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Environmental and Adaptive Changes Necessitate a Paradigm Shift for Indicators of Fecal Contamination

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ABSTRACT Changes in the occurrence, distribution, and seasonal variation of waterborne pathogens due to global climate change may increase the risk of human exposure to these microorganisms, thus heightening the need for more reliable surveillance systems. Routine monitoring of drinking water supplies and recreational waters is performed using fecal indicator microorganisms, such as *Escherichia coli*, *Enterococcus* spp., and coliphages. However, the presence and numbers of these indicators, especially *E. coli* and *Enterococcus* spp., do not correlate well with those of other pathogens, especially enteric viruses, which are a major cause of waterborne outbreaks associated with contaminated water and food, and recreational use of lakes, ponds, rivers, and estuarine waters. For that reason, there is a growing need for a surveillance system that can detect and quantify viral pathogens directly in water sources to reduce transmission of pathogens associated with fecal transmission. In this review, we present an updated overview of relevant waterborne enteric viruses that we believe should be more commonly screened to better evaluate water quality and to determine the safety of water use and reuse and of epidemiological data on viral outbreaks. We also discuss current methodologies that are available to detect and quantify these viruses in water resources. Finally, we highlight challenges associated with virus monitoring. The information presented in this review is intended to aid in the assessment of human health risks due to contact with water sources, especially since current environmental and adaptive changes may be creating the need for a paradigm shift for indicators of fecal contamination.

INTRODUCTION: WHY THIS REVIEW?

Current environmental changes, and subsequent adaptive alterations in microorganisms, are expected to adversely impact paradigms used in water quality safety criteria. Extreme weather events are foreseen to increase in intensity and frequency (1) due, in large part, to global warming, which may cause destruction of infrastructure needed to separate sewage and drainage from clean drinking and recreational waters and damage due to elevated water levels and high tides. For example, in 2010 during an unusual tropical storm in Madeira, Portugal, 108 mm of rainfall was recorded between

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6 and 11 a.m. at the Funchal weather station, and 165 mm at Pico do Arieiro (1,818 m altitude) (2). The south of the island was severely affected by flash floods and mudslides. The degree of devastation was substantial and costly, in terms of both lives lost and economics. As a result of the storm, numbers of the fecal indicator bacteria (FIB) *Enterococcus* spp. and *Escherichia coli*, and sporulating fungi, increased dramatically in both sand and water until September of the same year. Bathing water and sand quality monitoring months later revealed that FIB abundances in both water and sand were profoundly altered for 3 months following the event (3).

Besides extreme weather events, natural aging of drinking water distribution pipes may also cause significant water quality issues. In 2016 and in 2018, Finland reported two outbreaks of gastrointestinal illness affecting about 450 people each year, which in 2016 was caused by sapovirus, human adenovirus (HAdV), and *Dientamoeba fragilis*, and in 2018, by human astrovirus, norovirus (NoV), and enterotoxigenic *E. coli* (4). The outbreaks were determined to be a result of cross-contamination between untreated wastewater and drinking water due to aging of distribution pipes (4). Reports of contamination by wastewater like the ones described above are occurring more frequently due to extreme weather, aging of infrastructure, and operational errors (4–6). Therefore, it is important to be proactive and to adopt regulations to meet the needs of a changing world.

In 2015, a review of the United States of America's Recreational Water Quality Criteria (RWQC) pointed out alternative water safety parameters for recreational waters (7, 8). The rationale behind this review came from a discussion by experts on the use of traditional and alternative FIB. One of the main points of the discussion was that more appropriate fecal indicators are needed, such as *Bacteroidales*, since they are more exclusively related to fecal pollution than traditional FIB. This is particularly true for *Enterococcus* spp. and *E. coli* that may proliferate in the environment, especially in sands and sediments and on aquatic vegetation (9–11). Despite these limitations, FIB monitoring has been useful for assessing contamination due to significant fecal loading events such as large sewage spills. Use of alternative FIB such as *Bacteroides* or phages would likely provide improved health protection against contaminated waters. Fujioka et al. (7) described the use of human enteric viruses as an alternative to the currently used FIB, as they are the cause of most gastrointestinal illness associated with contaminated water (7, 12). One limitation of enteric virus monitoring is that it requires a large volume of sample, on the scale of 10 to 100 liters, for their detection because viruses tend to be at low concentrations in recreational waters. Human polyomavirus (HPyV) was suggested as a proxy for enteric viruses (13). This virus is excreted in human urine in greater numbers, making it more easily detectable in untreated sewage (i.e., mixture of feces, urine, and water) than other enteric viruses, such as NoV or enterovirus (EV), which are excreted by infected individuals only (14). Other sewage-associated viruses currently in use for surveillance are HAdV (15), pepper mild mottle virus (16), and crAssphage (17). Although viral markers such as HAdV and HPyV are highly host specific (13, 18, 19), their concentration in sewage may vary by 1 to 2 orders of magnitude (13, 20). It is worth mentioning that HPyV, pepper mild mottle virus, and crAssphage are indicators and not pathogens. Virus monitoring is not commonly used for routine water-quality monitoring throughout the world, although certain laboratories in the United States and Australia have been screening for enteric viruses. This approach needs to be expanded to other laboratories.

In the following sections, we focus on the main enteric viruses which are objects of frequent discussions at regulatory and scientific meetings. We recommend their expanded use in water quality assessment and monitoring, along with the methodologies currently available to detect and quantify viruses, and we review the challenges associated with virus monitoring. This review intends to serve as a learning resource about water regulations, since environmental and adaptive changes will require a paradigm shift of the current combination of indicators of fecal contamination and other pathogens and opportunists.

