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Estimating wastewater concentrations of norovirus and rotavirus from global data on community-level infection prevalence and viral shedding

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ABSTRACT

Water quality modelling offers the opportunity of estimating the magnitude of pathogen loads into wastewater by using data on disease prevalence and excretion. The objective of this paper is to reflect on the potential of using prevalence and excretion rate data from the literature to simulate wastewater concentrations for norovirus and rotavirus. Three systematic literature reviews were carried out to collect worldwide data. Firstly, targeting community-level prevalence data, secondly, viral excretion rates in faecal material, and thirdly, concentrations in wastewater. Data collected in the first two reviews were input to simulate concentrations in wastewater. Model results were compared with reported concentrations collected in the latest review. Of 2,193 studies, 97 were included. Reported community-level prevalence of infection ranged from 0.22 to 9.5% for norovirus and 0 to 3.3% for rotavirus. Mean viral excretion was 4.9×10^{10} and 9.7×10^8 GC/g stool for norovirus and rotavirus, respectively. Average reported wastewater concentrations were 1.5×10^7 and 2.4×10^7 GC/100 mL, respectively. Modelled concentrations were generally higher than observed values. This synthesis demonstrates the potential of integrating prevalence and excretion data through modelling to estimate pathogen loads in wastewater while highlighting major sources of variability and the need for more data collection on prevalence and excretion.

Key words: community-level prevalence, norovirus, rotavirus, virus shedding, wastewater surveillance, water quality modelling

HIGHLIGHTS

- Synthesised global data on community-level prevalence, viral shedding, and wastewater concentrations for norovirus and rotavirus.
- Identified a severe scarcity of community-level prevalence studies, limiting accurate estimation of viral loads in wastewater.
- Estimated average wastewater concentrations were higher than average observed values but remained within the overall reported range.

INTRODUCTION

Waterborne pathogens, rotavirus and norovirus, are a risk for human health all around the globe. Rotavirus is the main agent causing acute gastroenteritis in children under 5 years of age and is responsible for 28% of all gastroenteritis cases worldwide (Tate *et al.* 2012; da Silva *et al.* 2016), even after the introduction of vaccination campaigns in developing countries (Richardson *et al.* 2010). Norovirus has been associated with just under 20% of all acute gastroenteritis worldwide (Ahmed *et al.* 2014) and is the most common pathogen implicated in waterborne outbreaks (Payne *et al.* 2013; Moreira & Bondelind 2016). Norovirus is a genetically diverse species (*Norovirus norwalkense*) and has been subdivided into 10 genogroups, of which only GI, GII, GIV, GVIII, and GIX are infectious in humans (Vinjé *et al.* 2019). Rotavirus is classified into nine species, but only RVA, RVB, RVC, and RVH are known to infect humans (Matthijnsens *et al.* 2011; Matthijnsens *et al.* 2022). RVA is the most relevant species of rotavirus with about 79 genogroups described (da Silva *et al.* 2016).

Norovirus and rotavirus infections are spread through the faecal-oral route, for example, by consumption of contaminated food and water (Fankhauser *et al.* 1998; Patel *et al.* 2009; Pouillot *et al.* 2015). Just like many other human enteric pathogens,

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norovirus and rotavirus are excreted in high concentrations in faeces by infected individuals. Potential water contamination can occur when the viruses reach the water after passing through the sanitation system, for example, due to inefficient sanitation facilities and treatment technologies in developing countries (Adelodun *et al.* 2021).

Water quality modelling is a useful tool to simulate the load, transport, and in-stream concentrations of microbial pollutants. Furthermore, modelling facilitates the assessment of health risks under different scenarios and can provide insight into the effectiveness of measures to reduce these health risks, which is relevant for researchers and decision makers.

Water quality modelling typically accounts for the sources of contamination and simulates the load of pollutants into a water body (Hofstra *et al.* 2019). Researchers have modelled pathogen loads in wastewater using data on the fraction of the population connected to sewer facilities, prevalence of infection among the members of the community, and pathogen excretion rate by infected individuals (Sterk *et al.* 2016). Conversely, wastewater pathogen concentration data can be used together with sewered population data to estimate the viral load per person per day (Guo *et al.* 2022). Recently, this approach has gained momentum with the growth of wastewater-based surveillance to detect SARS-CoV-2 (Bivins *et al.* 2020), providing opportunities for understanding trends in prevalence of infection in the population for COVID-19 and other infectious diseases.

Two meta-analyses have summarised published concentrations of norovirus in wastewater. Eftim *et al.* (2017) summarised concentrations of norovirus in raw sewage and estimated an average of 4.6 log₁₀ gene copies per litre, noting that concentrations vary by genogroup, geographic area, and season. Guo *et al.* (2022) estimated the prevalence of infection for norovirus in the population using concentration data in raw sewage and excretion rates. However, no prevalence values were provided; instead, the study evaluated the sensitivity of the equation applied and concluded that calculated prevalence is more sensitive to analytical detection uncertainty than to excretion variability. For rotavirus, to the authors' knowledge, no studies were available that compile the literature on prevalence of infection, excretion rates, or concentrations in wastewater. Awere-Duodu & Donkor (2024) summarised the literature on the presence of rotavirus in untreated wastewater and showed that around 68% of the untreated wastewater samples contained rotavirus.

Therefore, there is a need to collect, analyse, and synthesise the available data on community-level prevalence of infection and viral excretion rates and to evaluate their usage as variables to estimate concentrations in wastewater. The objective of this paper was to reflect on the potential of using observed prevalence and excretion rate data from the literature to simulate wastewater concentrations for norovirus and rotavirus.

In this study, three systematic literature reviews were performed collecting worldwide scientific literature describing community-level prevalence of norovirus and rotavirus infection, excretion of both viruses via faeces, and concentrations of both viruses in wastewater treatment plant influents. Collected data were analysed for correlations with region, seasonality, and age group. Furthermore, wastewater concentrations were simulated using prevalence and excretion rates as input data and compared these estimations with the observed wastewater concentrations.

METHODS

Systematic literature review

Three systematic literature reviews were carried out using the search engine PubMed, aiming to find scientific articles on the prevalence, excretion, and concentration in wastewater of norovirus and rotavirus. Figure 1 displays the steps taken during the literature review process. Inclusion of papers was delimited up to the beginning of the COVID-19 pandemic to avoid methodological biases introduced by pandemic-related changes. During this period, the prevalence of infections, wastewater surveillance, and human mobility patterns underwent substantial shifts that were not representative of typical conditions. By ending the search by the start of the COVID-19 pandemic, the incomparability of observational data and underestimation of prevalence and excretion rates were prevented.

