







Virus detection in influent, activated sludge, and effluent from municipal wastewater treatment plants using composite and grab samples in Finland

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ABSTRACT

Wastewater-based surveillance has been commonly used as a monitoring tool for public health. Also, viruses present in wastewater can pose a health risk. In this study, we screened enterovirus, rhinovirus, norovirus GI and GII, pan-adenovirus, and gastroenteritis-causing adenovirus F40/41 in different wastewater sample types using reverse transcription quantitative polymerase chain reaction and quantitative polymerase chain reaction. We analyzed composite influent samples (N = 22), grab influent samples (N = 20), composite effluent samples (N = 78), grab effluent samples (N = 21), and activated sludge samples (N = 34) collected from six municipal wastewater treatment plants in the Pirkanmaa region of Finland. We detected the viruses in the influent to discover if they had the potential to be monitored using wastewater-based surveillance. In addition, we studied viruses in effluent and activated sludge to detect viruses that persisted in treatment processes. Furthermore, all sample types were compared to discover differences in the viral contents of different wastewater sample types. We detected all the studied viruses in influent, while in activated sludge, we detected enterovirus, pan-adenovirus, and adenovirus F40/41, and in effluent enterovirus, norovirus GI and GII, pan-adenovirus, and adenovirus F40/41 were identified. In addition, the relative amount of all the viruses was the highest in the influent. Our study also showed that composite sampling was a more representative and sensitive method for virus monitoring in wastewater than grab sampling, as the relative amount of the viruses present in composite samples was higher than in grab samples. Since we found abundant viruses in effluent, further studies are required to assess their infectivity and potential health risks as environmental pollutants.

1. Introduction

Wastewater surveillance has become an important public health surveillance tool used to monitor the COVID-19 pandemic globally. Both the European Commission and the United States Centers for Disease Control and Prevention have recommended that SARS-CoV-2 be systematically surveilled in wastewater (European Parliament, 2024; Keshaviah et al., 2021). In addition, the influenza A virus (IAV) and respiratory syncytial virus (RSV) have also been extensively studied in wastewater (Ando et al., 2023; Boehm et al., 2023; Hughes et al., 2022; Länsivaara et al., 2024a; Lehto et al., 2024; Wolfe et al., 2022) and have been added to the national wastewater surveillance system in the United States and Finland (Finnish Institute for Health and Welfare, 2024; United States Centers for Disease Control and Prevention, 2024).

Wastewater surveillance is an excellent supplementary tool for clinical surveillance, as it can detect subclinical cases, including people with mild symptoms, asymptomatic cases, and people who do not have access to or are not willing to access healthcare. It is also cost-effective, as the spread of a virus in a wastewater treatment plant (WWTP) catchment area can be assessed using composite samples. Wastewater surveillance can also be used as an early warning tool, as the spread of a virus can be monitored in real time and deviations can be detected and reported rapidly, allowing for timely intervention (Mao et al., 2020). For example, for norovirus, it was reported that the peak of the amount of the virus in wastewater occurred two weeks before the peak in clinical cases (Hellmér et al., 2014). Furthermore, for influenza, wastewater surveillance was reported to have a lead time of 17 days before a community outbreak (Mercier et al., 2022). In some instances, wastewater

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surveillance has shown the spread of a virus to be larger than expected, based on clinical cases (Wu et al., 2020).

Even though wastewater treatment removes a substantial amount of the viral load, some viruses may persist in effluent and thus become environmental pollutants (Okoh et al., 2010) upon discharge into bodies of water. Viruses, such as norovirus GI and GII and adenovirus, have been found in WWTP effluent (Corpuz et al., 2020). As many enteric viruses can cause waterborne epidemics, effluent can therefore pose a health risk upon release into bodies of water. Indicator organisms, such as enterococci and coliform bacteria, are typically monitored in effluent to indicate when pathogens are likely to be present (Fong and Lipp, 2005; Okoh et al., 2010). However, bacterial indicators have been shown to be inadequate when assessing the risk of viruses present in water, and viruses have been found in waters deemed safe based on bacterial indicators (Ehlers et al., 2005; Fong and Lipp, 2005). Thus, there is a need to study the viruses present in effluent.

Activated sludge derived from the activated sludge process used in secondary treatment at WWTPs may include viruses (Jebri et al., 2012). However, this wastewater sample type is much less studied than influent or effluent. Previous studies have mostly used flow cytometric quantification (Brown et al., 2015) or cell culture (Arraj et al., 2005) to detect viruses in activated sludge instead of reverse transcription quantitative polymerase chain reaction (RT-qPCR) or quantitative polymerase chain reaction (qPCR), which are often used when studying influent or effluent, as some viruses are challenging to culture. Since viruses might affect the microbial dynamics of the activated sludge process, it is important to study the viruses present in activated sludge (Brown et al., 2015).