BACTERIAL INDICATORS AS SURROGATES FOR ENTERIC VIRUSES

From a microbiological standpoint, waterborne pathogens, namely bacteria, viruses, protozoa, and parasitic helminths, are one of the main causes of gastrointestinal illnesses (21). The concentrations of each pathogen are not equal and may vary in the host and receiving environments due to dilution, decay, and factors such as environmental stress. In addition, a wide variety of pathogens can be present in the feces of humans and animals (22). Therefore, direct monitoring of all pathogens is not a technologically feasible option and can be costly. Thus, routine microbiological testing of drinking water supplies and recreational waters is performed using FIB or viral surrogates, such as somatic coliphages and F-specific phages (23). The most commonly used FIB are *E. coli* and *Enterococcus* spp. However, these FIB do not correlate well with human enteric virus numbers, especially viruses and protozoa in the environment, and can be found in the absence of pathogens, or vice versa (24). One of the major reasons why virus and FIB levels may not correlate is because FIB can replicate in the environment without a host; however, viruses need to be inside the host (or cells) to replicate. Moreover, viruses are more tolerant to disinfection or UV radiation than FIB, due in part to their size, surface charge, and other properties (25). In addition, due to the low infectious doses of viruses, they pose definite health risks to humans (26).

Chlorine is the most commonly used agent for water disinfection, since it rapidly inactivates most pathogens that cause disease in humans (27). However, enteric viruses have a moderate to high tolerance to chlorine compared to FIB (28, 29). Therefore, FIB are not a reliable indicator of the presence/absence of enteric viruses in the drinking water supply network or in recreational waters. For those reasons, there is a growing need for a surveillance system that can detect and quantify some of the most relevant viral pathogens directly in water sources and thus aid in the protection of human health.

WATERBORNE GASTROINTESTINAL ILLNESS (FECAL ORIGIN)

Population growth and urbanization put tremendous pressure on the quantity and quality of the planet's water resources (30, 31). About 90% of human deaths occur due to diarrheal illnesses that have been attributed to contaminated water and an inadequate or complete lack of sanitation (32, 33). Most enteric viruses present in wastewater treatment plants (WWTPs) originate from human feces (34). Over 200 enteric viruses known to infect humans are considered pathogens because of their high resistance to treatment processes and their low infectious doses (35). To appropriately eliminate these viruses, it is necessary to use tertiary treatment processes involving chlorine, ozone, and exposure to UV light in drinking water treatment plants and WWTPs (36–38). However, due mainly to cost issues, many of the rather complex treatments are not always available in most DWTPs or drinking water treatment plants worldwide. The viruses' ability to survive the water treatment processes and their subsequent discharge into fresh, marine, and estuarine water bodies pose serious health risks to humans (39).

ENTERIC VIRUSES OF PUBLIC HEALTH SIGNIFICANCE

The World Health Organization (WHO) has classified viruses as having a moderate to high significance on human health (40). The viruses HAdV and astrovirus are classified as having moderate health significance, whereas EV, hepatitis A (HAV) and E (HEV) viruses, NoV, rotavirus (RoV), sapovirus (SaV), and parechoviruses (PEVs) are classified as having high significance (40).

Enteric viruses require human hosts to propagate and are transmitted to humans via the fecal-oral route. They are frequently detected in contaminated food and water (41), and their transmission routes are quite diverse, ranging from consumption of contaminated food or water to contact with feces, vomitus, or aerosols to person-to-person spread (Fig. 1).

Enteric viruses are excreted in high numbers, up to 10^{10}/g of feces, by infected individuals, and their infectious doses may vary by many orders of magnitude (Table 1).

Human Adenovirus

HAdVs are members of the *Adenoviridae* family, classified in the genus *Mastadenovirus*, and have a DNA genome. HAdV classification comprises seven species (A to G) and over 80 genotypes, which can infect a wide range of tissues (42–44). HAdV can cause respiratory infections, gastroenteritis, conjunctivitis, and cystitis, as well as infections in other organs, e.g., the central nervous system and sexual organs (45–51). Their infective dose is low, and as little as 5 infectious units can cause disease in susceptible individuals (Table 1).

Transmission of HAdV occurs mainly through the fecal-oral route and aerosol inhalation (52, 53). Since they are persistent in the feces, urine, and respiratory secretions of infected individuals (48), they are highly prevalent in untreated wastewater (54, 55). Current wastewater treatment options have proven to be insufficient regarding HAdV removal, even with inactivation by UV treatment or at typical effluent-free chlorine concentrations (56–59). Therefore, their persistence in wastewater may be one of the key factors of HAdV transmission, which consequently leads to public health risks with a wide range of associated outbreaks (Table 2) (60–62). However, HAdV may not be the most suitable indicator microbe for NoVs and hepatitis viruses (63–65). This is mainly due to their high stability, ubiquitous distribution in many aquatic environments (including surface and groundwater, recreational water, drinking water, and wastewater [raw and treated]), considerable

