				Beef extract		MengoV 10-100%
150-510*			none	PBS, Antifoam A, Tween 80, NaPP	Somatic coliphages 3.6-20 %	
150-510*				PBS, Antifoam A, Tween 80, NaPP	F specific 0-24 %	
150-510*				PBS, Antifoam A, Tween 80, NaPP	Total coliphages (CB390PH) 0.5-24 %	
100	Treated potable water		Centrifugal ultrafiltration	Tween, NaPP, Antifoam Y	MS2 48 %	
0.1				Tween, NaPP, Antifoam Y	MS2 109 %	
10	Surface water	Flocculation-Centrifugation	none	none	'Not reported'	MengoV 46-50 %
10				none		MengoV 15-19 %
10	Treated potable water		none	none	'Not reported'	MengoV 24-50 %
10				none		MengoV 15-35 %

Australia's required treatment level is risk-based and depends on source water quality, with higher-risk categories requiring higher log reductions for protozoa, viruses and bacteria (NHMRC, 2011). Despite these standards, viral genomes can sometimes be detected in source and treated waters; therefore, continuous monitoring and robust operational verification remain important.

A key advantage of coliphages is that they can be quantified by relatively simple culture-based assays using *E. coli* host strains, and in

some contexts by molecular assays. Plaque assays directly enumerate infectious phage particles; where antibiotic-resistant host strains are used, samples can often be analysed without prior sterilization to suppress background bacteria. By contrast, mammalian cell-culture infectivity assays are generally more labour-intensive and typically require pre-sterilization (e.g., 0.22 μm filtration) to limit bacterial contamination before inoculation (Hamza et al., 2011; Haramoto et al., 2018; Singh et al., 2022). qPCR-based assays can be highly sensitive but are often strain- or marker-specific and are not yet standardised for broad enumeration of somatic or F-specific coliphage groups. In drinking water and other low-contamination matrices, the relatively high natural abundance of coliphages (compared with many human viruses) can improve the probability of detection and make them useful for assessing treatment and distribution system integrity. However, genome-to-PFU ratios can vary widely across phage types and environmental conditions, and culture and molecular results are not directly interchangeable; therefore, studies should report methods and units transparently and interpret molecular signals cautiously (Muniesa et al., 2018; Singh et al., 2022).

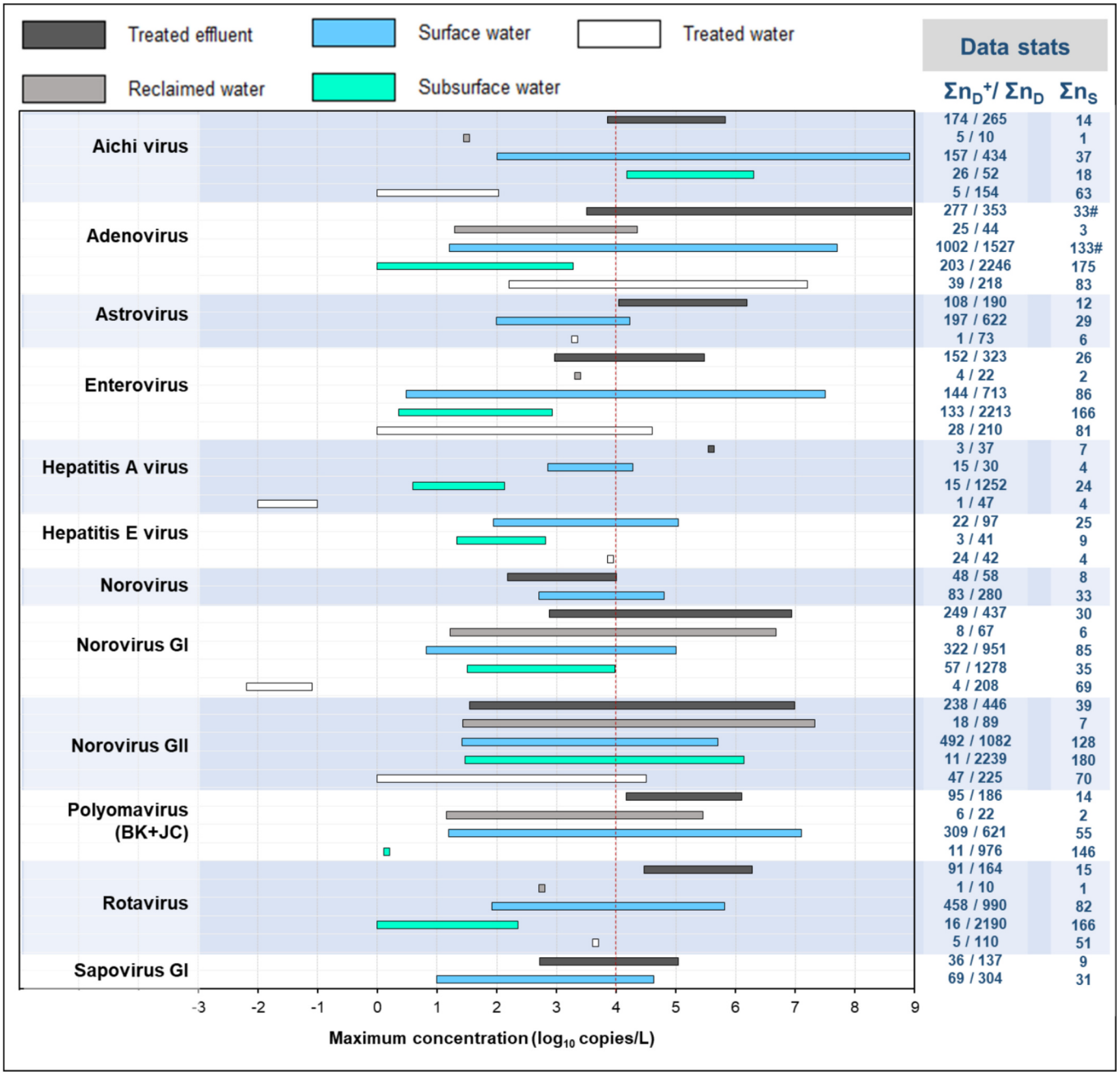


Fig. 4. The maximum concentration ranges of pathogenic viruses found in various raw and treated water matrices, as reported in multiple studies (see Table S1 for details). 'Treated effluent' refers to tertiary treated output from wastewater plants. 'Surface water' encompasses lakes, reservoirs, and rivers. 'Subsurface water' includes groundwater and well water. 'Treated potable water' covers treated drinking water from DWTP, distribution systems, and point-of-use supplies. ΣnD+ denotes the total number of positive detections, ΣnD the total number of sampling data points, ΣnS the total number of sampling sites, and nD+/nD the ratio of positive detections to total sampling data points. The symbol '#' indicates missing data points (either nD+, nD, or nS) due to unreported data in some publications (refer to Table S1). Because maxima are shown, single-study outliers can influence the upper-bound value; for HEV in treated potable water, the plotted maximum represents a single-study upper-bound observation.