In this study, the objective was to compare viruses present influent, activated sludge, and effluent to see how the amount of viruses changes along the wastewater treatment process, and to compare grab and composite sampling of wastewater. We studied several human pathogenic viruses, including enterovirus, rhinovirus, norovirus, and adenovirus. We included respiratory and enteric viruses, as well as RNA and DNA viruses, to test wastewater surveillance using influent for the monitoring of a wide array of different types of viruses and to make the results generalizable. In addition to studying pan-adenovirus, adenovirus F40/41 was included, as those genotypes are enteric and have been previously found to be the most frequently detected adenovirus genotypes in wastewater (Allayeh et al., 2022). Furthermore, we chose naked viruses because most of the studies on wastewater surveillance have been conducted on enveloped viruses, such as SARS-CoV-2, influenza A, and RSV. Furthermore, effluent was studied to see if it contained viruses as potential environmental contaminants. Activated sludge was included as a sample type, as the viruses in it have rarely been studied using qPCR and RT-qPCR (Jebri et al., 2012; Prado et al., 2014). In addition, the efficacy and representativeness of the grab and composite sampling methods were evaluated. Also, the effect of PCR inhibition on the detection of viruses in wastewater was studied.

This study showed that enterovirus, rhinovirus, norovirus, and adenovirus can be detected in Finnish influent, showing a possibility for these viruses to be monitored using wastewater surveillance in Finland. Furthermore, composite sampling was shown to be a more representative sampling method than grab sampling and should be preferred in wastewater surveillance. In addition, effluent was shown to include all the studied enteric viruses, in line with previous studies. Thus, effluent may impose a risk as an environmental pollutant. Lastly, the levels of PCR inhibition were shown to be high in wastewater, especially influent, showing a need for the evaluation of the levels of inhibition in wastewater studies, and the possible benefits of using inhibition removal methodologies.

2. Material and methods

2.1. Sampling

We took samples from six WWTPs in the Pirkanmaa region in Finland (WWTPs A, B, C, D, E, and F). These WWTPs serve 230,000 residents and apply conventional wastewater treatment processes (primary and secondary treatment). The population served by each WWTP and the flow (m^3/day) are reported in the supplementary file in [appendix B](#). The samples for the study were collected between 2016 and 2018. Samples were collected from different steps of the wastewater treatment process ($N = 175$) and included influent ($N = 42$) (WWTPs A and E), activated sludge from the activated sludge process ($N = 34$) (WWTPs A, E, and F), and effluent ($N = 99$) (all WWTPs). Influent and effluent samples were taken simultaneously as grab samples and as 24-h composite samples to compare the sampling methods. Activated sludge was collected as grab samples. In grab sampling, a single sample is taken at one point in time, whereas in composite sampling, a mixture of multiple grab samples are taken over 24 h in 1-h time intervals. A full sample list with collection dates is presented in [Appendix A](#). (See [Fig. 1](#) for the analysis pipeline)

The samples were taken directly into Falcon tubes at the collection sites. The 24-h composite samples were taken with automatic samplers. A sample volume of 50 ml was immediately frozen at $-20\text{ }^\circ\text{C}$ at the collection sites for later analysis and transferred to the analysis laboratory while frozen and stored at $-80\text{ }^\circ\text{C}$ before analysis.

2.2. Concentration of the samples

Before the samples were concentrated, they were melted in a $+4\text{ }^\circ\text{C}$ refrigerator. The samples were concentrated at $+4\text{ }^\circ\text{C}$ immediately after melting using Millipore Centricon Plus-70 3kDA cutoff filters (Millipore, Cork, Ireland). Each 50 ml sample was loaded into the filter and centrifuged at 3500 g for 60 min. Next, the concentrate was recovered in a collection cup attached to the filter and centrifuged at 1000 g for 2 min. The concentrate, including both the solid and liquid parts, was collected from the collection cup into a 1.5 ml tube. If the concentrate volume was less than 700 μl , the volume was adjusted to 700 μl with RNase-free molecular-grade water (Sigma-Aldrich, United States).