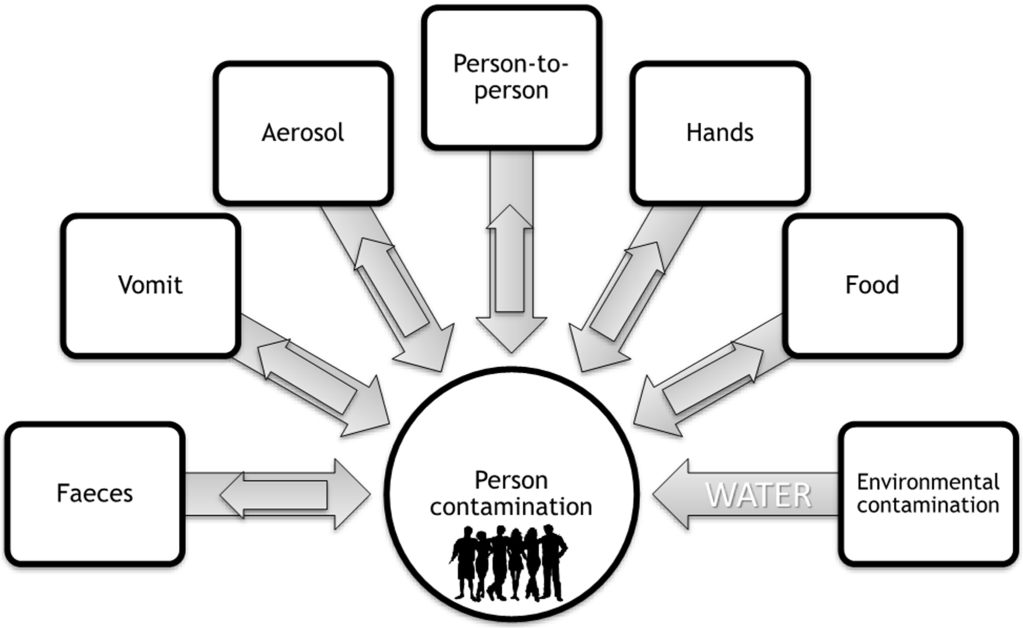

FIGURE 1 Transmission routes of enteric viruses.

TABLE 1 Symptoms, transmission pathways, and infectious doses of the most common waterborne enteric viruses in water sources

Viral pathogen		Transmission pathways	Detection in water	Infectious dose[a]	Related diseases	References
Human adenovirus (HAdV)		Fecal-oral route Aerosol inhalation Aspiration	Drinking water Ground water Surface water Wastewater	Low 5 IU can cause disease in susceptible individuals >150 IU (adenovirus serotype 7)	Acute respiratory diseases Cervicitis Cystitis Conjunctivitis Fever Gastroenteritis Hemorrhagic cystitis Pharyngoconjunctival fever Pneumonia Urethritis	40 203 54 204 45 46 52 205 47
Enterovirus (EV)	Polioviruses	Fecal-oral route Inhalation Aspiration Contact with contaminated oral secretions, vesicular fluid, surfaces, or fomites	Drinking water Ground water Seawater Surface water Wastewater	Low	Paralytic disease Poliomyelitis	40 68
	Coxsackievirus			<18 IU per inhalation ~10^5 to 10^6 IU	Herpangina Meningitis	206 207
	Echoviruses				Paralysis Myocarditis Hand-foot-and-mouth diseases	69 208 209 210
	EV68			Not known	Cranial neuropathy Flaccid paralysis Muscle weakness Flaccid paralysis	211 70 74 212
	EV71			Not known	Aseptic meningitis Cardiopulmonary dysfunction Neurogenic pulmonary edema Tachycardia	

(continued)

TABLE 1 Symptoms, transmission pathways, and infectious doses of the most common waterborne enteric viruses in water sources (*continued*)

Viral pathogen	Transmission pathways	Detection in water	Infectious dose[a]	Related diseases	References
Norovirus (NoV)	Fecal-oral route Direct contact with an infected individual	Drinking water Surface water Wastewater	Low ≥18 IU (50% human infectious dose, 18 to 1,015 to 1,320 to 2,800 genomic equivalents)	Abdominal cramps Chills Diarrhea Vomiting Nausea Headache Fever Muscle aches	36 40 95 213 82 76 214 215 116 216 83 94
Hepatitis A virus (HAV)	Fecal-oral route	Drinking water Ground water Seawater Surface water Wastewater	Low	Abdominal discomfort Anorexia Dark urine Diarrhea Fatigue Fever Hepatic cell damage Jaundice Malaise Nausea Vomiting Urticaria Pancreatitis Vasculitis glomerulonephritis	40 208 217 218 219 220 221 222 159 223 224 160
Hepatitis E virus (HEV)	Fecal-oral route Transfusions with contaminated blood Vertical transmission (maternal-fetal)	Seawater Surface water Wastewater	Not known	Jaundice Vomiting Loss of appetite Fatigue Fever Darkened urine Hepatalgia Hepatomegaly Neurological complications	40 159 168 225 178 226 227 228
Rotavirus[b]	Fecal-oral route Person-to-person transmission Inhalation of airborne human rotaviruses Aerosol inhalation	Drinking water Ground water Seawater Surface water Wastewater	Low (50% infectious dose, ~6)	Acute gastroenteritis Abdominal pain Dehydration Metabolic acidosis Diarrhea Fever Vomiting	40 209 229 230 231 232

[a] IU, infectious units.
[b] Rotavirus was excluded from the next sections because there is already a widely available vaccine for this agent.

thermal stability, and ability to survive for several months in the environment (Table 2). Thus, their survival rarely mimics that of pathogens that are present due to recent contamination (66).

Enterovirus

EVs are small, single-stranded RNA viruses of the *Picornaviridae* family. They have a nonenveloped icosahedral capsid with a diameter of 20 to 30 nm (40, 67). The EVs are divided into the polioviruses (3 serotypes), coxsackievirus A (23 serotypes), coxsackievirus B (6 serotypes), and echoviruses (28 serotypes) (40, 68). The most recently discovered EVs are not included in this classification and are designated by the serotype number, beginning with EV68 (40, 69, 70). The infectious doses of poliovirus and some other EVs are not known but are estimated to be low (Table 1). A previous study showed that infective rates vary with season, geography, and the age and socioeconomic status of the population sampled (71).