3.3. Prevalence of human pathogenic viruses in drinking water sources

Based on the compiled dataset, viral genomes (detected via (RT-) qPCR) belonging to the 10 target human enteric/pathogenic viruses were reported with varying levels across different water matrices (Fig. 4). Reported concentrations spanned wide ranges by water type: 1.5-7 log10 GC/L in tertiary treated effluent, 1.2-7.3 log10 GC/L in reclaimed water, 0.5-8.9 log10 GC/L in surface water, 1.2-7.3 log10 GC/L in subsurface water, and -1.0 to 4.6 log10 GC/L in treated potable water. PCR-based approaches can overestimate infectious virus abundance because they detect nucleic acids from both viable and inactivated viruses, as well as free nucleic-acid fragments; therefore, genome copy numbers do not directly indicate infective dose without additional viability/infectivity information (Ditommaso et al., 2014). Accordingly, this makes it challenging to compare datasets derived from different methods. Therefore, standardised multi-method monitoring is needed for source and treated waters.

In this study, the number of sampling data points (nD) for each virus varied widely, from as few as 10 to as many as 2246, while the number of sampling sites (nS) ranged from 1 up to 180. Marked variability emerged in the percentage of positive detections, from 0.5 % (for norovirus GII in groundwater) to 66 % (for adenovirus in surface water). Generally, subsurface waters (0.5-10 %) and treated drinking waters (1-20 %) showed lower positivity rates than other matrices. Groundwater exhibited the lowest frequency of detection and the narrowest viral concentration range among the source waters, likely reflecting confinement and natural filtration as water percolates into aquifers. An exception involved a Finnish outbreak setting where norovirus GII loads in subsurface water (6.2 log10 GC/L) approximated those in surface water (5.7 log10 GC/L), reflecting faecal ingress rather than typical background groundwater quality (Kauppinen et al., 2018). For hepatitis E virus (HEV), the treated-potable-water maximum shown in Fig. 4 (3.95 log10 GC/L) represents a single-study upper-bound observation from Portugal and should not be interpreted as a typical treated-water concentration; within that study, variability was wide and source-water maxima were higher (Salvador et al., 2020). Additional screened studies that reported non-detects did not alter the plotted upper bound because Fig. 4 and Table S1 synthesise maximum reported concentrations.

Of the viruses studied, five (adenovirus, enterovirus, hepatitis A virus, norovirus GI/GII, and rotavirus) were sampled and identified most frequently ($nS > 100$, $nD > 1000$) compared with aichivirus, astrovirus, hepatitis E virus, and sapovirus ($nS < 65$, $nD < 650$). This disparity likely reflects targeted monitoring of viruses with recognized public health significance, low infectious doses, or explicit regulatory interest (e.g., the US EPA drinking water contaminant candidate list and WHO drinking-water guidance) (USEPA, 2021; WHO, 2017a). Frequent detection should not, however, be equated with intrinsically higher prevalence; it also reflects research and monitoring priorities (Fig. 4).

Viral genomes (e.g., aichivirus, adenovirus and norovirus) may be reduced during wastewater treatment but can remain detectable in tertiary effluent and reclaimed water, which can contribute to surface or subsurface source waters via discharge or de facto reuse (Bailey et al., 2018; Prado et al., 2019). Viral genomes have also been reported in some studies of finished drinking water and distribution systems (Fig. 4); however, genome detection alone does not confirm infectivity and may reflect incomplete removal, post-treatment contamination, or persistence of non-infectious nucleic acids. Quantitative microbial risk assessment (QMRA) frameworks often use disability-adjusted life year (DALY) metrics to translate exposure into health burden (Havelaar and Melse, 2003; Medema, 2012). Because PCR-based methods detect genetic material regardless of infectivity, risk estimates based solely on genome copies can be conservative; where feasible, integrating infectivity-informed measurements (e.g., cell culture or validated capsid-integrity assays) alongside molecular detection improves interpretation (Omatola and Olaniran, 2022; Sylvestre et al., 2021; Richiardi et al., 2023).

3.4. Prevalence of coliphages and emerging indicators

Retrospective analyses of drinking-water-associated outbreaks – such as the 2007 multi-pathogen outbreak in Finland (Laine et al., 2011) and the 2010 norovirus outbreak in Sweden (Riera-Montes et al., 2011) - demonstrate that relying solely on bacterial indicators (e.g., *E. coli*, coliforms) does not consistently safeguard public health from enteric viruses, which can be present even when bacterial indicators are absent (Korajkic et al., 2018). These events have contributed to increasing interest in incorporating viral indicators (e.g., coliphages) and, where feasible, direct viral monitoring into water safety planning and risk-based operational monitoring, complementing traditional bacterial indicators (USEPA, 2006; EU Drinking Water Directive, 2020; NHMRC, 2011).

Despite progress in water safety approaches, significant disparities exist in data availability and distribution across various water matrices (Figs. 2, 3). Among 193 nations recognized by the UN, comprehensive viral occurrence data is substantially available only for the US, Spain, and Japan (UNEP, 2021). Additional data from 26 countries are sparse (UN-Water, 2021), and many lower-income nations have little or no data (Our World in Data, 2021; UNEP, 2021). This information gap is especially concerning where fast population growth and poor source water quality intensify viral risks (Soliman, 2024). Closing the knowledge gap in viral monitoring thus remains essential, particularly for low- and middle-income countries.

Reported maximum coliphage abundance ranged from 1.8 to 6.7 log10 PFU/L in tertiary treated effluent, 0.5-1 log10 PFU/L in reclaimed water, 1.6-5.6 log10 PFU/L in surface water, and 1.9-3.3 log10 PFU/L in subsurface water (Fig. 5). In several studies, coliphages and enteric viruses were detected concurrently, supporting their use as practical indicators of faecal contamination and potential viral pathways. In the dataset compiled here, culturable coliphages (somatic, F-specific, or F-specific RNA coliphages (FRNAPH)) were rarely detected in treated potable water (Fig. 5); however, non-detection does not prove absence and may reflect effective treatment, limited sampling, or analytical sensitivity constraints. For Fig. 5 and Table S1, infectious FRNAPH and other culture-based coliphage measurements are reported in PFU/L, whereas FRNAPH genogroups and molecular viral indicators are retained in genome copies/L as reported by the source studies; these endpoint types were not converted and should not be interpreted as directly interchangeable but are presented together to provide review of existing data. The number of treated-water observations remains relatively small (maximum detects $nD+/nD = 47/225$ from 70 sites), reinforcing the need for more systematic monitoring of final water and distribution systems.