The first systematic search aimed to find all existing scientific literature on community-level prevalence of infection of norovirus and rotavirus (registered in the International Prospective Register of Systematic Reviews PROSPERO ID: CRD42020172028). To avoid overrepresentation of infected individuals in the population, studies where participants were recruited after showing symptoms of gastroenteritis were excluded, such as hospital-based studies and outbreak case-studies. Results were limited to studies published in English between 2010/01/01 and 2019/12/31. The second search aimed at reports on norovirus and rotavirus human excretion rates in faeces. The third search aimed at scientific reports on norovirus and rotavirus concentrations in wastewater published between 2000/01/01 and 2019/12/31. Search criteria are detailed in the Supplementary Material S1.1.

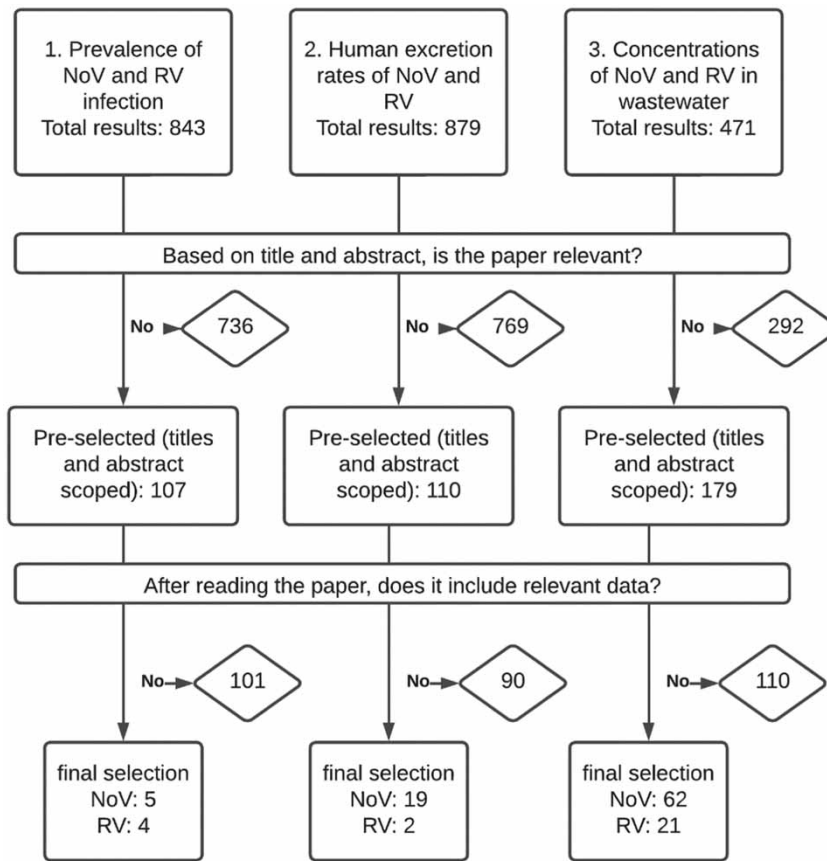


Figure 1 | Diagram showing the inclusion process in the three separate systematic literature reviews on norovirus (NoV) and rotavirus (RV).

For the first search, special attention was given to the recruiting of participants. Ideally, community-level studies recruit participants randomly without considering gender, age, health status, or socioeconomical background. Due to this criterium, several cohort studies that seemed relevant were excluded (Lopman *et al.* 2015; Platts-Mills *et al.* 2015; Harris *et al.* 2017), for example, because an equal number of healthy and symptomatic individuals were assessed or because only symptomatic participants were confirmed to be infected, meaning that the reported prevalence value did not include virus shedders that were not symptomatic. In the second search, original studies that indicated absolute quantification of norovirus or rotavirus in faecal material were included for detailed examination and data extraction. For the third search, original papers quantitatively reporting on the concentrations of both viruses in raw sewage and wastewater treatment plants (WWTPs) influents were included for data extraction.

Data such as first author, year of publication, targeted virus, virus genogroup, continent, country, time range of sampling, detection method, and DOI were extracted for the three searches. Norovirus genogroups were distinguished as GI, GII, and GIV, since previous reports have indicated the difference in some characteristics among genogroups, including their incidence, virulence, and stability (Zheng *et al.* 2006; Huhti *et al.* 2011; Kroneman *et al.* 2013). For rotavirus, where possible, different species (A or C) and genotypes (I and II) were distinguished.

Prevalence values and concentrations in either faecal material or wastewater were reported in different styles across the selected studies. When possible, concentration values were extracted per individual sample (either faecal or water sample); however, in multiple studies, only mean values over a group of samples were available. In studies where values were presented only graphically, data were extracted from published figures using 'WebPlotDigitizer' (Rohatgi 2025). When the author reported values for samples grouped by the inclusion of an independent variable, such as location, genogroup, or population age range, said group distinctions were preserved while collecting the data. All included values are available in detail in Supplementary Material S2.

For each collected record in search 1, the following data were extracted: the norovirus and rotavirus infection prevalence, total number of samples, number of samples positive for infection, and population age range. For search 2, the following data were extracted: the norovirus and rotavirus concentration in faecal material, presence of symptoms in participants, type of sample, number of participants, and population age range. For search 3, the following data were extracted: the concentration of viral particles in wastewater and type of wastewater sample (influent, domestic sewage, etc.).

Statistical analysis

Collected data showed different study designs and reporting styles. Estimates of prevalence and viral concentrations in wastewater and faeces were reported in sample groups that ranged in size from 1 to 180. To avoid bias from small groups, weighted means (Equation (1)) were calculated on concentration data reported in faeces or in wastewater. Sample subsets were defined for each publication based on region and viral genogroup. In reports on viral excretion rate, subsets were additionally defined by participants' age group (infants, 0–2 years old; children, 3–17 years old; adults, 18–60 years old; elders, older than 60 years) and symptomology (symptoms or no symptoms).

$$\text{Weighted mean} = \frac{\sum_{i=1}^n (w_i * x_i)}{\sum_{i=1}^n w_i} \quad (1)$$

where w_i is the weighting given to the i th subset, x_i is the arithmetic mean concentration (in faeces [GC/g stool] or in wastewater [GC/100 ml]) of the i th subset, and n is the number of subsets in the set. In this analysis, the weighting (w_i) was given by the number of samples reported in each subset. Estimated weighted means are provided in figures and Table 2 and detailed in the Supplementary Material S1.4, S1.5, and S2.