TABLE 2 Virus concentrations in wastewater, treated wastewater, surface water, groundwater, and seawater samples

Virus	Sample matrix	Concentration ($\log_{10}$)	Units[a]	References
Human adenovirus (HAdV)	Raw wastewater	4.97	Mean GC/100 ml	54
		5.70	Mean GC/100 ml	233
		4.8–6.0	Range GC/liter	234
		6.38	Mean GC/liter	55
		6.52	Median GC/liter	191
		7.07	Mean GC/liter	209
		7.00–8.23	GC/liter	192
		8.79	Mean GC/liter	57
	Secondary effluent	1.92–5.00	GC/liter	56
		3.26	Mean GC/liter	55
		4.11	Mean GC/liter	233
		4.0–4.2	Mean GC/liter	234
		6.52	Median GC/liter	191
		6.97	Average GC/liter	209
	Tertiary effluent	2.26	Mean GC/liter	55
		5.94	Median GC/liter	191
		6.78	Mean GC/liter	57
	Surface water	3.34	Maximum GC/100 ml	235
		0.00–6.95	Range GC/liter	236
		1.96–4.75	Range GC/liter	192
		2.11–5.20	Range GC/liter	219
		2.13–4.61	Range GC/liter	237
		3.30	Median GC/liter	238
		3.39	Median GC/liter	191
		5.18–7.96	GC/liter	220
		5.74	Mean GC/liter	209
	Groundwater	−1.15	Mean GC/liter	239
Enterovirus (EV)	Raw wastewater	2.95–7.90	Range GC/liter	240
		4.15	GC/liter	241
		4.60–5.20	Range GC/liter	242
		7.59	Mean GC /liter	209
	Secondary effluent	1.95–3.85	Range GC/liter	240
		3.1–3.2	Range GC/liter	234
		3.94	Median GC/liter	191
		7.15	Mean GC/liter	209
	Surface water	0.48–3.21	Range GC/liter	242
		1.00–6.30	Range GC/liter	236
		1.95–5.85	Range GC/liter	240
		2.65	Maximum GC/100 ml	235
		3.12	GC/liter	241
		3.77–4.69	Range GC/liter	237
		6.10	Mean GC/liter	243
		6.49	Mean GC /liter	209
	Groundwater	−0.10	Mean GC/liter	239
	Sea water	0.30–1.85	Mean GC/liter	242
Norovirus GI (NoV GI)	Raw wastewater	4.94	Maximum GC/ml	244
		3.32	Mean GC/100 ml	150
		5.51	Mean GC/100 ml	54
		5.16	Mean GC/100 ml	233
		1.50	Mean GC/liter	245
		2.66–6.36	Range GC/liter	246
		4.10–4.70	Mean GC/liter	234
		4.4	Mean GC/liter	247
		5.51	Mean GC/liter	248
		6.2	Mean GC/liter	143
		9.00	Maximum GC/liter	249
	Secondary effluent	2.53	Mean GC/100 ml	150
		3.85	Mean GC/100 ml	54
		5.34	Median GC/100 ml	250
		3.26	Mean GC/liter	233
		2.40–2.70	Mean GC/liter	234
	Tertiary effluent	5.23	Median GC/100 ml	250
	Surface Water	0.09	Mean GC/ml	251
	Groundwater	−0.22	Mean GC/liter	238

(continued)

TABLE 2 Virus concentrations in wastewater, treated wastewater, surface water, groundwater, and seawater samples (*continued*)

Virus	Sample matrix	Concentration ($\log_{10}$)	Units[a]	References
Norovirus GII (NoV GII)	Raw wastewater	3.11	Maximum GC/ml	244
		3.55	Mean GC/100 ml	150
		5.36	Average GC/100 ml	54
		6.90	Mean GC/100 ml	233
		3.58–7.85	Range GC/liter	246
		3.80–4.90	Mean GC/liter	234
		3.9	Mean GC/liter	245
		4.9	Mean GC/liter	247
		5.61	Mean GC/liter	248
		6.8	Mean GC/ liter	144
		7.78	Maximum GC/liter	249
	Secondary effluent	2.63	Mean GC/100 ml	150
		3.72	Average GC/100 ml	54
		3.61	Median GC/100 ml	250
		2.60–2.80	Mean GC/liter	234
		4.72	Mean GC/liter	233
	Tertiary effluent	3.61	Median GC/100 ml	250
	Surface water	3.33	Median GC/100 ml	250
		0.61	Mean GC/ml	251
Hepatitis A (HepA)	Raw wastewater	3.63	Mean GC/ml	252
		4.15	Maximum GC/liter	226
		5.55	Mean GC/liter	218
		3.63	Mean GC/liter	209
	Secondary effluent	1.63–5.09	Mean GC/liter	252
		<1.00	GC/liter	56
		3.76	Mean GC/liter	209
		5.40	Mean GC/liter	218
	Surface water	1.88–2.86	Range GC/liter	234
		1.18–5.28	Range GC/liter	39
		2.04–3.63	Range GC/liter	220
		3.35–4.99	Range GC/liter	253
		3.22–4.21	Range GC/liter	254
		3.89	Mean GC/liter	209
		5.23	GC/liter	219
	Groundwater	−2.22	Mean GC/liter	238
Hepatitis E (HepE)	Raw wastewater	1.65	GC/ml	255
		4.89	GC/liter	256
		2.60–3.30	Range GC/liter	226

[a] GC, $\log_{10}$ gene copy number.

Although the predominant form of transmission is via the fecal-oral route, EV can also be transmitted by contact with contaminated oral secretions, vesicular fluid, surfaces or fomites, and respiratory droplets (70, 71). These viruses cause over 30 million infections and several thousand hospitalizations per year in the United States (40, 69). In Portugal, a study carried out by Instituto Nacional de Saúde Doutor Ricardo Jorge using 625 fecal samples of suspected cases of EV infection (from 2010 to 2013) verified that 22.9% were positive for EV (72).