Indicator-based strategies can streamline viral monitoring by targeting abundant, consistently detectable markers. In addition to coliphages, emerging molecular indicators such as crAssphage (a human gut-associated bacteriophage group) and plant viruses such as pepper mild mottle virus (PMMoV) and tobacco mosaic virus (TMV) have been proposed as potential surrogates for human faecal contamination. In this meta-analysis, culturable coliphages were rarely reported in treated potable water, consistent with effective treatment and/or limited data; absence of detection should not be interpreted as proof of absence of infectious human viruses. By contrast, PMMoV genomes were detected in some treated potable waters (2.9-4.9 log10 GC/L), illustrating that molecular targets can persist even when infective particles are reduced below detection by culture-based indicators. Maximum crAssphage counts were 5.3-8.5 log10 GC/L in tertiary effluent and 5.7-8.3 log10 GC/L in surface water. TMV and PMMoV were also highly abundant in tertiary effluent and surface waters. Importantly, PMMoV and TMV are plant viruses; their presence is influenced by dietary inputs and can also reflect crop-derived or agricultural runoff sources, meaning they are not strictly human-specific. Therefore, pairing plant virus markers with

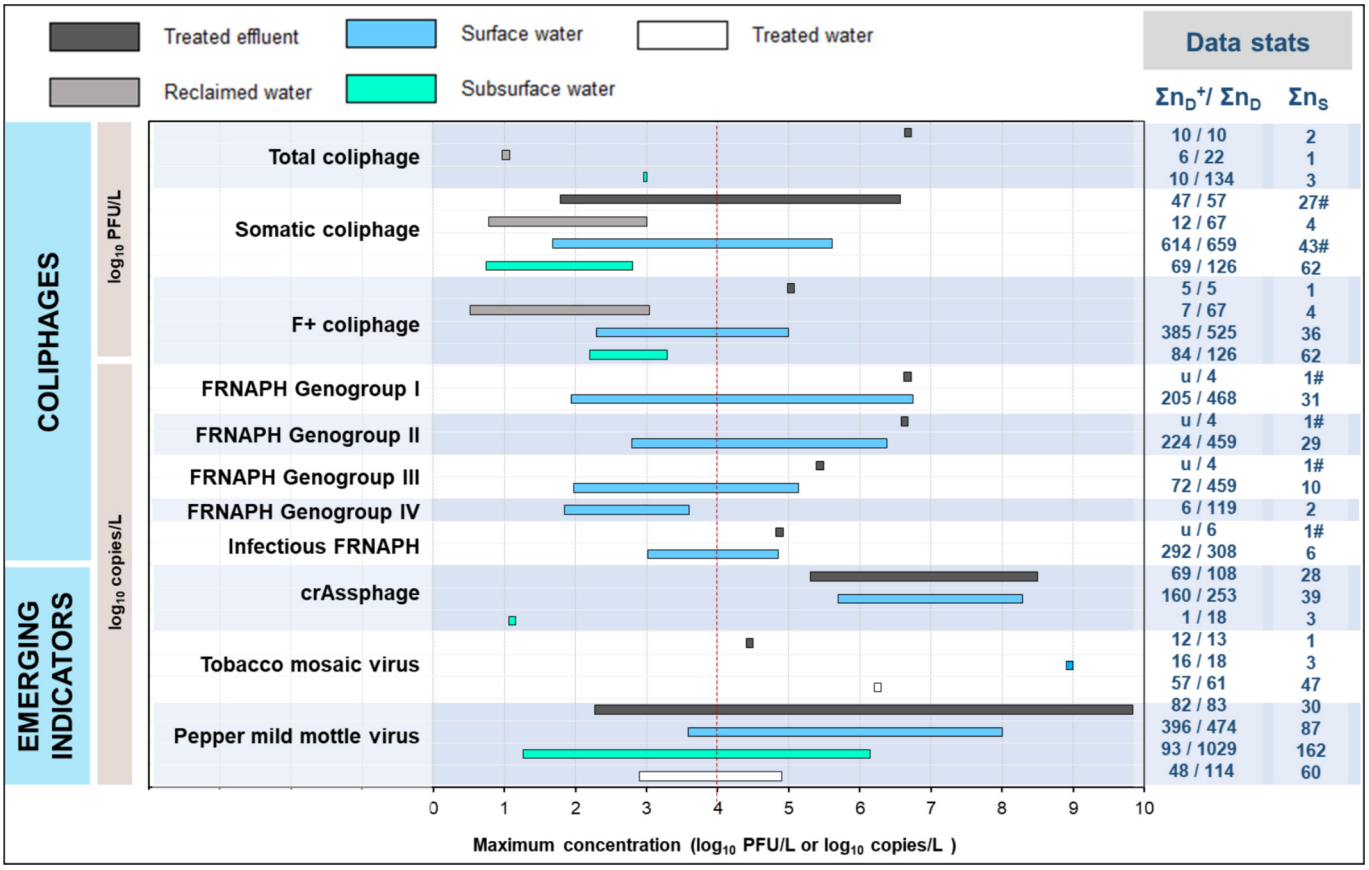


Fig. 5. The maximum ranges of coliphages and viral surrogates in different raw and treated water types, as reported in several studies (details in Table S1). 'Treated effluent' encompasses tertiary treated wastewater from treatment facilities. 'Surface water' includes lakes, reservoirs, and rivers. 'Subsurface water' denotes groundwater and well water. 'Treated water' refers to drinking water processed at treatment plants, distributed via systems, and supplied at point-of-use. FRNAPH - F-specific RNA bacteriophages; TMV - tobacco mosaic virus; PMMoV - pepper mild mottle virus; ΣnD+ - cumulative number of positive detects; ΣnD - cumulative number of sampling data points; ΣnS - cumulative number of sampling sites; nD+/nD - ratio of positive detects to total sampling data points. # - some missing data points (nD+ or nD or nS) since data not reported in all publications (refer to Table S1). All FRNAPH concentrations (genogroups I-IV and infectious FRNAPH) are expressed as RNA-genome copies per litre (copies L^-1). Plaque-forming-unit values are shown in their reported PFU L^-1 units on the same log10 axis for visual comparison and were not converted to genome equivalents.

human-associated markers (e.g., crAssphage and/or bacterial microbial source tracking (MST) markers) can improve source attribution and reduce misinterpretation in catchments with agricultural activity. In practice, coliphages provide a stronger operational measure of treatment process effectiveness (infective / culturable particles), whereas plant viruses and crAssphage provide high-sensitivity molecular signals that can support tracking of faecal contamination and persistence; the optimal combination depends on the monitoring objective (treatment validation, pathogen detection, or source tracking) (Ahmed et al., 2019a, 2019b; Betancourt et al., 2014; Canh et al., 2021; Gu et al., 2018; Hata et al., 2021; Tandukar et al., 2018) but PMMoV/TMV need careful interpretation – especially in drinking water studies.

3.5. *Infectious human enteric viruses: detection method development*

Measuring viral infectivity typically involves culturing water samples on mammalian cell lines, since no single line can support all viruses within a family (Hamza et al., 2011). Cell culture requires specialised laboratories, equipment and skilled personnel, thus adding complexity and cost. Recently, capsid-integrity qPCR has emerged to differentiate infectious from non-infectious viruses. It relies on intercalating azo dyes, such as ethidium monoazide (EMA) and propidium monoazide (PMA), or related treatments that preferentially suppress amplification from damaged particles (Canh et al., 2021). These approaches can shorten turnaround time relative to cell culture, but their performance is virus- and strain-dependent and they do not replace direct infectivity assays (Leifels et al., 2021; Oristo et al., 2018). Additional approaches including surfactants, platinum compounds, enzymes, and receptor- or antibody-based capture (e.g., porcine gastric mucin magnetic beads, PGM-MBs) have also been explored to improve discrimination of intact or host-binding particles (Farkas et al., 2018; Oristo et al., 2018).