Excretion rates between groups were compared using one-way ANOVA followed by Tukey's honestly significant difference between continents, countries, viral genogroup, age group, and symptomatic and asymptomatic groups of participants. Concentrations in wastewater were compared between continents, countries, and viral genogroups. All statistical analyses were performed in R version 3.6.1

Simulation of viral concentrations in wastewater

Nation-wide concentrations in wastewater were simulated using collected data on community-level prevalence of infection and viral excretion rates, as shown in Equation (2), where C_{ww} is the simulated viral concentration in wastewater [GC/100 ml], P is the total population in a country, P_s is the fraction of the total population connected to a sewer, P_v is the community-level prevalence of infection of a virus, S is the stool production per person per day [g/person · day], ER is the excretion rate of a virus in stool [GC/g stool], and W_c is the total volume of wastewater collected per country per day [L/day].

$$C_{ww} = \left(\frac{(P * P_s) * P_v * S * ER}{W_c} \right) / 10 \quad (2)$$

In this study, P_v was defined as the percentage of the population, regardless of symptoms, infected by norovirus or rotavirus. Although the prevalence data originated from studies with varying study designs and sampling frequencies, they were assumed to represent daily prevalence.

For countries with observational wastewater concentration data (Supplementary Material S1.4 and S1.5), viral concentrations in wastewater were simulated. Population data (P) were obtained from the World Development Indicators (World Bank 2022), the fraction connected to a sewer (P_s) was taken from the WASH database (WHO/UNICEF 2020), the stool production per person per day (S) was taken from Rose *et al.* (2015), and total daily wastewater volume (W_c) was taken from Jones *et al.* (2020). These data have been used together with values from the literature for P_v and ER and compared with the observed concentrations in wastewater.

A one-factor-at-a-time sensitivity analysis was performed using Equation (2) to test the sensitivity of the simulated viral concentrations in wastewater to variations in the input variables (Supplementary Material S1.3). For this purpose, each input: prevalence, excretion rate, stool production, and fraction population connected to sewer, was varied individually by $\pm 10\%$ relative to its baseline value and by using minimum and maximum values found in literature. Additionally, to evaluate

the potential reduction in pathogen loading due to toddlers wearing nappies and the potential reduction in loading due to vaccination for rotavirus, the population that contributes to the pathogen loading has been adjusted in the sensitivity analysis. The resulting simulated concentration values were compared with those obtained with baseline input values.

RESULTS

Literature review on norovirus and rotavirus community-level prevalence

Out of the initial pool of 843 research papers, just 6 were deemed suitable for inclusion for the prevalence of norovirus and rotavirus at the community level (Table 1). In studies where prevalence values were not directly reported, as was the case for all rotavirus studies, prevalence was estimated based on the reported number of infected people divided by the total sampled population.

Of the six population studies included, three were conducted in Europe, specifically in the Netherlands and Sweden. The remaining studies took place in Australia, India, and Peru. It is noteworthy that all community-level prevalence studies were centred on children under the age of 5 years. The only reported infection prevalence among adults was performed in the Netherlands (Heusinkveld *et al.* 2016).

Table 1 highlights the varying prevalence rates of norovirus genogroups. In children, reported norovirus GI prevalence ranged from 0.22 to 9.0%, while adults exhibited a 3.3% prevalence rate. For norovirus GII, the range fell between 0.45 and 6.6%, with a sole study reporting a 2.6% prevalence in adults. Notably, the highest combined-genogroup norovirus prevalence of 9.5% among children was observed in the Netherlands.

In the case of rotavirus, only four studies met the inclusion criteria (Table 1). These studies revealed a 3.3% prevalence among infants and children in the Netherlands. In Sweden, no infections were observed in the 438 participants. Australia reported a 0.4% prevalence of rotavirus infection among infants. Data on adult prevalence were scarce, with only one study available, which identified a 1.1% infection rate among 915 Dutch participants.

Human excretion rates of norovirus and rotavirus

Nineteen studies out of 879 met the inclusion criteria for search 2. The included studies reported excretion rates of norovirus in human faeces in 12 countries: Australia, Brazil, Burkina Faso, China, Germany, Japan, Korea, Spain, Taiwan, Thailand, USA, and Venezuela (Table 2). All papers report viral concentration as gene copies in a gram of stool (GC/g). Asymptomatic

Table 1 | Studies reporting community-level prevalence of infection (percentage of infected in the sampled population) of norovirus and rotavirus collected in search 1

Norovirus	Prevalence (%)			Population	Author
	GI	GII	Combined GI and GII		
India		6.6		76 children under 5 years old	Berendes <i>et al.</i> (2017)
Netherlands			9.5	5,197 infants 0–24 months	Enserink <i>et al.</i> (2014)
Netherlands	4.3	6.5		928 children under 5 years old	Heusinkveld <i>et al.</i> (2016)
	3.3	2.6		915 adults	
Sweden	0.2	0.4		438 children under 5 years old	Kaarme <i>et al.</i> (2016)
Peru	2.8			5,185 infants 0–5 months of age	Saito <i>et al.</i> (2014)
	9.0			5,185 infants 6–11 months of age	
Rotavirus					
Country	Prevalence (%)		Population	Author	
Netherlands	3.3		5,197 infants 0–24 months	Enserink <i>et al.</i> (2014)	
Netherlands	1.3		928 Children under 5 years old	Heusinkveld <i>et al.</i> (2016)	
	1.1		915 Adults		
Sweden	0.0		438 Children under 5 years old	Kaarme <i>et al.</i> (2016)	
Australia	0.4		158 infants 0–24 months	Ye <i>et al.</i> (2018)	

Note: Prevalence of norovirus is reported for genogroups GI, GII separately, and both genogroups clustered.

Table 2 | Norovirus and rotavirus excretion rates reported by the nineteen studies included

