# **Wastewater Fecal Pollution Management**

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Global water scarcity has led to significant dependence on reclaimed or recycled water for potable uses. Effluents arising from human and animal gut microbiomes highly influence water quality. Wastewater pollution is, therefore, frequently monitored using bacterial indicators (BI).

wastewater indicators management

pepper mild mottle virus

enteric viruses

Public health

## 1. Introduction

The wastewater virome is a distinct subset of the microbiome owing to frequent infection of humans and domestic/companion animals <sup>[1][2][3][4]</sup>. For instance, a typical healthy human is estimated to harbor more than 10 virus-mediated chronic infections and occasionally more <sup>[5]</sup>. In particular, the human gut virome composition is intensively studied because of the continuous introduction of newly pathogenic agents and altered pathogenesis patterns, along with changes in immune responses owing to selection pressure imposed on the existent virome <sup>[6]</sup>. Gastroenteritis displays the potential pathogenesis of acquired gut virome <sup>[3][8]</sup>. For instance, rotavirus A, noroviruses and astroviruses are considered as the major causes of acute gastroenteritis worldwide and mainly result in infantile acute diarrhea <sup>[9][10][11]</sup>.

Exposure to wastewater represents a common transmission route of enteric viruses via recreation water, surface water usage, wastewater/greywater-mediated irrigation and toilet flushing <sup>[12][13][14][15][16]</sup>. Therefore, wastewater reuse guidelines were suggested for safe use of wastewater. Moreover, fecal contamination indicators were proposed to ensure compliance with these guidelines. Coliform members represent the frequently used fecal indicators; however, other indicators have also been proposed involving bacteriophages, enterococci and sulfite-reducing bacteria <sup>[17][18][19][20]</sup>. These have not, however, met the expected sensitivity of enteric viruses' detection <sup>[21]</sup>. On the contrary, microbial source tracking (MST) tools provided higher specificity. Currently, MST professionals adopt a toolbox approach, i.e., implementing numerous MST markers, such as using pepper mild mottle virus (PMMoV) together with cross-assembly phage crAssphage <sup>[22][23][24]</sup>.

## 2. Viral Contamination of Treated Wastewater

Viruses are obligatory intracellular parasites of both eukaryotic and prokaryotic cells, and considered as the smallest microorganisms capable of replication <sup>[25]</sup>. Since they are unable to metabolize, they cannot engage in any energy-dependent processes like growth, respiration or reproduction on their own <sup>[26]</sup>. The main component of viruses is nucleic acid (DNA or RNA), which is shielded by a protein capsid that, in some cases, is surrounded by a lipid envelope <sup>[27]</sup>. Despite their apparent simplicity, viruses can nevertheless penetrate host cells using a number of physical and chemical mechanisms that are part of both the virus and the cell's structure. Moreover, viruses can also manipulate cellular processes to produce progeny viruses using various routes of entry <sup>[28]</sup>. Of particular interest is that enteric viruses are transmitted via the fecal–oral pathway. Enteric viruses are considered as the most persistent fecal microorganisms even during treatment approaches of contaminated raw water owing to their unique characteristics <sup>[29]</sup>. These viruses have an icosahedral structure, are between 20 and 100 nm in size, and primarily show a negative charge at neutral pH <sup>[30]</sup>. They are principal causes of viral gastroenteritis, hepatitis and poliomyelitis, displaying their adverse effect on public health. In addition, some enteric viruses, including polyomaviruses, have been linked to cancer <sup>[31]</sup>. Moreover, enteric viruses are highly efficient at surviving outside the gut for long periods, thus are easily disseminated through water resources <sup>[32]</sup>.

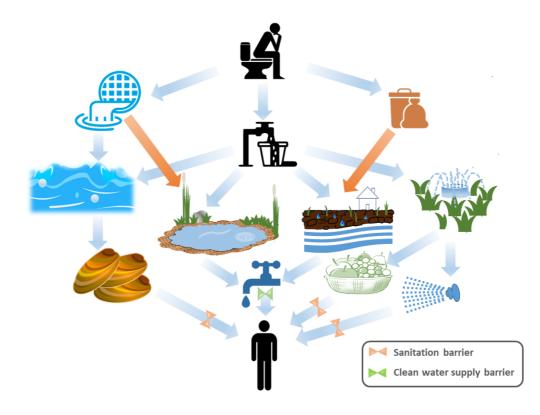
Inappropriate wastewater treatment has caused viral contamination of shellfish, fresh produce and recreational waterways <sup>[33]</sup>. Many developing nations struggle with this ongoing problem because they lack the resources for effective wastewater treatment <sup>[34]</sup>. It is not surprising that, in current US frameworks, viruses demand substantially bigger reductions than bacteria or protozoan parasites when wastewater is reused for potable reasons. Target log<sub>10</sub> removal value (LRV) attributions are computed using a risk-modelling methodology, assuming the worst-case scenario (very high viral concentrations, as would be the situation during a big epidemic), and a final risk of less than one illness in 10,000 exposures, per year <sup>[35]</sup>. Currently, states are choosing between one of these three LRV programs that were previously developed <sup>[36]</sup>. Although Texas' requirements seem to be less stringent than those of California or the National Water Research Institute (NWRI), it should be emphasized that Texas only counts LRVs from treated effluent toward final product water, excluding wastewater treatment reduction <sup>[37]</sup>. The wastewater treatment plant must exhibit its ability to eliminate pathogens to the levels required by the state prior to earning an LRV attribution, typically through a pilot demonstration <sup>[38]</sup>.