Table 1 summarises genome-positive samples alongside results from infectivity-informed methods and cytopathic-effect (CPE) observations. Culture-based assays provide a direct measure of infectivity for viruses that can be propagated in available host systems, whereas capsid-integrity or host-binding methods are proxy approaches that infer whether particles are more likely to remain intact. Across studies, reported ratios between infectivity-informed positives and genome positives span wide ranges and should be interpreted cautiously. Ratios above 100 % most likely reflect methodological uncertainty and differences between assays (e.g., variability in culture versus qPCR, differential recovery, and inhibition or underestimation in molecular assays). Conversely, very low ratios indicate that only a small fraction of detected genomes are infectious under the assay conditions. Importantly, infectivity assays are host- and strain-dependent, and absence of culture positivity does not necessarily mean absence of infectious virus; combining molecular and infectivity-informed approaches therefore improves interpretation of treatment performance and risk (Shaffer et al., 2022; Leifels et al., 2021).

Examining norovirus GI and GII across different matrices highlights

how strongly results depend on the infectivity-related method applied (Table 1). For norovirus GI in surface waters, genome-positive detections ranged from 4/52 to 16/20, while infectivity-informed positives ranged from 1/52 using porcine gastric mucin magnetic-bead capture (PGM-MBs) to 15/20 using EMA-(RT-)qPCR and 6/20 using SD-CDDP-(RT-)qPCR. For treated effluent, norovirus GI remained detectable by both genome and host-binding proxy methods (14/39 and 8/39, respectively), while in treated water both norovirus GI and GII were occasionally genome-positive but were not detected by EMA-(RT-)qPCR in the available 49-sample treated-water datasets. Overall, norovirus GII was commonly detected by (RT-)qPCR but generally showed lower infectivity-informed positivity than genome positivity across several datasets, reinforcing that molecular assays often detect a broader pool of non-infectious or damaged particles and/or free genomes. Methods such as EMA-(RT-)qPCR, SD-CDDP-(RT-)qPCR and PGM-MBs have been explored to enrich for potentially intact or host-binding particles, but performance remains virus- and strain-dependent (Prevost et al., 2016; Leifels et al., 2021).

Adenovirus and enteroviruses are frequently highlighted as potential infectivity threats because they are relatively persistent and have been detected in source waters and occasionally in treated waters (Betancourt et al., 2014; Borchardt et al., 2012; Stokdyk et al., 2020). Although overall viral loads in treated potable water are typically low, some studies report that residual adenovirus and enterovirus signals can retain infectivity, with highly variable infectivity ratios depending on matrix, treatment and assay conditions (Table 1). Several studies note that inactivation is not universal and that performance can vary with water quality and treatment conditions (Canh et al., 2021; Dong et al., 2010; Fongaro et al., 2013; Prevost et al., 2016; Simmons and Xagoraraki, 2011; Sylvestre et al., 2021). Consistent with this broader evidence, only a small number of studies in the screened dataset reported infectious enteric viruses in finished drinking water, and these detections were at very low levels. For example, Lee and Kim (2002) detected infectious enteroviruses and adenoviruses in tap water in urban areas in Korea (2×10^{-3} to 2.9×10^{-2} MPN infectious units per litre), and Ali et al. (2004) detected enteroviruses by plaque assay in drinking water treatment facilities and parts of the distribution system in Egypt, highlighting that operational performance and distribution integrity can influence virological quality.

Capsid-integrity assays assume that genomes protected within intact capsids are more likely to represent potentially infectious particles, yet decay kinetics for capsids and nucleic acids may differ and integrity dyes/enzymes are not universally effective across all viruses and strains (Yezli and Otter, 2011; Leifels et al., 2021; Oristo et al., 2018). Culture-based infectivity assays also have limitations: no single mammalian cell line is permissive to all enteric viruses, and a given cell line may support replication of multiple virus types, meaning additional steps (e.g., qPCR or sequencing of culture lysates) are needed to identify which virus caused a cytopathic effect. Despite these limitations, infectivity remains the gold standard for risk assessment because dose-response is driven by infectious particles; some enteric viruses (e.g., rotavirus) have extremely low infectious doses compared with many bacteria or protozoa (Ward et al., 1986; Table 1). Quantitative microbial risk assessment (QMRA) and water safety plans (WSPs) therefore benefit from integrating occurrence data with dose-response models and from using operational indicators (e.g., coliphages) to verify treatment barriers. Because routine cell culture and higher-containment work are resource-intensive, operators often rely on simpler indicators, but in higher-consequence contexts such as potable reuse, more detailed infectivity-informed verification may be warranted (Gerba et al., 2017; Gerrity et al., 2023).

Virus persistence and transport are influenced by environmental conditions (temperature, pH, turbidity, organic matter and disinfectant residuals), which can create gaps between perceived and actual contamination risk if monitoring relies on a single method. High virus shedding by infected individuals can lead to substantial loads in sewage and impacted waters (Anderson and Weber, 2004; Boehm et al., 2019; Sarmento et al., 2021). Because (RT-)qPCR can detect genomes from non-infectious particles, combining molecular detection with infectivity-informed approaches (e.g., cell culture, integrated cell culture-qPCR, or validated capsid-integrity assays) improves risk interpretation. For context on potential magnitudes, Gerba et al. (2017) summarised historical reports of maximum virus concentrations in untreated wastewater measured by cell culture, including values up to approximately 1.1×10^{6} PFU/L using BGM cell lines. These observations reinforce the importance of robust multi-barrier treatment, transparent reporting of analytical methods and continued development of practical tools that distinguish viable from non-viable viruses for routine monitoring.

4. Future perspectives on viruses in drinking water

Mounting evidence of human enteric virus occurrence in drinking-water sources necessitates robust strategies for monitoring and mitigating these pathogens (Singh et al., 2022). Virus detection and analysis in drinking water are increasingly important amid heightened risks from climate change and wastewater impacts on river water quality (Levy et al., 2018; Semenza and Paz, 2021; Exton et al., 2023). This review highlights the need for refined, standardised methods to quantify low-level viral signals in source and treated waters. A multi-indicator approach is advisable: coliphages remain central for verifying treatment effectiveness using an infective particle signal, while molecular markers such as crAssphage and plant viruses (PMMoV/TMV) can provide sensitive supplementary information for tracking faecal contamination and persistence. However, PMMoV/TMV are not strictly human-specific and can be influenced by dietary and agricultural inputs; therefore, interpretation should consider catchment context and ideally include human-associated markers. Standardised protocols are needed to concentrate viruses from large sample volumes, particularly for on-site application, and to manage inhibition and recovery losses (Cashdollar and Wymer, 2013; Haramoto et al., 2018).