First author	Country	Age group	Weighted mean excretion rate as GC/g stool [min-max]	
			Symptomatic	Asymptomatic
Norovirus				
<i>Norovirus GI</i>				
Chan <i>et al.</i> (2015)	China	All ages	8.4×10^5	
Ozawa <i>et al.</i> (2007)	Japan	Adults	4.4×10^7	3.8×10^6
Cheon <i>et al.</i> (2010)	Korea	Infants		1.3×10^5 [$2.7 \times 10^4 - 3.2 \times 10^5$]
Gonzalez <i>et al.</i> (2011)	Venezuela	Infants	3.2×10^9	
<i>Norovirus GII</i>				
Huynen <i>et al.</i> (2013)	Burkina Faso	Children	1.5×10^5	8.9×10^5
Lee <i>et al.</i> (2007)	China	Children	8.9×10^8	
Chan <i>et al.</i> (2006)	China	All ages	3.0×10^8	
Ozawa <i>et al.</i> (2007)	Japan	Adults	3.3×10^8	5.5×10^8
Cheon <i>et al.</i> (2010)	Korea	Infants		5.1×10^7 [$4.3 \times 10^7 - 7.2 \times 10^7$]
Lai <i>et al.</i> (2013)	Taiwan	Adults	6.3×10^7	
		Elders	3.5×10^9	
Khamrin <i>et al.</i> (2016)	Thailand	Infants	2.5×10^7 [$6.2 \times 10^4 - 5.0 \times 10^7$]	1.8×10^6
Sabrià <i>et al.</i> (2016)	Spain	Adults	3.2×10^7	3.1×10^6
Costantini <i>et al.</i> (2016)	USA	Adults	7.9×10^{11} [$3.5 \times 10^5 - 4.5 \times 10^{13}$]	8.5×10^{11} [$1.1 \times 10^7 - 3.9 \times 10^{12}$]
Barreira <i>et al.</i> (2010)	Brazil	Infants	2.5×10^8	1.4×10^7
Fumian <i>et al.</i> (2013)	Brazil	Infants	2.3×10^{11}	
Reymão <i>et al.</i> (2018)	Brazil	Infants	3.9×10^8	
Ferreira <i>et al.</i> (2008)	Brazil	N/A	5.3×10^8 [$5.5 \times 10^4 - 1.1 \times 10^9$]	
Pratte-Santos <i>et al.</i> (2019)	Brazil	Children	1.1×10^3	
Gonzalez <i>et al.</i> (2011)	Venezuela	Infants	9.3×10^{10}	
<i>Norovirus GI and GII</i>				
Aoki <i>et al.</i> (2010)	Japan	Elders	2.4×10^6 [$1.1 \times 10^4 - 5.8 \times 10^6$]	4.3×10^1
Henke-Gendo <i>et al.</i> (2009)	Germany	all ages	2.9×10^7	
Dabilla <i>et al.</i> (2017)	Brazil	Infants	1.5×10^8 [$4.3 \times 10^7 - 2.7 \times 10^8$]	
Marshall <i>et al.</i> (2001)	Australia	Elders	5.0×10^5	
<i>Norovirus GI and GIV</i>				
Huynen <i>et al.</i> (2013)	Burkina Faso	Children	3.2×10^6	1.9×10^5
First author	Country	Genogroup	Concentration	
Rotavirus				
Nordgren <i>et al.</i> (2010)	Sweden	-	4.3×10^9	
	Sweden	A-II	5.2×10^8	
	Nicaragua	A-I	8.3×10^8	
	Nicaragua	A-II	1.1×10^9	
Pratte-Santos <i>et al.</i> (2019)	Brazil	-	2.6×10^8	

Note: Weighted mean, minimum, and maximum (when available) excretion rates are presented to facilitate comparison among groups.

and symptomatic participants of all ages were recruited in different settings across studies, with most data coming from hospitals and medical care facilities. Most reported data corresponded to the norovirus genogroup GII, followed by GI. Four articles reported concentrations of genogroups GI and GII combined; one article reported a combination of genogroups GI and GIV.

Globally, norovirus was found to be excreted at a rate of 4.9×10^{10} GC/g stool (weighted mean), and the excretion rate ranged from 4.3×10^1 to 4.5×10^{15} GC/g stool. As shown in Figure 2(b), norovirus GI was found to be excreted at a rate (weighted mean) of 1.6×10^7 GC/g stool (ranging from 2.7×10^4 to 4.4×10^7) and norovirus GII at 5.9×10^{10} GC/g stool (ranging from 1.1×10^5 to 4.5×10^{15} GC/g stool). Given the variability in reported excretion rates, no significant differences were found between genogroups. Norovirus excretion rates were compared among participants in different continents (Figure 2(a)), and significant differences were found between excretion rates in North America and Africa ($p < 0.01$) and between North America and Asia ($p < 0.05$). Weighted mean excretion of norovirus in infants was 3.1×10^{10} GC/g stool ($2.7 \times 10^4 - 3.9 \times 10^{11}$ GC/g stool), while children excreted 1.7×10^8 ($1.1 \times 10^5 - 8.9 \times 10^8$), adults 7.7×10^{10} ($3.5 \times 10^5 - 4.5 \times 10^{15}$), and elders 1.3×10^9 ($4.3 \times 10^1 - 3.5 \times 10^9$) (Figure 2(c)). No significant differences were found among age groups ($p > 0.05$). Norovirus excretion rates among participants displaying gastroenteritis symptoms (Figure 2(d)) were observed to have a weighted mean of 6.9×10^{10} GC/gram stool, with a range spanning from 1.1×10^5 to 4.5×10^{15} GC/g stool. For asymptomatic participants, the excretion rate (weighted) averaged 1.2×10^{10} GC/g stool, ranging from 4.3×10^1 to 3.9×10^{12} GC/g stool. No significant differences were found between these two groups.

Rotavirus was found to be excreted at a weighted mean rate of 9.7×10^8 GC/g stool across studies, spanning from 1×10^6 to 3×10^{10} GC/g stool. Table 2 shows data from the two papers meeting the inclusion criteria for rotavirus excretion. Nordgren *et al.* (2010) found rotavirus A to be excreted at similar rates across two different genogroups and two locations with different settings, Sweden and Nicaragua. However, no distinctions were provided between the age of participants or symptomatology. Pratte-Santos *et al.* (2019) found excretion rates in the same order of magnitude among children showing symptoms of gastroenteritis in Brazil.