EV infections occur mainly in children younger than 10 years old (71), and infections may be asymptomatic or present a mild to severe symptomatology. When illness occurs, it usually takes the form of a febrile illness lasting only a few days, often accompanied by symptoms of upper respiratory tract infection or gastrointestinal illness. Although less frequent, the symptoms can become more serious according to the type of EV (62, 68, 73). Polioviruses can cause paralytic disease and poliomyelitis. Coxsackieviruses and echoviruses can cause herpangina, meningitis, paralysis, myocarditis, or hand-foot-and-mouth diseases (62, 68, 69). The most recently discovered EVs have also been associated with disease in humans. EV68 has been associated with respiratory diseases and neurological complications, namely, muscle weakness, polio-like acute flaccid paralysis, and cranial neuropathy (69). Moreover, EV71 has been associated with aseptic meningitis, cardiopulmonary dysfunction, neurogenic pulmonary edema, and tachycardia (70). EVs are fairly contagious, and while they can be transmitted by inhalation, aspiration, contact with contaminated oral secretions, vesicular fluid, and surfaces or fomites, most cases are acquired by the fecal-oral route through the drinking of contaminated water (Table 1) (40). Over the years, EVs have been found in surface water, drinking water, treated and untreated wastewater, and seawater in several countries (Table 2). Symonds et al. (74) detected culturable EV in Bolivian wastewater samples from different stages of the treatment process at WWTPs. These viruses maintain the capacity to infect human cells, unlike nonculturable viruses that are not infectious. The concentration of total culturable EVs in untreated wastewater ranged from 42 to 62 infectious units/ml, while the concentration of total culturable EVs in treated wastewater ranged from 0.037 to 20 infectious units/ml.

Norovirus

NoVs are members of the *Caliciviridae* family, classified in the genus *Norovirus* (75). The NoVs are nonenveloped icosahedral viruses (28 to 30 nm diameter) with a single-stranded RNA genome (7.5 to 7.7 kb long) encoding three open reading frames (ORFs) (76). ORF 1 codes for seven nonstructural proteins involved in replication of the genome, while ORFs 2 and 3 code for the major and minor capsid proteins, respectively, whose functions remain undetermined. NoVs can be classified into six genogroups (GI to GVI), of which only GI, GII, and GIV are known to infect humans, and the GII group causes >95% of NoV infections (76–79). Infection with NoV represents a serious and global economic problem, with an estimated economic burden of $4.2 billion in direct health care costs and $60.3 billion in societal costs per year (80).

NoVs are a major cause of acute nonbacterial gastroenteritis among adults (81), most cases being mild with symptoms characterized by diarrhea and vomiting, followed by nausea, abdominal cramps, headache, fever, chills, and muscle aches, all of which usually last 2 to 4 days (76, 82, 83). Morbidity and mortality rates of NoV infection are significant in young children, the elderly, and immunosuppressed and immunocompromised patients (84–88).

Globally, NoV is estimated to be responsible for almost 20% of all cases of acute gastroenteritis (89), accounting for 677 million cases each year and over 213,000 deaths (90). NoV incidence is different in the Northern and Southern Hemispheres, presenting a peak of occurrence in the winter months, corresponding to December to February in the Northern Hemisphere and June to August in the Southern Hemisphere (91). Although mortality risks are expected to be much higher in developing countries, NoV is a problem in both low- and high-income countries, with an estimated 71,000 child deaths every year, as the high incidence of disease appears to be universal (92, 93).

NoVs have an estimated 50% human infectious dose of 18 to 1,015 genomic equivalents (94) to 1,320 to 2,800 genomic equivalents (95) (Table 1), although this difference may result from different statistical methods used rather than from biological differences (96). A recent analysis indicates that host genetic susceptibility to NoV may result from the virus strain specificity (97).

Pang et al. (98) used a multiplex PCR assay and identified NoVs as the most common agents in sporadic gastroenteritis (98). Immunity after NoV infection is incomplete, and recurrent infections from the same agent are common (99), suggesting that long-term immunity may be absent (100, 101). Several volunteer studies also showed that immunity to NoV appears to be short lived and that resistance to one particular strain does not provide protection from other heterogeneous strains (102–104). Currently, there are no vaccines available for NoV, although a bivalent NoV vaccine is in clinical trials, and other vaccines are in several stages of development (105–107).

NoV exposure, and possible outbreaks, may occur in numerous places, such as hospitals, schools, nursing homes, swimming pools, cruise ships, and restaurants (100, 108–111). Transmission of NoV primarily takes place via the fecal-oral contamination route, via direct contact with an infected individual and (contaminated) water and food consumption (94, 112–115). Exposure to the virus may occur in drinking water (116) and irrigation water (117), with NoV genogroup GI strains being more frequently associated with waterborne outbreaks (118–120).

The consumption of several food products has also been associated with foodborne disease outbreaks, chiefly, bivalve shellfish (121–126). Most of these episodes are associated with sewage pollution due to system failure or incapacity, stormwater runoff, wet and dry weather overflows (127, 128), and boating activity (121). Moreover, fruit and vegetables may also be a source of foodborne outbreaks (129, 130), linked to contamination in irrigation, packaging, processing, or cooking (112, 114, 131–133).

The vast number of contaminated water sources—surface water, groundwater, and wastewater (raw and treated)—linked to NoV indicates a ubiquitous distribution of the virus, with a natural dispersion in water systems that needs to be thoroughly explored (134). In addition, the coexistence of different NoV genotypes of each genogroup can expose individuals to multiple NoV strains. Moreover, the risk of recombination within multiply infected individuals may lead to the emergence of new and possibly more virulent and pathogenic strains, with greater capacity to infect susceptible individuals or superior survival ability in the environment (135, 136).

NoV is highly resistant to environmental degradation in water (137–139). Thus, its presence in surface and groundwater intended for human consumption raises a potential health risk. The incidence of NoV in surface waters is directly linked to possible contaminated sources, with environmental conditions affecting the survival of NoV (140, 141). Although the molecular detection of NoV does not suggest infectivity, the virus remains detectable in mineral and tap water for over 2 months (138) and for 728 days in groundwater (142). In a more recent study, NoV was shown to remain detectable for over 3 years and infectious for at least 61 days in groundwater (139). Seasonal variation of NoV in source water and wastewater has been documented, with higher occurrence in colder periods (143–145), with the prevalence of NoV GII being greater than GI during colder periods (143, 146). This reflects the GII infection incidence during this season (147).