Coliphages and other viral indicators can aid source apportionment and catchment-level viral load estimation. Yet, specific contamination events introduce complex mixtures of viruses and indicators that are shaped by site-specific hydrology and physicochemical conditions. For instance, FRNAPH GII was more common during riverbed sediment resuspension, whereas surface runoff elevated GI levels (Fauvel et al., 2016). Combined sewer overflows, continuous discharges, rainfall and snowmelt can shift the water matrix and viral loads, emphasising the importance of recognising contamination drivers to anticipate load fluctuations (Grøndahl-Rosado et al., 2014). Because rainfall patterns and turbidity differ by region and season, pairing hydroclimatic data with pathogen/indicator monitoring strengthens predictions of viral fate and treatment challenge periods (Xagoraraki et al., 2014). A persistent limitation across the literature is infrequent sampling at individual sites, which hinders understanding of temporal and spatial variability. Expanded, repeated monitoring (including event-based sampling) can improve the evidence base for risk assessment and long-term water quality management.

A key challenge lies in the wide variability of virus detection across strains, matrices and analytical methods. Comprehensive risk assessment benefits from catchment-scale data on flow dynamics and turbidity, alongside virus occurrence and supporting faecal indicator data such as *E. coli* (Ahmed et al., 2020). Multiple indicators and surrogates can improve risk quantification and treatment verification (Singh et al., 2022). Future research should refine dose-response models for QMRA by considering pathogen-specific infectivity in different water matrices. Meanwhile, the EU Drinking Water Directive includes somatic coliphages as a risk-based operational parameter (EU Drinking Water Directive, 2020), reinforcing their role in assessing viral removal and operational control. Expanding monitoring frameworks to better capture pathogen diversity and persistence—while remaining pragmatic for routine laboratories—may improve treatment optimisation and public

health protection.

Advanced PCR methods, particularly those that attempt to differentiate intact from degraded viruses, hold promise. Capsid-integrity qPCR combined with PMA or EMA can help indicate whether detected genomes are associated with intact particles; however, performance is virus- and strain-dependent and does not replace infectivity assays (Leifels et al., 2021). Digital droplet PCR (ddPCR) can improve quantification precision in inhibitor-rich matrices, but requires specialised instrumentation and expertise. For routine water assessments in smaller laboratories, a tiered approach may be most practical: use standardised concentration and (RT-)qPCR and/or culture-based coliphage assays for routine surveillance, with periodic confirmatory or investigative testing using ddPCR, capsid-integrity methods or cell culture in specialist laboratories. Ultimately, developing and validating tools that more reliably distinguish viable from non-viable virus particles—and linking molecular signals to infectivity under realistic environmental conditions—remain priorities for improving QMRA and strengthening water safety planning.

5. Conclusions

This review evaluated viral prevalence in both source and treated drinking water, drawing on a sizable dataset from numerous studies. Coliphages emerged as powerful indicators of potential viral contamination routes, especially when pathogenic viruses were not directly detected. Nonetheless, enteric virus detection varied by strain and water matrix, revealing critical gaps in our grasp of viral behaviour under diverse environmental and treatment conditions. Such variation reinforces the need for further investigation into coliphage-virus comparisons to enhance contamination tracking and optimize water treatment.

Differences in viral detection across regions and matrices highlight the necessity for broader and more region-specific research initiatives. Expanded monitoring should encompass source waters (surface and subsurface) to pinpoint viral hotspots accurately and guide risk evaluations. This is underscored by variability in coliphage versus virus data, indicating one-size-fits-all solutions are inadequate. As climate change and other environmental pressures increasingly impact drinking water sources, understanding viral transport, persistence, and removal becomes imperative. Findings herein suggest that water treatment protocols must adapt to effectively inactivate or remove the range of viruses that pose public health threats.

Evolving regulations (e.g., EU Drinking Water Directive) underscore the importance of standardised methods for virus detection and concentration across multiple water types. Future work should emphasize validating these approaches through collaborative studies to ensure consistency and applicability. Though qPCR remains valuable for detecting viral genomes in treated water, including potable supplies, genome presence alone does not signify infectivity. This review reiterates that viral infectivity demands a multi-faceted approach, factoring in decay rates and environmental conditions. In summary, key findings include:

- Coliphages effectively indicate viral contamination routes even when pathogenic viruses are not detected.
- Enteric virus detection differs significantly by strain and matrix, revealing knowledge gaps.
- Region-specific monitoring is crucial due to the variability in virus occurrence across regions and matrices.
- Water treatment processes need refinement to address virus persistence, especially under environmental changes like climate change.
- Standardised detection protocols, as advocated by the EU Drinking Water Directive, must be validated through coordinated research.

CRediT authorship contribution statement

Suniti Singh: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **John Haley:** Writing – original draft, Project administration, Funding acquisition, Conceptualization. **John Fawell:** Writing – review & editing, Validation, Conceptualization. **Francis Hassard:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2026.181784.

Data availability

Data associated with this manuscript is available at: 10.17862/cranfield.rd.20227497

References

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764.

Ahmed, W., Hamilton, K., Toze, S., Cook, S., Page, D., 2019b. A review on microbial contaminants in stormwater runoff and outfalls: potential health risks and mitigation strategies. Sci. Total Environ. 692, 1304–1321.

Ahmed, W., Payyappat, S., Cassidy, M., Besley, C., 2019a. Enhanced insights from human and animal host-associated molecular marker genes in a freshwater lake receiving wet weather overflows. Sci. Rep. 9 (1), 12503.

Ali, M.A., Al-Herrawy, A.Z., El-Hawaary, S.E., 2004. Detection of enteric viruses, Giardia and Cryptosporidium in two different types of drinking water treatment facilities. Water Res. 38 (18), 3931–3939.

Anderson, E.J., Weber, S.G., 2004. Rotavirus infection in adults. Lancet Infect. Dis. 4 (2), 91–99.

Bailey, E.S., Casanova, L.M., Simmons, O.D., Sobsey, M.D., 2018. Tertiary treatment and dual disinfection to improve microbial quality of reclaimed water for potable and non-potable reuse: a case study of facilities in North Carolina. Sci. Total Environ. 630, 379–388.

Bartsch, S.M., O'Shea, K.J., Lee, B.Y., 2020. The clinical and economic burden of norovirus gastroenteritis in the United States. J. Infect. Dis. 222 (11), 1910–1919.

Betancourt, W.Q., Kitajima, M., Wing, A.D., Regnery, J., Drewes, J.E., Pepper, I.L., Gerba, C.P., 2014. Assessment of virus removal by managed aquifer recharge at three full-scale operations. J. Environ. Sci. Health A 49 (14), 1685–1692.

Bibby, K., Crank, K., Greaves, J., Li, X., Wu, Z., Hamza, I.A., Stachler, E., 2019. Metagenomics and the development of viral water quality tools. NPJ Clean Water 2 (1), 9.

Boehm, A.B., Silverman, A.I., Schriewer, A., Goodwin, K., 2019. Systematic review and meta-analysis of decay rates of waterborne mammalian viruses and coliphages in surface waters. Water Res. 164, 114898.

Borchardt, M.A., Spencer, S.K., Kieke Jr., B.A., Lambertini, E., Loge, F.J., 2012. Viruses in nondisinfected drinking water from municipal wells and community incidence of acute gastrointestinal illness. Environ. Health Perspect. 120 (9), 1272–1279.

Canada, Health, 2019. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document- Enteric Viruses. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Canh, V.D., Torii, S., Furumai, H., Katayama, H., 2021. Application of capsid integrity (RT-) qPCR to assessing occurrence of intact viruses in surface water and tap water in Japan. Water Res. 189, 116674.