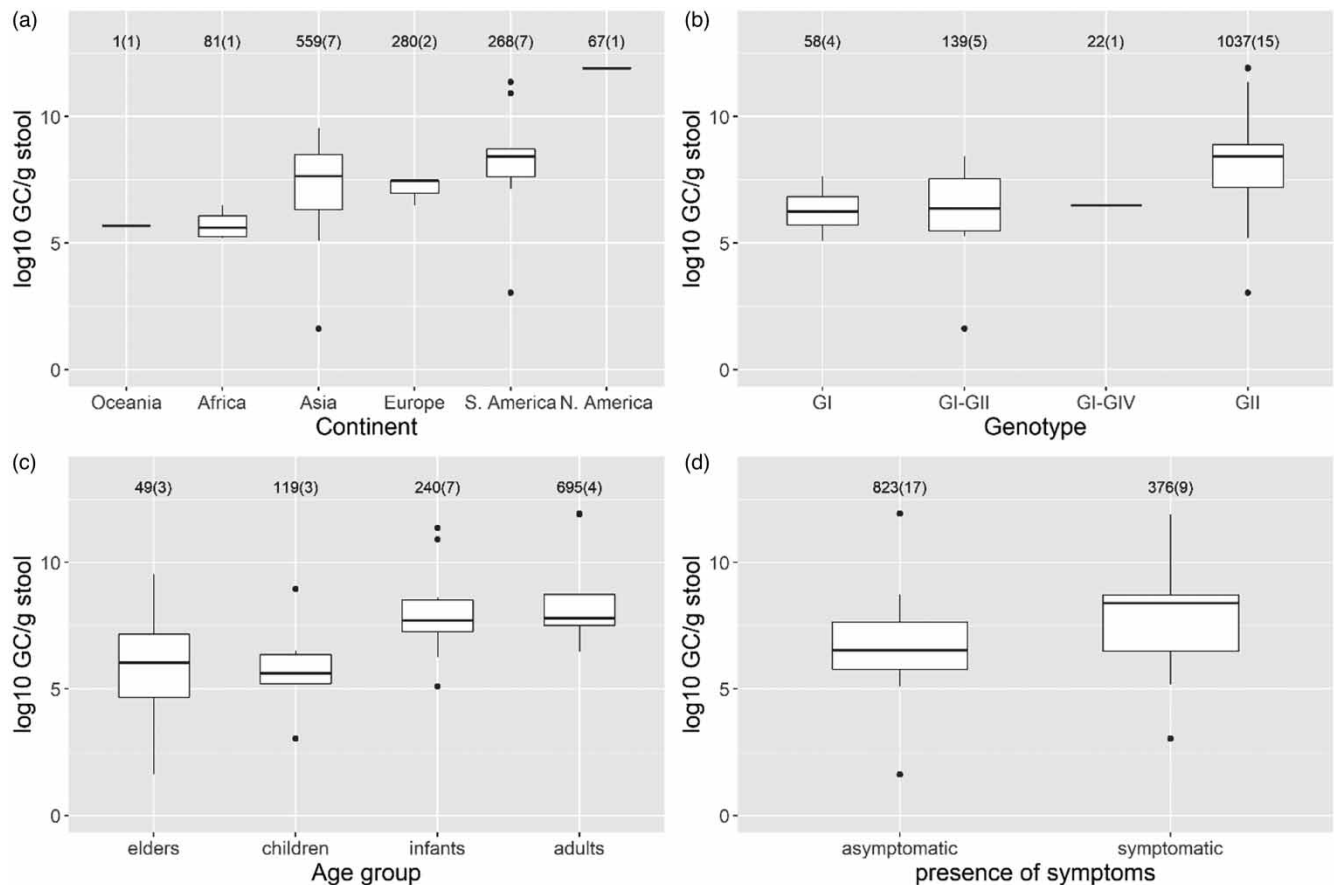


Figure 2 | Box plots (5, 25, 50, 75, and 95th percentiles) displaying weighted mean norovirus excretion rates [GC/g stool] for entries collected in Search 2, grouped by continent (a), genogroup (b), age group (c), and presence of symptoms (d). The number of samples and studies (in parentheses) included in each group is presented above the boxes.

Norovirus and rotavirus reported concentrations in sewage

Out of a total of 471 papers assessed, 72 were eligible for inclusion in this literature review, focusing on either norovirus (62 papers) or rotavirus (21 papers). Norovirus studies reported on the genogroups GI, GII, and GIV. However, a handful of studies reported norovirus concentrations in wastewater without specifying the genogroup, as detailed in Supplementary Material S1.4. Across all the studies, the weighted average norovirus concentration was 1.5×10^7 GC/100 ml (Supplementary Material S2.3). Specifically, genogroup GI exhibited a weighted average concentration of 2.9×10^7 GC/100 ml, GII presented an average of 6.4×10^6 GC/100 ml, whereas when GI and GII were reported combined, the weighted average concentration was 1×10^4 GC/100 ml. The weighted average concentration for genogroup GIV was 3.0×10^4 GC/100 ml. No statistical significance was detected among these genogroups ($p > 0.05$).

Considering weighted average norovirus concentrations by continent, the highest concentrations were demonstrated in Europe and Asia, in contrast to studies from Africa, Oceania, and the Americas (Figure 3(a)). The differences in concentrations between Asia-Africa, Europe-Africa, Oceania-Asia, and Oceania-Europe were statistically significant ($p < 0.05$).

Twenty-one papers reported rotavirus concentrations in wastewater (Supplementary Material S1.5), revealing a focus on rotavirus species A (13 papers) and C (2 papers), while in some instances, the rotavirus species remained unspecified (7 papers). The global weighted average concentration for rotavirus, encompassing all species, was 2.4×10^7 GC/100 ml. Specifically, for rotavirus A, the weighted average concentration was 2.6×10^7 GC/100 ml, whereas for rotavirus C, it was 1.2×10^6 GC/100 ml. The weighted average concentration was 6.9×10^5 GC/100 ml in cases where the species was not specified. Across all continents, rotavirus concentrations were reported in 17 countries. Upon combination of data by continent (Figure 3(b)), the highest weighted average concentrations of rotavirus in wastewater were found in Africa and Asia, while the lowest was observed in Oceania. Importantly, no statistically significant distinctions were found among continents ($P > 0.05$).

Simulation of concentrations in wastewater

Norovirus concentrations were simulated for the 25 countries, and rotavirus concentrations for the 17 countries where they were reported (Supplementary Material S1.4 and S1.5). All reported community-level prevalence values (Table 1) and 27 reported excretion rate values (Table 2, including both genogroups GI or GII, as no statistical differences between the two were reported) were used for this calculation and influenced the spread of the simulated concentrations in wastewater. Simulated nation-wide wastewater concentrations were pooled by continent (Figure 4(a)), showing a high variability (range in Africa $7.4 \times 10^{-2} - 6.9 \times 10^9$; Asia $5.6 \times 10^{-2} - 4 \times 10^{11}$; Europe $4.3 \times 10^{-2} - 9.3 \times 10^9$; North America $5.3 \times 10^{-2} - 2 \times 10^9$; South America $1.4 \times 10^{-1} - 4.2 \times 10^{10}$; and Oceania $5.3 \times 10^{-2} - 3.9 \times 10^9$ GC/100 ml). Variability among simulated