NoV can be present in extremely high concentrations in feces, up to 10^{11} $\log_{10}$ gene copy numbers (GC)/g (148). The reported median viral load of NoV GII is >100-fold higher than that of GI (149). Due to their association with feces, NoVs may therefore be found in high concentrations in wastewater (Table 2). A reduction of NoV concentrations is observable in wastewater treated with mechanical systems and chlorine disinfection, but still, significant concentrations have been reported for GI and GII genogroups, e.g., 5.3 and 5.9 $\log_{10}$ GC/liter, respectively (143), and 2.53 $\log_{10}$ and 2.63 $\log_{10}$ GC/100 ml, respectively (150) (Table 2).

Hepatitis Viruses

Hepatitis viruses can cause inflammation of the liver (151). Six different viruses have been identified that can provoke this infection, referred to as hepatitis A, B, C, D, E, and G viruses (152). HAV and HEV are the two agents responsible for enteric infection, the main source of contamination being feces from infected individuals. Infection mainly occurs via the fecal-oral route. In contrast, hepatitis B, C, and D viruses are transmitted by the blood and are the agents responsible for parenteral infection, caused mainly by breaches in the skin or mucosa. All viral hepatitis infections have acute characteristics, but hepatitis B, C, and D can also result in chronic disease (151, 152).

It has been estimated that about 4 million cases of HAV and HEV occur each year globally, with about 40,000 deaths and 40,000 cases of chronic liver damage. Infection is frequently due to the consumption of raw or undercooked bivalves/shellfish harvested from contaminated coastal waters. As these mollusks are filter feeders, they can effectively concentrate the enteric viruses from harvesting waters contaminated with sewage (153).

Hepatitis A

HAV is a small nonenveloped single-stranded RNA virus in the genus *Hepatovirus* (family *Picornaviridae*) (62, 154, 155). These viruses have an icosahedral capsid with a diameter of 27 to 28 nm (154) and are often responsible for causing infectious hepatitis. Infections can be easily spread through person-to-person contact, especially in families, day care centers, schools, and other institutions. In addition, parenteral HAV transmission may occur through the use of blood products or needles during transfusions, although this is rare (151). There was a fairly widespread person-to-person outbreak reported in 2017 that occurred in several countries in Europe and the United States (156, 157). This outbreak was addressed by vaccination of susceptible at-risk individuals, and so far, to the best of our knowledge, there have been no waterborne follow-up events.

In approximately 90% of the cases of infection by this virus, especially in children, there is no lesion formation in the liver cells, and when lesions occur, they are reduced and no clinical symptoms are observed. In general, the severity of the disease increases with age (40, 158). When the virus enters the body via ingestion, it multiplies in the intestine and then diffuses into the liver through the bloodstream, where it can cause damage to liver cells (40, 62). HAV has a long incubation period, about 4 weeks, with a range of 2 to 6 weeks (62). This virus is not detected by routine cell culture procedures, and therefore, its epidemiology, incidence, and behavior in the environment are not well understood. Thus, the infectious dose in humans is not known (159, 160). However, it is thought to be as low as a single viral particle (Table 1). Hepatic cell damage results from the release of liver-specific enzymes (e.g., aspartate aminotransferase). These lesions may also result in the liver's inability to remove bilirubin from the bloodstream, which accumulates and causes jaundice and dark urine. After the incubation period (about 28 to 30 days), symptoms begin, such as fever, malaise, nausea, vomiting, anorexia, abdominal discomfort, fatigue, fever, and possibly jaundice, diarrhea, and urticaria (40, 161). In severe cases, HAV

TABLE 3 Advantages and disadvantages of currently used virus detection methods

Method	Advantages	Disadvantages
Cell culture	Provides information on the infectivity of viruses Large volume of samples can be processed	Cell lines not available for all viruses Not suitable for slow-growing viruses Analysis time is lengthy Need to use multiple cell lines Expensive
PCR	Detection/nondetection Sensitive Specific Can be multiplexed Results can be obtained in 4–6 h	Does not differentiate between infectious and noninfectious viruses Nonquantitative Can be affected by inhibition of the PCR
Real-time PCR or quantitative PCR (qPCR/RT-qPCR)	Quantitative Sensitive Specific Can be multiplexed Results can be obtained in 2–4 h	Does not differentiate between infectious and noninfectious viruses Prone to PCR inhibition
Long-target-region PCR (LTR-PCR)	May provide information on viral infectivity	May reduce detection sensitivity
Integrated cell culture PCR	Provides information on the infectivity of viruses More sensitive than conventional cell culture	Cell line not available for all viruses Not suitable for slow-growing viruses Analysis time is lengthy Need to use multiple cell lines Expensive Time-consuming
Digital PCR	Does not require standard curve 1–2 orders more sensitive than qPCR Less prone to PCR inhibition	Platform is expensive Limited dynamic range of detection Limited number of samples can be processed compared to qPCR format
Nucleic acid sequence-based amplification	Rapid compared to PCR Can be multiplexed Does not require a thermal cycler Portable and easy-to-use detection method; suitable for a laboratory with basic instruments	Sample pretreatment is required RNA handling may require attention
Microarray	Multiple viral targets can be detected Powerful diagnostic tool	Assay designing requires significant bioinformatics knowledge Random amplification is required Sensitivity is questionable for environmental waters Nonquantitative
Metagenomics	Does not involve culturing or cloning Novel or unknown viruses can be detected	Requires removal of cellular organisms Preamplification is required Complex process; requires significant expertise in sequence analysis Taxonomic assignment is poor due to unknown sequences Absolute quantification is not possible

can lead to death when fulminant hepatitis associated with chronic liver disease occurs. In addition, it can lead to extrahepatic complications, including pancreatitis, vasculitis, and glomerulonephritis (151).

While the mortality rate from HAV is generally low, repair of liver damage is a slow process that can keep patients disabled for more than 6 weeks. The estimated mortality rate is 0.1% in children <15 years old, 0.3% for adults 15 to 39 years old, and about 2.1% in adults ≥40 years old (30, 161). After infection, HAV can continue to be excreted in feces for 6 months. The concentration of HAV may range from 10^6 GC/ml in serum to more than 10^8 GC/ml in stool (160).