Validating virus elimination is necessary for LRV attribution in reuse schemes. However, pathogenic virus concentrations in sewage and treated effluent vary, and frequently are not at levels that might effectively verify an 8–12 LRV <sup>[39]</sup>. It is frequently not practical or safe to spike pathogenic viruses at each phase to confirm overall LRV. Non-pathogenic viruses are frequently used as a process indicator <sup>[40]</sup>.

### 3. Human Health Risk of Virus-Associated Water Pollution

On an annual basis, there are over 4 billion instances of waterborne diarrheal illnesses, which cause 2 million deaths, with under-five year olds the majority <sup>[41]</sup>. Enteric viral infections account for a sizable fraction of these diseases <sup>[42]</sup>. The most crucial way for enteric viruses to spread is through direct contact with infected individuals. Enteric viruses are spreading via the fecal–oral pathway as shown in **Figure 1** <sup>[43]</sup>. However, the majority of enteric viruses remain persistent in areas where residential wastewater discharges exist and are frequently linked to

waterborne epidemics <sup>[43]</sup>. Although typical wastewater treatment techniques can be comparatively inefficient at eliminating enteric viruses, wastewater is frequently treated before being released into the environment [44]. Fecal matter pollutes the environment and drinking water sources in poor countries since many locations lack suitable sanitary infrastructure and wastewater treatment facilities [45]. Additionally, significant amounts of untreated wastewater may be released by combined sewer overflows (CSOs) during periods of high rainfall as well as through dry water overflows, such as those caused by snowmelt, tidal infiltration, system failures and obstructions [46][47][48]. Consequently, people who come into direct or indirect contact with contaminated waters are prone to the risk of contracting viral infections as a result of these events, which allow enteric pathogens to contaminate the environment directly <sup>[49]</sup>. Enteric viruses are extremely contagious in ambient waters and can stick to particles in the water column or accumulate in sediment <sup>[50]</sup>. They might subsequently be consumed by aquatic organisms, such as bivalve shellfish harvested for human consumption <sup>[51]</sup>. Additionally, wastewater is regularly used for irrigation in areas with a shortage of freshwater; as a result, enteric viruses may directly contaminate fruit and salad vegetables, and result in foodborne outbreaks [47]. The typical duration of gastroenteritis caused by enteric viruses is 2-5 days <sup>[52]</sup>. In certain circumstances, the infection goes asymptomatic or causes symptoms in the skin, neurological system or respiratory system <sup>53</sup>. The Picornaviridae, Caliciviridae, Reoviridae, and Adenoviridae families make up the majority of those responsible for gastroenteritis (Table 1). For instance, noroviruses (family Caliciviridae) account for a sizable portion of gastroenteritis infections worldwide, causing 685 million cases and roughly 200,000 fatalities <sup>[54]</sup>, with a total direct cost to the healthcare system of USD 4.2 billion and associated societal costs of USD 60.3 billion annually <sup>[54]</sup>. The main etiological agents of gastroenteritis in newborns and young children are rotaviruses (family Reoviridae) and group F mastadenoviruses (AdVs; family Adenoviridae) [55]. The three most frequent viral pathogens linked to waterborne and water-associated foodborne outbreaks are noroviruses, hepatitis A virus (family Picornaviridae) and AdVs [56]. Infection can cause significant illness, such as acute hepatitis <sup>[57]</sup>.



**Figure 1.** Diagrammatic representation of the fecal–oral route for transmission of enteric viruses. Human excreta go through land runoff and sewage that contaminate oceans, rivers, lakes and ground water. Moreover, sewage can contaminate irrigation water. The contaminated oceans, rivers and lakes influence filter feeders (shellfish) and recreation water, whereas the direct water supply would be affected by the improperly decontaminated ground water and rivers. Crops and irrigation-based aerosols are also contaminated by inadequately treated irrigation water. On the other hand, the human excreta give rise to solid wastes that affect the groundwater, leading to unclean water supply. Absence of a sanitation barrier and a properly clean water supply barrier lead to enteric virus infection of a new human host.