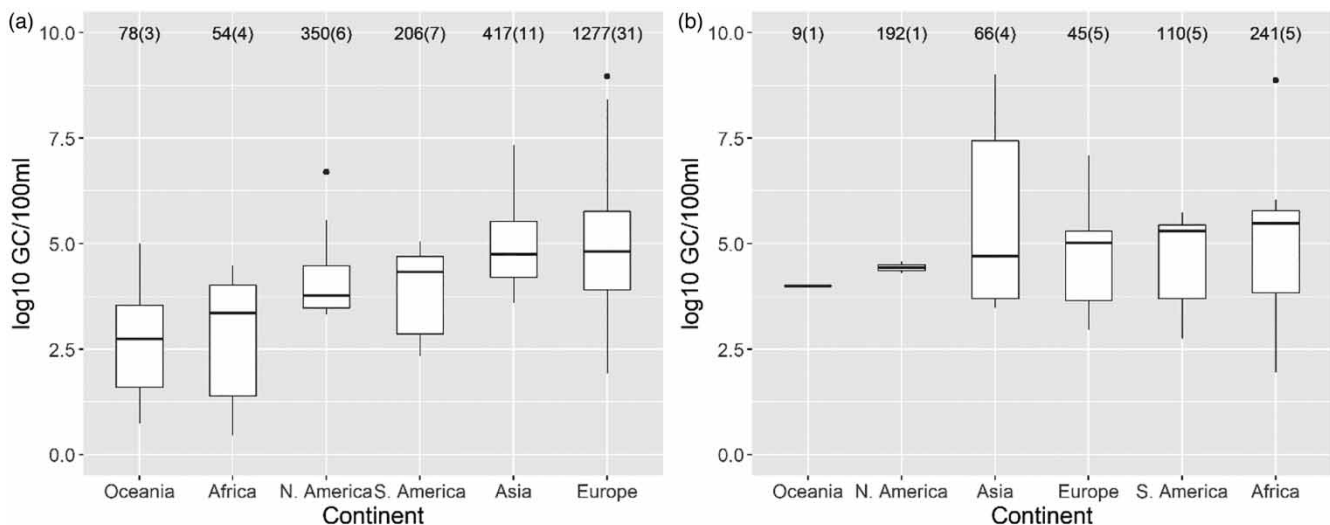


Figure 3 | Norovirus (a) and rotavirus (b) concentrations in wastewater per continent. Virus concentrations are reported in GC/100 ml of wastewater. Sample size and number of papers included in parentheses are indicated above each box.

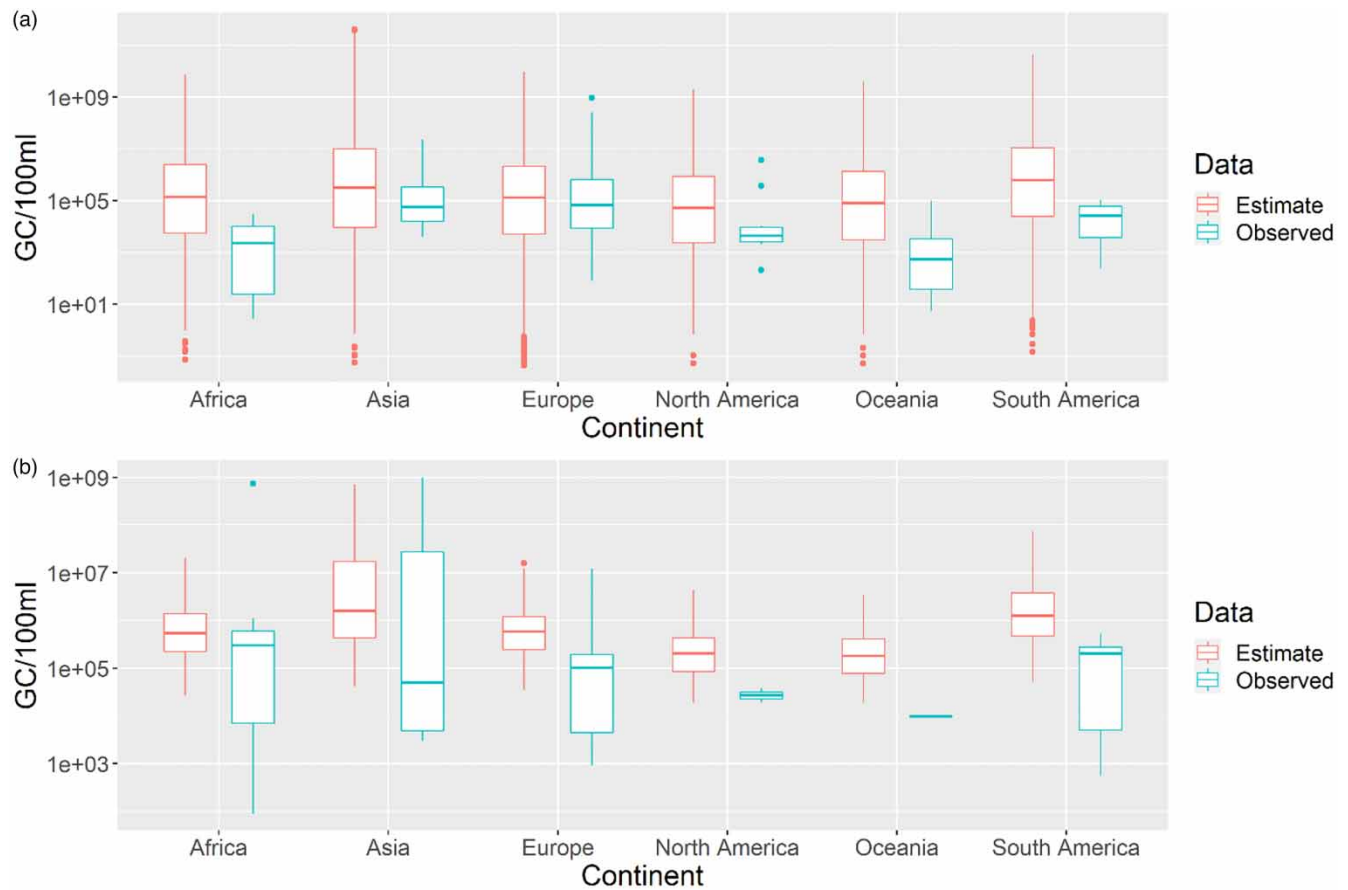


Figure 4 | Estimated wastewater concentrations (in red) of norovirus (a) and rotavirus (b). Estimates are compared with observational concentrations in wastewater (in blue) across all five continents. Concentrations reported for different genogroups were pooled for each continent. Data are presented as gene copies in 100 ml.

concentrations was larger than among observed wastewater concentrations. Median values in simulated concentrations were overall higher than the medians of the observed concentrations.

Rotavirus concentrations were simulated for wastewaters in the 17 countries presented in Supplementary Material S1.5. The five reported prevalence values and five reported excretion rates in studies mentioned in Tables 1 and 2 were used as input data. Country estimates were combined per continent, showing a wide variability as displayed in Figure 4(b). Estimates per continent ranged in Africa $2.6 \times 10^4 - 2.1 \times 10^7$; Asia $4.1 \times 10^4 - 7 \times 10^8$; Europe $3.6 \times 10^4 - 1.6 \times 10^7$; North America $1.9 \times 10^4 - 4.3 \times 10^6$; South America $5.2 \times 10^4 - 7.5 \times 10^7$; and Oceania $1.9 \times 10^4 - 3.4 \times 10^6$ GC/100 ml. The median calculated concentrations were overall higher than the median of the observed concentrations.

Sensitivity analysis

Results of the sensitivity analysis showed that increases or decreases in prevalence, excretion rate, and fraction of population connected to sewer and stool production influence the resultant concentration values proportionally. Altering the input values for collected wastewater per day showed an inverse proportional effect on resultant concentrations. Additionally, the changing population contributing to the pathogen loading due to the use of nappies in children and the effectiveness of vaccines influences the results proportionally. When minimum and maximum literature values have been used in the sensitivity analysis, it becomes clear that Equation (2) is particularly sensitive to changes in the excretion rate (minimum and maximum values result in a difference of 12 orders of magnitude), and to a much lesser extent, the wastewater collected per day (difference of 6 orders of magnitude).