HAV has a global distribution and is a major worldwide cause of acute viral hepatitis, with approximately 1.4 million clinical cases reported each year globally (161, 162). However, the highest endemicity of HAV occurs in regions with poor sanitation and wastewater treatment practices, particularly in countries of South Asia, Africa, Central and South America, and the Middle East. In these regions the seroprevalence of HAV immunoglobulin G antibodies reaches 90% in adults, and most children have been infected by 10 years of age (162–164). In regions of intermediate endemicity, including North America, western Europe, Australia, and Japan, 50 to 60% of adults and 20 to 30% of 10-year-old children are infected with HAV. In regions with low endemicity, such as some northern European countries, better hygiene conditions and socioeconomic status have led to a decrease in infection rates during childhood (155, 162).

Most HAV infections are transmitted via the fecal-oral route (about 95%), mainly through ingestion of contaminated water and food (151, 160, 162, 165, 166). In recent years, this virus has been detected in drinking water, surface water, groundwater, and treated and untreated wastewater in many countries (Table 2).

This virus has been shown to be resistant to several water treatment processes, including concentrations of free residual chlorine of 0.5 to 1.5 µg/ml for 1 h, at temperatures between 60 and 80°C for 1 h, freeze/thaw, relatively low moisture (±25% for 7 days), and low pH (pH 1) (160). Several studies have shown the presence of HAV in influent samples but also in a considerable number of treated effluent samples (Table 2).

The foods most commonly associated with the transmission of HAV are shellfish, vegetables, and fruits. However, bivalves (shellfish) are more easily contaminated with these viruses due to filtration of large amounts of water during their natural feeding (162, 167). Polo et al. (167) reported that reverse transcriptase quantitative PCR (qPCR) analyses indicated that ~10% of bivalve samples obtained from 10 harvesting areas in estuarine inlets on the coast of Galicia, Spain, an important bivalve production area in Europe, contained HAV and NoV in 2011 to 2012.

Hepatitis E

HEV is a small, nonenveloped, single-stranded RNA virus of the genus *Hepevirus* in the family *Hepeviridae*. The virions are spherical particles with a diameter of 27 to 34 nm (168, 169). HEVs infecting humans are divided into four genotypes (genotypes 1 to 4), with several subgenotypes (170). Genotypes 1 and 2 infect only humans and are responsible for many outbreaks, mainly in developing countries with poor sanitation conditions (170, 171). Genotypes 3 and 4 infect humans and other animals. Genotype 1 is prevalent in South Asia, Central Asia, and North Africa; genotype 2, in Mexico and West Africa; genotype 3, in the Americas, Europe, and Japan; and genotype 4, in China and Southeast Asia (168, 171, 172). In addition to waterborne transmission, HEV can be transmitted through ingestion of contaminated food, transfusions with contaminated blood, and vertical transmission (maternal-fetal) (168, 172–175).

After entering the body, the virus mainly targets the liver. It accumulates in bile and reaches the intestine through the bile duct, being found in the feces of the host about two weeks after initial infection (176). The incubation period of the HEV ranges from 15 to 60 days, but depending on the genotype in question, it may take longer, reaching 6 months for genotype 3 (174). The infectious dose of HEV in humans is not known (159).

The symptoms of acute infection caused by HEV are similar to those of HAV. After the virus enters the body, there is an increase in liver enzymes, such as alanine aminotransferase, aspartate transaminase, and gamma-glutamyl transpeptidase. The resulting symptoms include jaundice, vomiting, loss of appetite, fatigue, fever, darkened urine, hepatalgia, and hepatomegaly (175, 177). HEV infection may also be associated with neurological complications, notably Guillain-Barré syndrome, neuralgic amyotrophy, inflammatory polyradiculopathy, ataxia/encephalitis, and peripheral neuropathy (178, 179).

The mortality rate associated with HEV is about 2% in the general population, but in pregnant women, the mortality rate is much higher, reaching 20% due to fulminant hepatic failure (177, 180). The number of reported HEV infections is probably an underestimation of the actual worldwide burden (170). In 2005, it was estimated that 20.1 million people were infected with HEV (genotypes 1 and 2), resulting in 3.4 million symptomatic cases, 70,000 deaths, and 3,000 stillborn infants (171).

In regions of endemicity, the seroprevalence rates of antibodies to HEV range from 15 to 16%. The peak of incidence of HEV occurs in young people between 15 and 35 years of age. In countries with a low incidence of HEV, the prevalence of antibodies to HEV varies from 3% in Tokyo, Japan, 3.2% in central France, 7.3% in Catalonia, Spain, 16.6% in southwest France, 16% in southwest England, 20.2% in Portugal (181), to 21.3% in U.S. blood donors (180). Initially, it was thought that HEV was not an important public health threat in industrialized countries because infections were generally reported in people who had traveled to areas where the virus is endemic. However, in recent years, a number of sporadic cases or small clusters of cases have been reported in developed countries in the absence of travel to countries of HEV endemicity, suggesting the existence of HEV reservoirs in these areas. Sporadic reports of cases have emerged in the United States, Europe (United Kingdom, France, the Netherlands, Austria, Spain, Italy, and Greece), and developed Asia-Pacific countries (Japan, Taiwan, Hong Kong, and Australia) (182, 183).

HEV is the only enteric virus with a large animal reservoir, which includes domestic animals such as swine, cattle, goats, and rodents (30, 175, 178, 184). HEV is mainly transmitted by the fecal-oral route, through ingestion of contaminated or insufficiently treated water (168, 172–174, 184), and in undercooked pork products (185) (Table 1). This virus has been detected in environmental water and treated and untreated wastewater from several countries (Table 2).