Cashdollar, J.L., Wymer, L., 2013. Methods for primary concentration of viruses from water samples: a review and meta-analysis of recent studies. J. Appl. Microbiol. 115 (1), 1–11.

CDPH, 2014. Regulations related to recycled water. Titles 22 and 17 California code of regulations.
Coudray, C., Merle, G., Martin-Latil, S., Guillier, L., Perelle, S., 2013. Comparison of two extraction methods for the detection of hepatitis a virus in lettuces using the murine norovirus as a process control. J. Virol. Methods 193 (1), 96–102.
Ditommaso, S., Ricciardi, E., Giacomuzzi, M., Rivera, S.R.A., Ceccarelli, A., Zotti, C.M., 2014. Overestimation of the Legionella spp. load in environmental samples by quantitative real-time PCR: pretreatment with propidium monoazide as a tool for the assessment of an association between Legionella concentration and sanitary risk. Diagn. Microbiol. Infect. Dis. 80 (4), 260–266.
Dong, Y., Kim, J., Lewis, G.D., 2010. Evaluation of methodology for detection of human adenoviruses in wastewater, drinking water, stream water and recreational waters. J. Appl. Microbiol. 108 (3), 800–809.
EU Drinking Water Directive, 2020. Directive (EU) 2020/2184 of the European Parliament and of the Council on the quality of water intended for human consumption. Official Journal of the European Union, L 435, 23.12.2020, pp. 1-62.
Exton, B., Hassard, F., Medina Vaya, A., Grabowski, R.C., 2023. Polybacterial shift in benthic river biofilms attributed to organic pollution-a prospect of a new biosentinel? Hydrol. Res. 54 (3), 348–359.
Farkas, K., Cooper, D.M., McDonald, J.E., Malham, S.K., de Rougemont, A., Jones, D.L., 2018. Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine and estuarine receiving waters. Sci. Total Environ. 634, 1174–1183.
Fauvel, B., Cauchie, H.M., Gantzer, C., Ogorzaly, L., 2016. Contribution of hydrological data to the understanding of the spatio-temporal dynamics of F-specific RNA bacteriophages in river water during rainfall-runoff events. Water Res. 94, 328–340.
Fong, T.T., Lipp, E.K., 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiol. Mol. Biol. Rev. 69 (2), 357–371.
Fongaro, G., Nascimento, M.A.D., Rigotto, C., Ritterbusch, G., da Silva, A.D.A., Esteves, P.A., Barardi, C.R., 2013. Evaluation and molecular characterization of human adenovirus in drinking water supplies: viral integrity and viability assays. Virol. J. 10, 1–9.
Gerba, C.P., Betancourt, W.Q., Kitajima, M., 2017. How much reduction of virus is needed for recycled water: a continuous changing need for assessment? Water Res. 108, 25–31.
Gerrity, D., Crank, K., Steinle-Darling, E., Pecson, B.M., 2023. Establishing pathogen log reduction value targets for direct potable reuse in the United States. AWWA Water Science 5 (5), e1353.
Goodwin, D., Raffin, M., Jeffrey, P., Smith, H.M., 2015. Applying the water safety plan to water reuse: towards a conceptual risk management framework. Environ. Sci.: Water Res. Technol. 1 (5), 709–722.
Grøndahl-Rosado, R.C., Yarovitsyna, E., Trettenes, E., Myrmel, M., Robertson, L.J., 2014. A one year study on the concentrations of norovirus and enteric adenoviruses in wastewater and a surface drinking water source in Norway. Food and Environmental Virology 6, 232–245.
Gu, X., Tay, Q.X.M., Te, S.H., Saeidi, N., Goh, S.G., Kushmaro, A., Thompson, J.R., Gin, K. Y.H., 2018. Geospatial distribution of viromes in tropical freshwater ecosystems. Water Res. 137, 220–232.
Gunnarsdottir, M.J., Gardarsson, S.M., Figueras, M.J., Puigdomènech, C., Juárez, R., Saucedo, G., Arnedo, M.J., Santos, R., Monteiro, S., Avery, L., Pagaling, E., 2020. Water safety plan enhancements with improved drinking water quality detection techniques. Sci. Total Environ. 698, 134185.
Guo, Y., Sivakumar, M., Jiang, G., 2022. Decay of four enteric pathogens and implications to wastewater-based epidemiology: effects of temperature and wastewater dilutions. Sci. Total Environ. 819, 152000.
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.
Haramoto, E., Kitajima, M., Hata, A., Torrey, J.R., Masago, Y., Sano, D., Katayama, H., 2018. A review on recent progress in the detection methods and prevalence of human enteric viruses in water. Water Res. 135, 168–186.
Hassard, F., Sharp, J.H., Taft, H., LeVay, L., Harris, J.P., McDonald, J.E., Tuson, K., Wilson, J., Jones, D.L., Malham, S.K., 2017. Critical review on the public health impact of norovirus contamination in shellfish and the environment: a UK perspective. Food and Environmental Virology 9, 123–141.
Hata, A., Shirasaka, Y., Ihara, M., Yamashita, N., Tanaka, H., 2021. Spatial and temporal distributions of enteric viruses and indicators in a lake receiving municipal wastewater treatment plant discharge. Sci. Total Environ. 780, 146607.
Havelaar, A.H., Melse, J.M., 2003. *Quantifying public health risk in the WHO guidelines for drinking-water quality: a burden of disease approach*. RIVM rapport 734301022. Bilthoven: Rijksinstituut voor Volksgezondheid en Milieu (RIVM). Available at: http://hdl.handle.net/10029/9098.
Heffron, J., Mayer, B.K., 2021. Virus isoelectric point estimation: theories and methods. Appl. Environ. Microbiol. 87 (3), e02319–e02320.
Hill, V.R., Kahler, A.M., Jothikumar, N., Johnson, T.B., Hahn, D., Cromeans, T.L., 2007. Multistate evaluation of an ultrafiltration-based procedure for simultaneous recovery of enteric microbes in 100-liter tap water samples. Appl. Environ. Microbiol. 73 (13), 4218–4225.
Hill, V.R., Polaczyk, A.L., Hahn, D., Narayanan, J., Cromeans, T.L., Roberts, J.M., Amburgey, J.E., 2005. Development of a rapid method for simultaneous recovery of diverse microbes in drinking water by ultrafiltration with sodium polyphosphate and surfactants. Appl. Environ. Microbiol. 71 (11), 6878–6884.
IESWTR, 1998. National Primary Drinking Water Regulations: interim enhanced surface water treatment. Fed. Regist. 63 (241), 69478.
Ikner, L.A., Gerba, C.P., Bright, K.R., 2012. Concentration and recovery of viruses from water: a comprehensive review. Food and Environmental Virology 4, 41–67.