2.3. Nucleic acid extraction

Nucleic acids were extracted from the samples immediately after concentration. The nucleic acid extraction was performed using a QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The sample volume was 140 μl , and the elution volume was 60 μl . A positive cell culture control, including adenovirus, enterovirus, and rhinovirus, and a negative control were included in each nucleic acid extraction. The nucleic acids were frozen in a $-80\text{ }^\circ\text{C}$ freezer for later analysis.

2.4. Detection of viruses using RT-qPCR and qPCR

The viruses studied included enterovirus, rhinovirus, norovirus GI and GII, pan-adenovirus, and adenovirus F40/41. RT-qPCR was performed for enterovirus, rhinovirus, and norovirus GI and GII using the one-step QuantiTect Probe RT-PCR assay (Qiagen, Hilden, Germany) and qPCR was performed for pan-adenovirus and adenovirus F40/41 using the one-step Qiagen QuantiTect Probe PCR assay. The reaction volume of the RT-qPCR and qPCR reactions was 10 μl , and the template volume was 2 μl . The equivalent sample volume representing the amount of original sample that was included in every qPCR reaction was 0,3 ml. The sequences and concentrations of the primers and probes (Macrogen, Amsterdam, the Netherlands) used are shown in [Table 1](#). The specificity and sensitivity of the primers and probes were validated with Quality Control for Molecular Diagnostics panels. The RT-qPCR reaction was run using Applied Biosystems™ 7500 Real-Time PCR



Fig. 1. Analysis pipeline for the study. Created in Biorender.

Table 1

The names of the primers and probes of the viral targets, their oligonucleotide sequences, and the concentrations used in the PCR assays.

Name	Oligonucleotide Sequence (5'-3')	Concentration (nM)	Reference
Enterovirus forward primer	CGGCCCTGAATGCGGCTAA	900	Honkanen et al., 2013a
Enterovirus reverse primer	GAAACACGGACACCCAAAGTA	900	
Enterovirus probe 1	TCTGTGGCGGAACCGACTA	300	
Enterovirus probe 2	TCTGCAGCGGAACCGACTA	300	
Rhinovirus forward primer	CYAGCCTGCGTGCC	900	Honkanen et al. (2013b)
Rhinovirus reverse primer	GAAACACGGACACCCAAAGTA	900	
Rhinovirus probe	TCCTCCGGCCCTGAATGYGGC	300	
Norovirus GI forward primer	CGYTGATGCGNTTYCATGA	900	Krogvold et al. (2014)
Norovirus GI reverse primer	CTTAGACGCCATCATCATTYAC	900	
Norovirus GI probe	AGATYGGGATCYCCTGTCCA	250	
Norovirus GII forward primer	CARGARBCNATGTTYAGRTGGATGAG	900	Krogvold et al. (2014)
Norovirus GII reverse primer	TCGACGCCATCTTCATTACACA	300	
Norovirus GII probe	TGGGAGGGCGATCGCAATCT	300	
Adenovirus forward primer	CCGGCCGAGAAGGGCGTGCCGAGGTA	900	Modified from Cinek et al., (2006)
Adenovirus reverse primer	CATGACTTTTGAGGTGGAYC	300	
Adenovirus probe	ATGGATGAGCCCACCCT	200	
Adenovirus F40/41 forward primer	CTGACATGACTTTTGAGGT	900	Inhouse oligos
Adenovirus F40/41 reverse primer	CCGGCCGAGAAGGGCGTGCCG	900	
Adenovirus F40/41 probe	ATGGATGAGCCCACACTTC	250	

instrument with the program—that is, for 30 min at 50 °C for reverse transcription; 15 min at 95 °C of RT-inactivation and initial denaturation; 2-step cycling, including 15 s denaturation at 94 °C and 1 min annealing; and extension with fluorescence data collection at 60°C—and the qPCR reaction was run in the same way but without reverse transcription. The number of cycles was 50. To minimize potential inter-assay variation, different sample types were combined in the same runs. qPCR negative control and nucleic acid extraction negative and positive control were included in each RT-qPCR and qPCR run. All samples were run as triplicate reactions, and those with Ct-values below 40 were considered positive. The viruses were detected using relative quantification, and the resulting relative amounts of the viruses were compared using Ct-values.