ENTERIC VIRUS DETECTION METHODS

Virus concentrations in water systems are expected to increase with escalating frequencies of flooding and sewage contamination events, in large part due to changes in extreme weather and climate change combined with shortages of potable water sources. The standard methods of detection of infectious viruses in water require the use of susceptible cell lines within which the viruses can propagate and produce cytopathic effects. However, these methods are time-consuming and are not suitable for the detection of some nonculturable/noncytopathic enteric viruses; e.g., several of these viruses cannot be grown easily (e.g., adenovirus serotypes 40 and 41) or at all (e.g., HAV) in cell culture (186, 187). In the case of NoV, recent advances have been made for its cultivation (188).

Recently, there has been an investment in developing molecular methods for microbiological risk assessment through the detection and quantification of viral nucleic acids in food and water samples (56, 189, 190). Due to their high sensitivity, these methods also overcome the problems of low density of viruses in water samples, making the virus detection not strictly dependent on concentration methods to detect pathogens. Real-time qPCR (RT-qPCR) has been increasingly used for microbiological risk assessment through the detection and quantification of viral nucleic acids in food and water samples (56, 189, 190).

Aside from HAdV having a DNA genome, most waterborne viruses with significant public health implications have RNA genomes. The evolution of the PCR method greatly increased the capacity to detect pathogenic viruses in the environment, with high sensitivity and specificity. However, this method has the limitation of not allowing the evaluation of virus viability and infectivity, defined as the capacity of the virus to infect host cells and to use their resources to produce new infectious virus particles. This aspect is important in assessing and understanding the risks to public health, because even if viral nucleic acids are detected by the PCR technique in water samples, they may not be able to infect human cells (191–193). Based on statistical correlations between genome copy numbers and infectious enteric viral particles in wastewater samples, Donia et al. (194) proposed a cutoff value of 200 GC/liter to be used as an indication of EV survival in environmental monitoring (194).

Table 3 summarizes the currently used methods for detection/quantification of virus, as well as the advantages and disadvantages of each of them (based on reference 195).

CONCLUSIONS

Paradigm Shift Due to Climate Changes

The United Nations Intergovernmental Panel on Climate Change (IPCC) has forecasted that carbon dioxide emissions will result in an increase in the frequency, duration, and severity of extreme weather events (https://www.ipcc.ch/). Severe droughts, heatwaves, floods, and destructive storms are expected with growing intensity and frequency. These often compromise the integrity of infrastructure built to supply homes with commodities and to drain and treat our liquid wastes (196). Included in these extreme weather scenarios are the scarcity of potable water and the possible cross-contamination between inlet and outlet water systems (http://www.unwater.org/water-facts/scarcity/).

To mitigate climate change and global warming effects and to protect water sources and human health, the United Nations Framework Convention on Climate Change agreed in 2016 to maintain the increase in global temperature of “well under 2°C” above the preindustrial era. This covenant, which became known as the Paris agreement, aims to strengthen global responses to threats of climate change and to increase the ability of countries to deal with the impacts of climate change (https://unfccc.int/process-and-meetings/the-paris-agreement/the-paris-agreement). While these goals are ongoing, the rise in global temperature has been melting ice in glaciers, resulting in a global sea level rise of ~23 cm since 1880 according to the U.S. Environmental Protection Agency’s 2016 report (197), and the rate of sea rise is accelerating. Countries located away from the poles will see their territorial limits regress and will suffer from salt introgression, a phenomenon where potable water becomes contaminated by invading sea water, rendering it useless unless desalinated. The WHO recommends that countries develop drinking water safety plans for preparedness against depletion of potable water sources, and these are currently in implementation throughout Europe and the United States (40).

New Challenges for Water Management and Alternative Sources

Currently, there is an increasing need for alternative water sources due in large part to a shortage of potable water supplies. Some of the alternative water sources are located far underground, where viral particles are small enough (under 100 nm) to pass through soils and sediments without being adsorbed, thus contaminating these water sources. Future drinking water safety measures need to be modified to include particles that readily indicate the actual contamination of water, rather than fecal indicator organisms.

In times of scarcity, water reuse programs will face strong demand from agriculture, industrial, and urban sectors (198). Taking into account that most pathogenic viruses are also resistant to several of the most commonly used water treatment technologies (disinfection, heating, pressure, and low pH [56, 199]), it is essential that the treatment of water for human consumption or water reuse be effective and regulated (200). However, some studies indicate that these treatments are sometimes not completely effective in eliminating the enteric viruses discussed here, either in drinking water or wastewater (38, 56, 189, 190, 201). Moreover, about 90% of all wastewater is released into the environment without complete disinfection. This represents a risk of exposure to these viruses, not only through direct consumption of water, but also through indirect pathways such as recreation or the consumption of vegetables, bivalves, or animal meat that comes into contact with contaminated water (167, 187, 189).

Despite the fact that there has been great investment and advancement in water/wastewater treatment technologies for use in developing countries, these solutions are not available for every community and in every country. This leads to situations where populations depend on untreated surface water for their immediate water needs, resulting in waterborne outbreaks which remain a significant threat to human health worldwide (202). The underreporting of outbreaks related to enteric viruses, mostly in countries where these viruses are not endemic, hinders the establishment of a universal screening system to track the distribution and frequency of these outbreaks.

In this review, we have presented the main enteric viruses that we believe should start being used as additional fecal indicators in water quality assessment and monitoring. We can monitor for viruses that cause illness, surrogates of viruses, and/or viruses that indicate the possible presence of human fecal waste. The presence and prevalence of several of the viruses addressed here in surface and groundwater intended for human consumption raises a potential health risk. Despite the shortcomings of FIB monitoring, it can be useful, particularly for significant sewage contamination events. However, since enteric viruses are resistant to WWTP treatments and their concentrations can be low but highly variable in treated wastewater, more research is needed on the use of indicator viruses as proxy for enteric viruses. More data comparing the fates of FIB and enteric viruses in various stages of wastewater treatment processes are also required to support their application for regulatory use.

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