Virus	Size of Viral Particle	Zoonotic Transmission	Aquatic Environment	References
Mastadenovirus A–F	70–90 nm	No	Wastewater	[ <u>58][59]</u>
Torque teno virus	30 nm	Yes	River	[58][59][60]
Astrovirus	28–30 nm	Potentially	Sewage water	[58][61][62]
Norovirus GI, GII	35–40 nm	No	River	[ <u>58][63]</u>
Sapovirus GI, GII		No	Wastewater and river	[58][64]
Human-associated circovirus	15–25 nm	No	Sewage	[65][66]
Hepatitis E virus type 1– 4	27–34 nm	Yes	Tap and bottled water	[ <u>67][68]</u>
Assorted papillomaviruses	55 nm	No	Wastewater	[ <u>69][70]</u>
Human bocavirus type 1–4	22 nm	No	Recycled water and sewage	[ <u>58][71]</u>
Aichivirus A–B	30–32 nm	No	Sewage and surface water	[72]
Cosavirus A		No	River and waste water	[ <u>65][73</u> ]
Coxsackievirus B		No	Sewage water	[ <u>73</u> ][ <u>74</u> ]
Enterovirus A–D		No	Groundwater	[ <u>73][75</u> ]
Poliovirus type 1–3		No	Wastewater	[ <u>73][76</u> ]
Hepatitis A virus	40–45 nm	No	Wastewater	[73][77]

#### Table 1. Human pathogenic viruses detected in the aquatic environment.

Virus	Size of Viral Particle T	Zoonotic ransmission	Aquatic Environment F	References
BK polyomavirus		No	River and sewage water	[ <u>78]</u>
JC polyomavirus	[ <u>81][82][83][84</u> ]	No	Wastewater	[ <u>79</u> ]
Rotavirus A	60–80 nm	Potentially	Drinking water	<u>32</u> ] [ <u>58][80]</u>

norovirus, sapovirus, astrovirus and rotavirus have also been reported <sup>[83]</sup>. Hepatitis E virus was linked to the greatest viral waterborne outbreak in Kanpur, India, which affected almost 80,000 individuals <sup>[84]</sup>.

Environmental waterways have recently been found to include both recently discovered viruses and well-known viruses that weren't previously connected to wastewater (Table 1). Infected people's feces and urine have only lately been found to include human polyomaviruses (PyVs) and papillomaviruses, which were initially identified in the 1970s and 1950s, respectively <sup>[85]</sup>. High concentrations of several PyVs, such as BKPyV, WUPyV, KIPyV, MCPyV and JCPyV, have been found in wastewater, river and ocean, silt, swimming pools, and tap water (up to 10<sup>8</sup> genome copies (gc)/l) [86][87]. Although the method of transmission of these viruses is not yet known because healthy persons frequently show no symptoms, aquatic infections are most likely [88]. On the other hand, the first description of Bocaviruses (family Parvoviridae), which cause gastroenteritis and respiratory tract infections, was made in 2005 [89]. Since then, human bocaviruses have been discovered in wastewater at quantities of 10<sup>3</sup>-10<sup>5</sup> genome copies (gc)/l in both untreated and treated wastewater <sup>[90]</sup>. Additionally, sewage and contaminated river waters have been shown to contain the gastroenteritis-causing torgue teno virus (family Anelloviridae). Similar to bocaviruses, the torque teno virus has much lower concentrations (up to 10<sup>6</sup> gc/l) than other, more prevalent enteric viruses (10<sup>4</sup>–10<sup>9</sup> gc/l) [64]. Additionally, human picobirnaviruses (family Picobirnaviridae) have been found in contaminated rivers and wastewater with concentrations ranging from 10<sup>3</sup> to 10<sup>6</sup> gc/l [91]. Wastewater has also been shown to contain the entire or partial genomes of circoviruses (family Circoviridae), cardioviruses (family Picornaviridae), and enveloped viruses (coronaviruses, influenza virus) <sup>[92]</sup>. Human infections from aquatic coronaand influenza viruses (such as SARS-CoV-2) are uncommon since enveloped viruses break down quickly in water [<u>93</u>]

## 4. Management Strategies for Wastewater Pollution

### 4.1. Traditional Fecal Bacterial Indicators

The microbiological safety of irrigation water is monitored using indicator organisms <sup>[94]</sup>. *E. coli* is classified as specifically having fecal origin and is a member of the coliform subgroup known as the fecal coliforms <sup>[95]</sup>. The primary indicator of fecal contamination of water is frequently *E. coli* <sup>[96]</sup>. There are several problems with *E. coli* as a fecal indicator. To begin with, the presence of viral infections is not correlated with *E. coli* which is not host-specific. Moreover, *E. coli* also decays in the environment more quickly compared to other foodborne bacteria <sup>[97]</sup>. In contrast, the standard fecal indicator should show environmental survival and movement across the matrix that are equal to or greater than those of the pathogen, exist at higher concentrations than the pathogen, and provide

source specificity <sup>[98]</sup>. Also, the indicator organism assay method should be accurate, specific, quick, quantitative, sensitive, widely applicable and indicative of infectivity <sup>[99]</sup>.