International Committee on Taxonomy of Viruses (ICTV), 2024. Virus Taxonomy: ICTV Online (taxonomy browser / Master Species List). Available at: https://ictv.global/taxonomy (accessed: December 2024).
ISO - International Standardization Organization. 1995. Water quality. Detection and enumeration of bacteriophages. Pt. 1: enumeration of F-specific RNA bacteriophages. ISO 10705-1:1995. International Standardization Organization, Geneva Switzerland.
ISO - International Standardization Organization. 2000. Water quality. Detection and enumeration of bacteriophages. Pt. 2: Enumeration of somatic coliphages. ISO 10705-2:2000. International Standardization Organization, Geneva Switzerland.
Jamal, R., Mubarak, S., Sahulka, S.Q., Kori, J.A., Tajammul, A., Ahmed, J., Mahar, R.B., Olsen, M.S., Goel, R., Weidhaas, J., 2020. Informing water distribution line rehabilitation through quantitative microbial risk assessment. Sci. Total Environ. 739, 140021. https://doi.org/10.1016/j.scitotenv.2020.140021.
Jones, K.R., Eftim, S., Lindahl, A.J., Black, S., Nappier, S.P., 2022. Occurrence of coliphage in effluent: a systematic literature review and meta-analysis. Hygiene and Environmental Health Advances 3, 100014.
Kauppinen, A., Pitkänen, T., Miettinen, I.T., 2018. Persistent norovirus contamination of groundwater supplies in two waterborne outbreaks. Food and Environmental Virology 10, 39–50.
Korajkic, A., McMinn, B.R., Harwood, V.J., 2018. Relationships between microbial indicators and pathogens in recreational water settings. Int. J. Environ. Res. Public Health 15 (12), 2842.
Laine, J., Huovinen, E., Virtanen, M.J., Snellman, M., Lumio, J., Ruutu, P., Kujansuu, E., Vuento, R., Pitkänen, T., Miettinen, I., Herrala, J., 2011. An extensive gastroenteritis outbreak after drinking-water contamination by sewage effluent. Finland. *Epidemiology & Infection* 139 (7), 1105–1113.
Lee, S.H., Kim, S.J., 2002. Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. Water Res. 36 (1), 248–256.
Leifels, M., Cheng, D., Sozzi, E., Shoults, D.C., Wuertz, S., Mongkolsuk, S., Sirikanchana, K., 2021. Capsid integrity quantitative PCR to determine virus infectivity in environmental and food applications-a systematic review. Water Research X 11, 100080.
Levy, K., Smith, S.M., Carlton, E.J., 2018. Climate change impacts on waterborne diseases: moving toward designing interventions. Current environmental health reports 5, 272–282.
Mackowiak, M., Leifels, M., Hamza, I.A., Jurzik, L., Wingender, J., 2018. Distribution of escherichia coli, coliphages and enteric viruses in water, epilithic biofilms and sediments of an urban river in Germany. Sci. Total Environ. 626, 650–659.
Medema, G., 2012. Microbial risk assessment of pathogens in water. In Environmental Toxicology: Selected Entries from the Encyclopedia of Sustainability Science and Technology (pp. 361-401). New York, NY: Springer New York.
Merrett, H., Porter, J., Jewell, K., Long, R., Lowther, J.A., 2013. Viruses in Raw and Partially Treated Water: Targeted Monitoring Using the Latest Methods. Department for Environment, Food and Rural Affairs: Drinking Water Inspectorate, Project WT 1227, Report No. 5001-UA001104-NER-01. Hyder Consulting (UK) Ltd, Cardiff, UK.
Michen, B., Graule, T., 2010. Isoelectric points of viruses. J. Appl. Microbiol. 109 (2), 388–397.
Morsy El-Senousy, W., Guix, S., Abid, I., Pintó, R.M., Bosch, A., 2007. Removal of astrovirus from water and sewage treatment plants, evaluated by a competitive reverse transcription-PCR. Appl. Environ. Microbiol. 73 (1), 164–167.
Muniesa, M., Ballesté, E., Imamovic, L., Pascual-Benito, M., Toribio-Avedillo, D., Lucena, F., Blanch, A.R., Jofre, J., 2018. Bluephage: a rapid method for the detection of somatic coliphages used as indicators of fecal pollution in water. Water Res. 128, 10–19.
Nappier, S.P., Hong, T., Ichida, A., Goldstone, A., Eftim, S.E., 2019. Occurrence of coliphage in raw wastewater and in ambient water: a meta-analysis. Water Res. 153, 263–273.
NC OAH, 2011. North Carolina environmental quality. North Carolina Adm. Code 15A NCAC 2U reclaimed water.
NHMRC, 2011. Australian Drinking Water Guidelines v3.8 (2011) - Updated September 2022. (National Health and Medical Research Council and National Resource Management Ministerial Council).
Olivieri, A.W., Pecson, B., Crook, J., Hultquist, R., 2020. California water reuse-past, present and future perspectives. In: Advances in Chemical Pollution, Environmental Management and Protection, vol. 5. Elsevier, pp. 65–111.
Omatola, C.A., Olaniran, A.O., 2022. Epidemiological significance of the occurrence and persistence of rotaviruses in water and sewage: a critical review and proposal for routine microbiological monitoring. Environ Sci Process Impacts 24 (3), 380–399.
Oristo, S., Lee, H.J., Maunula, L., 2018. Performance of pre-RT-qPCR treatments to discriminate infectious human rotaviruses and noroviruses from heat-inactivated viruses: applications of PMA/PMAxx, benzonase and RNase. J. Appl. Microbiol. 124, 1008–1016.
Our World in Data, 2021. Clean Water and Sanitation. Available at: https://ourworldindata.org/clean-water-sanitation (Accessed: 02 August 2024).
Pang, X.L., Lee, B.E., Pabbaraju, K., Gabos, S., Craik, S., Payment, P., Neumann, N., 2012. Pre-analytical and analytical procedures for the detection of enteric viruses and enterovirus in water samples. J. Virol. Methods 184 (1–2), 77–83.
Pang, X., Qiu, Y., Gao, T., Zurawell, R., Neumann, N.F., Craik, S., Lee, B.E., 2019. Prevalence, levels and seasonal variations of human enteric viruses in six major rivers in Alberta, Canada. Water Res. 153, 349–356.
Prado, T., de Castro Bruni, A., Barbosa, M.R.F., Garcia, S.C., Moreno, L.Z., Sato, M.I.Z., 2019. Noroviruses in raw sewage, secondary effluents and reclaimed water produced by sand-anthracite filters and membrane bioreactor/reverse osmosis system. Sci. Total Environ. 646, 427–437.