DISCUSSION

This study synthesised the literature on norovirus and rotavirus infection prevalence, excretion rates, and wastewater concentrations. The findings revealed a notable lack of available data, particularly for community-level prevalence, and significant variability in wastewater concentrations and excretion rates for both viruses.

In this review, the PubMed database was exclusively utilised, omitting Web of Science, Scopus, and Google Scholar. Pilot searches across the different databases confirmed that limiting to the PubMed database did not influence the relevant papers found. Procedures followed the Cochrane Handbook for Reviews (Lefebvre *et al.* 2022) and adhered as closely as possible to PRISMA guidelines, as detailed in Supplementary Material S1.2.

To ensure comparability under standard epidemiological conditions, inclusion of studies was restricted to the end of 2019, excluding those conducted during the COVID-19 pandemic. During the COVID-19 pandemic, major disruptions in human mobility and other social behaviours such as a reduction in social interaction and an increase in hygiene practices altered the routes of disease transmission of diverse enteric pathogens. Reduction in incidence of foodborne diseases during this period was reported in the UK and US (Ray *et al.* 2021; Love *et al.* 2022). Although during the pandemic, wastewater surveillance was intensified worldwide, generating important volumes of pathogen concentration data, the mentioned social behaviour disruptions make it inadequate to compare data generated before and during the COVID-19 pandemic. Future literature reviews could include studies that measured the prevalence of infection, excretion rates, and wastewater concentrations after the end of the pandemic.

Other authors have previously collated data on norovirus and rotavirus prevalence, excretion rate, and concentrations in wastewater. However, the current study includes 47 more scientific publications than those discussed by Eftim *et al.* (2017), who employed a more stringent selection approach for norovirus in wastewater and focused on municipal sewage systems with the requirement of documenting controls and PCR inhibition. The criteria applied here span a longer time frame. However, both studies reported similar norovirus concentration ranges ($2.83 - 9.3 \times 10^8$ in the current study versus $1.05 - 1.5 \times 10^9$ in Eftim *et al.* (2017)). Data collected on norovirus excretion were consistent with Guo *et al.* (2022), although the present study centres on simulating viral environmental loading, in contrast to Guo *et al.*'s focus on reporting excretion rates and wastewater concentrations.

The primary source of variability in this literature review was the difference in study design and analytical methods among the collected studies. For example, although most studies quantified viral particles using qPCR, other techniques were also employed (MPN and cell culture in 3 out of 56 papers). Furthermore, among studies using the same type of assay, variability emerges due to the differences in methodological and analytical approaches. Gerba *et al.* (2018) identified variability in quantitative molecular methods arising from the diverse ways in which three main analytical components are handled: first, the presence of inhibitors, non-target genetic material, and varying DNA purity in samples; second, material loss during sample processing; and third, molecular detection error tied to qPCR optimisation. Additional variability stems from data analysis, including interpretation of process controls, limits of quantification, statistical analysis, and the software used.

Community-level prevalence

The five community-level prevalence reports included exhibited significant variations in their study designs, making direct comparisons of prevalence values challenging. Although there is an extensive body of literature (843 papers found) that reports on the prevalence of norovirus and rotavirus infections, these studies often focus on symptomatic individuals seeking medical care.

In the case of norovirus, Inns *et al.* (2017) and Qi *et al.* (2018) represent previous attempts to collate community-level prevalence data. However, both papers employed different participant-selection methods, which complicates the comparison of their results. Qi *et al.* (2018) estimated, using a random-effect model with data from 71 studies, that approximately 7% of participants without symptoms could be infected with norovirus. Inns *et al.* (2017) found that norovirus prevalence could range from 0.024 to 60 cases per 1,000 person-years. However, both studies suffered from selection biases. Qi *et al.* (2018) relied on studies that focused on asymptomatic participants, while Inns *et al.* (2017) was based on papers that recruited participants through medical facilities during outbreaks, introducing a healthcare-access bias, which is known to lead to underestimations of infections (Delgado-Rodríguez & Llorca 2004; Harris 2016).

As for rotavirus, extensive research into its epidemiology was conducted in the decades preceding the study's defined time limit. However, prevalence studies were limited to recent times (2010 to pre-COVID-19), because the use of improved quantitative methods became the norm, and recent studies can better represent current trends, resulting in limited data to compare

rotavirus prevalence among genogroups, countries, or time periods. More studies are needed to assess community-level rotavirus prevalence, particularly after the introduction of rotavirus vaccination in various countries over the past decades.

In this study, community-level data were deliberately selected to minimise selection biases and represent the daily prevalence of infections in a population under standard conditions. Such data is useful for water quality modelling as it approximates the conditions within a community where the prevalence is assumed to be spatially and temporally consistent, even though in reality it is fluctuating due to disturbances in disease transmission, for example, during outbreaks. The current data does not represent such variability, and more research is required to incorporate it.

The existing variability in prevalence values can be attributed to both biological and environmental factors. Within a population, individuals may have varying underlying health conditions and differing levels of susceptibility to pathogens. Environmental conditions play a crucial role, with changes in seasonality (Heymann 2005), impacting the spread of infections. Furthermore, variations in the prevalence of rotavirus have been observed among G-types and P-types serotypes, with the distribution of these serotypes varying within countries and across years (Beards *et al.* 1989).

Most studies on community-level prevalence have predominantly focused on children under 5 years of age, a group with generally high prevalence of enteric diseases and sometimes elevated morbidity rates. Consequently, there is an overrepresentation of this high-prevalence group in the prevalence data. One study did report prevalence data for both children and adults for both viruses (Heusinkveld *et al.* 2016). While the difference in prevalence between children (1.3%) and adults (1.1%) was relatively small for rotavirus, it was more pronounced for norovirus (6.5% in children and 2.6% in adults). This suggests that the prevalence of norovirus in the general population may be overestimated when using the currently presented data. Moreover, the discrepancies in reporting styles among authors due to differing study objectives, complicate comparisons among the few existing reports.