Levels of fecal contamination in water have conventionally been assessed using fecal indicator bacteria (FIB; including coliform bacteria, Enterococcus, *E. coli* and *Streptococcus* spp.) <sup>[100]</sup>. Bacterial pathogens, like fecal coliforms, can survive for up to 15 days on the surface of food and up to 30 days in water and sewage <sup>[101]</sup>. However, bacteria have been demonstrated to be substantially less persistent in the environment and significantly less resistant to wastewater treatment than enteric viruses <sup>[25]</sup>. Consequently, FIB are subpar predictors of the risk of viral infection, which implies that current water-quality monitoring programs based only on FIB are insufficient <sup>[102]</sup>.

### 4.2. Viral Indicators

Human enteric viruses come in about 100 different varieties and the number is growing due to newly discovered and emerging strains <sup>[103]</sup>. Surrogates and indicators are frequently employed to study the fate and transport of pathogenic strains in the environment owing to the high diversity of viral pathogens <sup>[104]</sup>. An indicator may be useful for evaluating pathogen abundance, persistence, adsorption and transit in the aquatic environment, as well as for making a general assessment of the effectiveness of wastewater and drinking water treatment <sup>[38]</sup>. Therefore, a good viral indicator should ideally have comparable inactivation and retention of the target pathogens and should be present year-round in wastewater and habitats impacted by wastewater <sup>[105]</sup>. This would allow for ongoing monitoring and provide information on the degree of pollution and the probability that pathogens are present <sup>[106]</sup>. **Table 2** lists some enteric viruses that are connected to wastewater and may be utilized as indicators, but not all of these viruses meet the criteria. High concentrations of influenza, corona-, circo- and papillomaviruses have been found in wastewater but not in contaminated areas, which may be because of how quickly they degrade in water <sup>[107]</sup>.

Organism	Habitat	Temperature	Duration (Days)	Log Reduction	Reference	
Adenovirus	Groundwater	4	132	1.00	[108]	
Adenovirus	Groundwater	20	36	1.00		
Adenovirus 40	Seawater	15	28	1.40		
		15	85	2.00	[109]	
	Drinking water	4	60	0.49		
	Drinking water	4	92	2.00		
Adenovirus 41	Seawater	15	28	1.60	[109]	

**Table 2.** Survival of enteric viruses in various water environments.

Organism	Habitat	Temperature	Duration (Days)	Log Reduction	Reference
		15	77	2.00	
	Drinking water	4	60	1.00	
		4	304	2.00	
	Fresh water	20	10	2.00	[ <u>110]</u>
		4	32	2.00	
Rotavirus	Seawater	37	7	5.00	[ <u>111]</u>
	Soil	37	7	1.70	[112]
	Drinking water	20	64	2.00	[113]
	Groundwater	25	1266	1.79	[114]
	Mineral water	25	80	1.30	
Norovirus		4	80	0.89	[ <u>115]</u>
	Tap water	25	80	0.80	[]
		4	80	3.00	
	Seawater	20	28	4.00	[ <u>116</u> ]
Hepatitis A virus		25	11	1.00	[ <u>117]</u>
	Artificial seawater	24	19	1.00	
	Drinking water	4	60	1.60	[ <u>109]</u>
		4	56	2.00	1703
	Bottled water	21	21	1.99	[ <u>118]</u>
Astrovirus	Tap water	20	30	2.00	[ <u>119</u> ]
		4	60	2.00	

be zoonotic; as a result, their occurrence in the environment may be caused by things other than human waste, such as agricultural operations <sup>[120]</sup>. Although the hepatitis A and E viruses are widespread in less developed countries, they only sometimes cause epidemics in more developed areas <sup>[121]</sup>. Furthermore, in temperate regions, enteroviruses, noroviruses and sapoviruses all exhibit distinct seasonality, peaking either in the summer (for enteroviruses) or the winter (for noroviruses and sapoviruses) <sup>[45]</sup>. Consequently, these viruses are not constantly present in contaminated environments and wastewater throughout the year <sup>[122]</sup>. On the other hand, it has been proposed that human adenovirus, polyomaviruses and Aichi viruses can serve as accurate fecal markers because they are frequently found in sewage and other contaminated areas without any discernible seasonality <sup>[123]</sup>.

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