Prevost, 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 Res. 91, 68–76.
Qiu, Y., Li, Q., Lee, B.E., Ruecker, N.J., Neumann, N.F., Ashbolt, N.J., Pang, X., 2018. UV inactivation of human infectious viruses at two full-scale wastewater treatment plants in Canada. Water Res. 147, 73–81.
Rhodes, E.R., Huff, E.M., Hamilton, D.W., Jones, J.L., 2016. The evaluation of hollow-fiber ultrafiltration and celite concentration of enteroviruses, adenoviruses and bacteriophage from different water matrices. J. Virol. Methods 228, 31–38.
Richiardi, L., Pignata, C., Fea, E., Bonetta, S., Carraro, E., 2023. Are indicator microorganisms predictive of pathogens in water? Water 15 (16), 2964.
Riera-Montes, M., Sjölander, K.B., Allestam, G., Hallin, E., Hedlund, K.O., Löfdahl, M., 2011. Waterborne norovirus outbreak in a municipal drinking-water supply in Sweden. Epidemiol. Infect. 139 (12), 1928–1935.
Rusiñol, M., Fernandez-Cassi, X., Timoneda, N., Carratalà, A., Abril, J.F., Silvera, C., Figueras, M.J., Gelati, E., Rodó, X., Kay, D., Wyn-Jones, P., 2015. Evidence of viral dissemination and seasonality in a Mediterranean river catchment: implications for water pollution management. J. Environ. Manag. 159, 58–67.
Salvador, D., Neto, C., Benoliel, M.J., Caeiro, M.F., 2020. Assessment of the presence of hepatitis E virus in surface water and drinking water in Portugal. Microorganisms 8 (5), 761.
Sandmann, F.G., Shallcross, L., Adams, N., Allen, D.J., Coen, P.G., Jeanes, A., Kozlakidis, Z., Larkin, L., Wurie, F., Robotham, J.V., Jit, M., 2018. Estimating the hospital burden of norovirus-associated gastroenteritis in England and its opportunity costs for nonadmitted patients. Clin. Infect. Dis. 67 (5), 693–700.
Sarmento, S.K., de Andrade, J.D.S.R., Miagostovich, M.P., Fumian, T.M., 2021. Virological and epidemiological features of norovirus infections in Brazil, 2017-2018. Viruses 13 (9), 1724.
Schijven, J., Teunis, P., Suylen, T., Ketelaars, H., Hornstra, L., Rutjes, S., 2019. QMRA of adenovirus in drinking water at a drinking water treatment plant using UV and chlorine dioxide disinfection. Water Res. 158, 34–45.
Sedji, M.I., Varbanov, M., Meo, M., Colin, M., Mathieu, L., Bertrand, I., 2018. Quantification of human adenovirus and norovirus in river water in the north-east of France. Environ. Sci. Pollut. Res. 25 (30), 30497–30507.
Semenza, J.C., Paz, S., 2021. Climate change and infectious disease in Europe: impact, projection and adaptation. The Lancet Regional Health-Europe 9.
Shaffer, M., Huynh, K., Costantini, V., Bibby, K., Vinjé, J., 2022. Viable norovirus persistence in water microcosms. Environ. Sci. Technol. Lett. 9 (10), 851–855.
Simmons, F.J., Xagoraraki, I., 2011. Release of infectious human enteric viruses by full-scale wastewater utilities. Water Res. 45, 3590–3598. https://doi.org/10.1016/j.watres.2011.04.001.
Singh, S., Pitchers, R., Hassard, F., 2022. Coliphages as viral indicators of sanitary significance for drinking water. Front. Microbiol. 13, 941532.
Soliman, A., 2024. Unacceptable: a staggering 4.4 billion people lack safe drinking water, study finds. Nature. https://doi.org/10.1038/d41586-024-02621-0. Available at:
Stokdyk, J.P., Firnstahl, A.D., Walsh, J.F., Spencer, S.K., de Lambert, J.R., Anderson, A.C., Rezania, L.I.W., Kieke Jr., B.A., Borchardt, M.A., 2020. Viral, bacterial, and protozoan pathogens and fecal markers in wells supplying groundwater to public water systems in Minnesota, USA. Water Res. 178, 115814.
Suttle, C.A., 2007. Marine viruses—major players in the global ecosystem. Nat. Rev. Microbiol. 5 (10), 801–812.
SWTR, 1989. Surface Water Treatment Rule.
Sylvestre, É., Prévost, M., Burnet, J.B., Pang, X., Qiu, Y., Smeets, P., Medema, G., Hachad, M., Dorner, S., 2021. Demonstrating the reduction of enteric viruses by drinking water treatment during snowmelt episodes in urban areas. Water Research X 11, 100091.
Tandukar, S., Sherchand, J.B., Bhandari, D., Sherchan, S.P., Malla, B., Ghaju Shrestha, R., Haramoto, E., 2018. Presence of human enteric viruses, protozoa, and indicators of pathogens in the Bagmati River. Nepal. Pathogens 7 (2), 38.
UNEP (2021) 'Globally, 3 billion people at health risk due to scarce data on water quality', United Nations Environment Programme. Available at: https://www.unep.org/news-and-stories/story/globally-3-billion-people-health-risk-due-scarce-data-water-quality (Accessed: 19 August 2024).
UN-Water, 2021. Billions of People Will Lack Access to Safe Water, Sanitation and Hygiene in 2030 unless Progress Quadruples. UN-Water, 1 July 2021. Available at: https://www.unwater.org/news/billions-people-will-lack-access-safe-water-sanitation-and-hygiene-2030-unless-progress. (Accessed 19 August 2024).
USEPA - United States Environmental Protection Agency, 2001a. Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure. USEPA Office of Water, Washington, DC. EPA 821-R-01-030.
USEPA - United States Environmental Protection Agency, 2001b. Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure. USEPA Office of Water, Washington, DC. EPA 821-R-01-029.
USEPA - United States Environmental Protection Agency, 2006. National Primary Drinking Water Regulations: Ground Water Rule, 40 CFR Parts 9, 141, and 142.
USEPA - United States Environmental Protection Agency, 2018a. Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure. USEPA Office of Water, Washington, DC. EPA 820-R-18-001.
USEPA - United States Environmental Protection Agency, 2018b. Method 1643: Male-specific (F+) and Somatic Coliphage in Secondary (No Disinfection) Wastewater by the Single Agar Layer (SAL) Procedure. USEPA Office of Water, Washington, DC. EPA 820-R-18-003.
USEPA - United States Environmental Protection Agency, 2021. Drinking Water Contaminant Candidate List 5-Draft.
Wang, H., Neyvaldt, J., Enache, L., Sikora, P., Mattsson, A., Johansson, A., Lindh, M., Bergstedt, O., Norder, H., 2020. Variations among viruses in influent water and effluent water at a wastewater plant over one year as assessed by quantitative PCR and metagenomics. Appl. Environ. Microbiol. 86 (24) pp.e02073-20.
Ward, R.L., Bernstein, D.I., Young, E.C., Sherwood, J.R., Knowlton, D.R., Schiff, G.M., 1986. Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. J. Infect. Dis. 154 (5), 871–880.
WHO, 2017a. Fourth Edition Incorporating the First Addendum, Guidelines for Drinking-Water Quality. World Health Organization, Geneva.
WHO, 2017b. Potable Reuse Guidance for Producing Safe Drinking Water, 4th edition. World Health Organization, Geneva.
Xagoraraki, I., Yin, Z., Svambayev, Z., 2014. Fate of viruses in water systems. J. Environ. Eng. 140 (7), 04014020.
Yezli, S., Otter, J.A., 2011. Minimum infective dose of the major human respiratory and enteric viruses transmitted through food and the environment. Food and Environmental Virology 3 (1), 1–30.



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Selected human enteric viruses and viral indicators in drinking-water sources and treated waters: a review on prevalence and monitoring methods

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