Viral excretion data

The excretion rates of norovirus exhibited a wide range, spanning from 4.3×10^1 and 4.5×10^{15} GC/gram of stool. Notably, excretion rates in North America were significantly higher than those observed in Africa and Asia. These continental disparities could be linked to the emergence of distinct norovirus GII variants. In North America, individuals shedding high viral loads of norovirus were found to be infected by emerging strains, such as GII.4 New Orleans and GII.4 Sydney (Costantini *et al.* 2016). GII.4 New Orleans was also prevalent in 90% of participants from Brazil, as reported by Fumian *et al.* (2013), who documented the highest excretion rates in South America among the studies included in this analysis. In a study conducted in Venezuela, no significant correlations were identified between high viral loads in stools and variables such as age group, gender, or symptomatology (Gonzalez *et al.* 2011). While strains were not distinguished in this study, it remains a plausible explanation for the elevated excretion rates observed. Furthermore, the data collected suggest that the excretion rates of norovirus are not statistically different between symptomatic and asymptomatic individuals, aligning with findings from other literature reviews that employed varying approaches (Milbrath *et al.* 2013).

In the case of rotavirus, limited data exist on the viral excretion rate in stool specimens. This study included two reports indicating that rotavirus was excreted in similar concentrations by participants in Brazil, Nicaragua, and Sweden. However, due to the scarcity of data, drawing definitive conclusions remains challenging.

The synthesis of excretion rate data is not without its uncertainties. One notable source of uncertainty stems from variations in the timing of excretion rate assessment relative to the moment of infection. Most of the collected data did not specify the period between infection and the quantification of viral load. Studies have reported significant changes in excretion rates as the infection progresses, with concentrations typically being higher during the initial 10 days of the infection, particularly in the case of norovirus (Aoki *et al.* 2010). Very little data are available regarding shedding just before and after the onset of illness, mainly due to the lag between disease onset and cases seeking medical attention.

Seasonal trends represent another source of uncertainty. For instance, Chan *et al.* (2006) reported that the viral excretion of norovirus GII was approximately 100-fold higher than that of GI, and the prevalence of norovirus GII increased more during winter months than that of GI. Nevertheless, variations in study designs and reporting styles limited the ability to explore seasonal differences within the collected excretion data.

Viral concentrations in wastewater

Among the papers reporting high concentrations of norovirus in wastewater, one stands out. La Rosa *et al.* (2010) reported particularly high concentrations up to 9.3×10^8 GC/100 ml for GI in Italian wastewaters. However, the reasons behind these

exceptionally high concentrations remain elusive. On the other hand, a particularly high concentration of norovirus GII in wastewater was reported in Spain by [Rusiñol *et al.* \(2015\)](#) of 2.5×10^7 GC/100 ml during the winter, aligning with the documented seasonality of norovirus by other researchers ([Nordgren *et al.* 2009](#); [Kitajima *et al.* 2012](#); [Eftim *et al.* 2017](#)). However, analysis of collected data in this study did not reveal a significant disparity between winter and spring concentrations. This discrepancy could be attributed to substantial data variability and the inclusion of data from regions with non-comparable seasonal patterns, like areas characterised by only wet and dry seasons.

It is important to consider sources of variability among sewer systems when interpreting the concentration of pathogens in wastewater. For example, in sewer networks that collect more than domestic wastewater, the input of water from other sources directly impacts the pathogen concentrations due to dilution. Furthermore, network characteristics influence viral decay, sedimentation, and resuspension during transport, influencing the quantity of microorganisms reaching the WWTP influent.

Simulation of concentrations in wastewater using community-level prevalence and excretion rates

Several assumptions were made in the simulation of wastewater concentrations. First, uniform daily stool production was assumed worldwide, irrespective of regional or health variations. Second, no differentiation was made between symptomatic and asymptomatic infection prevalence because both groups exhibit similar viral excretion rates, as shown by the comparisons made here and supported by previous research ([Ozawa *et al.* 2007](#)). Third, decay rates or losses during transport were assumed to be negligible, a justifiable assumption as per [Guo *et al.* \(2022\)](#). Additionally, the current approach does not account for various factors that could influence observed viral concentrations in wastewater, such as unidentified outbreaks, sanitation system disruptions, methodological errors in sampling and quantification, among others.

Simulated wastewater concentrations displayed significant variability, primarily driven by the widely varying excretion rates used as input. Previous studies on population and flow-normalised virus loads in wastewater demonstrated less variability ([Wilder *et al.* 2021](#); [Langeveld *et al.* 2023](#); [Rainey *et al.* 2023](#)). A sensitivity analysis was conducted to gauge the impact of variability in input parameters in Equation (2). Despite the linearity of the equation, alteration in input variables did not always translate to directly proportional changes in simulated wastewater concentrations. For instance, stool amount may only moderately differ (e.g., double or halve), while the excretion rate can vary significantly over more than 10 log units ([Figure 2](#)). High shedders, especially, play a crucial role in wastewater concentrations. Notably, a thousand infected individuals excreting at 1×10^4 GC/g are required to match the impact of one person excreting at 1×10^7 GC/g. Therefore, it is imperative to employ excretion rates that align with the central tendency or arithmetic mean. [Langeveld *et al.* \(2023\)](#) demonstrated that in populations exceeding 6,000 people, the flow and population-normalised virus (CrAssphage) load in wastewater exhibited minimal variability, despite considerable differences in shedding rates. This indicates that the observed virus load at the inlet of WWTP reasonably represents the central tendency of excretion. However, gaining a deeper understanding of the proportion of the population shedding high values is crucial to improving water quality models.

CONCLUSIONS

This work synthesises the published work over the last decade, up to the beginning of the COVID-19 pandemic, on community-level prevalence of norovirus and rotavirus, and of the last two decades, up to the beginning of the COVID-19 pandemic, on their excretion rate and concentrations in wastewater. The potential of using these types of data in modelling loads of enteric pathogens to wastewater was explored, and the following conclusions were drawn.

Community-level data on norovirus and rotavirus prevalence of infection are extremely scarce. There is a need for monitoring studies where epidemiological estimates represent entire communities and not only population subsets. Only two studies reporting rotavirus excretion rates were found while norovirus data were more abundant and widely variable. Comparisons in norovirus excretion rates showed no statistically significant difference between genogroups GI and GII, across study regions, between symptomatic and asymptomatic individuals, or between age groups. On the other hand, articles reporting norovirus or rotavirus concentrations in wastewater were abundant. Norovirus was reported to occur in a wide range of concentrations across continents, while rotavirus concentrations presented a narrower variability.

Viral concentrations in wastewater for both pathogens were modelled through the input of prevalence of infection and excretion rate values. Consistent with empirical data, estimated concentrations in wastewater were found to be widely variable. Norovirus concentrations ranged between 13 (estimated) and 8 (observed) orders of magnitude, while rotavirus ranged across eight orders of magnitude in both estimated and observed data. The modelled concentration values reflect variability in

the input data arising from inherent biological variability, as well as differences in sampling and analytical methods. Extensive monitoring efforts need to take place in the future to close the knowledge gap, especially on the community-level prevalence of infection. Consistent study designs and reporting are needed to reduce data uncertainties and strengthen water quality modelling that contributes to the understanding of current and future pathogen concentrations in wastewater and communities worldwide.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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