2.5. PCR inhibition testing

Possible PCR inhibition present in the samples was evaluated using the semliki forest virus (SFV), which is not present in Finnish wastewater. Each extracted nucleic acid sample was spiked with 0.5 µl of SFV. Molecular grade H₂O was used as an inhibition-free control, which was also spiked with 0.5 µl of SFV. To evaluate how the inhibition affected the PCR reactions in each sample type, the Ct-values of SFV in the

samples were compared to the Ct-value of an inhibition-free control. The potential inhibition rate of each sample was calculated by subtracting the Ct value of the SFV in the sample from the Ct value of the SFV in the control (i.e., inhibition present in the sample = Ct-value of SFV in the control – Ct-value of SFV in the sample) (Länsivaara et al., 2024a). To evaluate the effect of inhibition, the Ct-values of the samples were corrected for the inhibition present in the sample. Corrected Ct-value of a sample = Ct-value of the sample + (Ct-value of SFV in the control – Ct-value of SFV in the sample).

2.6. Statistics

All statistical analyses were performed on IBM® SPSS® software version 28.0.1.0 (142). For the differences in the relative amounts of the viruses, statistical significance was tested using a Mann–Whitney *U* test when two groups were compared and a Kruskal–Wallis *H*-test when more than two groups were compared. The null hypothesis was that there is no difference between the relative amounts of the viruses. The statistical significance of the differences between the positivity rates in different sample types and viruses was tested using a Z-test and the null hypothesis that there are no differences between the positivity rates in different sample types and viruses.

3. Results

3.1. Detected viruses

Almost all of the samples (94.8 %) were positive for at least one of the viruses. Adenovirus was the most common virus in wastewater, with adenovirus F40/41 detected in 84.6 % and pan-adenovirus in 69.7 % of the samples (Table 2). Enterovirus was the second most detected virus, detected in 45.1 % of the samples. Norovirus was the third most detected virus, with the norovirus GI genogroup detected in 25.7 % and norovirus GII detected in 14.9 % of the samples. Rhinovirus was the least detected virus, detected in only 2.9 % of the samples.

3.2. Influent

All influent samples (N = 42) were positive for at least one of the viruses (Table 2). All composite influent–wastewater samples and 85 % of the grab influent samples were positive for F40/41 adenovirus, making it the most detected target in influent (Table 2). Adenoviruses detected by pan-adenovirus assay were almost as common, detected in 86.4 % of the composite influent–wastewater samples and 85 % of the grab influent samples. Enterovirus was also frequently detected, being found in 81.8 % of the composite influent samples and 80 % of the grab influent samples. The norovirus GI genogroup was detected more often than the GII genogroup, with the norovirus GI genogroup found in 50 % of the composite influent samples and 30 % of the grab influent samples and the GII genogroup found in 27.3 % of the composite influent samples and 20 % of the grab influent samples. Rhinovirus was found only in 13.6 % of the grab influent samples and 10 % of the composite influent samples.

A comparison of the relative amounts of the viruses present in various sample types showed that the highest median relative amount of a virus was the norovirus GI genotype in both composite and grab influent (Fig. 2). The lowest median relative amount of a virus was the norovirus GII genotype in composite influent and rhinovirus in grab influent. The differences in the relative amounts of the viruses in influent were statistically significant ($p < 0.001$). Furthermore, the relative amount of the viruses was higher in influent than in activated sludge ($p < 0.001$) or effluent ($p < 0.001$).

A comparison of the composite and grab influent sampling methods showed that the positivity rate of each viral target was higher in the composite samples than the grab samples, but the differences were not statistically significant (Table 2). The relative viral load of the composite samples was higher than the relative viral load of the grab samples ($p =$

0.004).

PCR inhibition was evaluated from the samples spiking the same amount of SFV in the samples and in the noninhibition control (molecular biology grade water). The Ct-values of the samples were compared to the Ct-value of the noninhibition control, and the inhibition is presented as the difference between the Ct-cycles. The average inhibition was -1.9 cycles (standard deviation [SD] = 1.5) for the composite influent and -1.3 cycles (SD = 1.3) for the grab influent.

3.3. Activated sludge samples

All activated sludge samples (N = 34) were positive for at least one of the viruses. Adenovirus F40/41 genotypes were detected in all activated sludge samples. The pan-adenovirus was also a commonly detected target, as it was detected in 82.4 % of the samples. Enterovirus was detected in 26.5 % of the samples. Neither rhinovirus nor noroviruses were detected in any activated sludge samples. The highest relative amount of a virus in activated sludge was adenovirus F40/41, while the lowest relative amount of a virus was pan-adenovirus. The differences in the relative amounts of the viruses in activated sludge were statistically significant ($p < 0.001$). The average PCR inhibition was -1.1 cycles (SD = 1.3) for activated sludge.

3.4. Comparison of influent and activated sludge

To assess the prevalence and relative loads of the viruses in the wastewater treatment process, the composite influent and activated sludge samples taken on the same day and from the same WWTP (N = 20 pairs) were compared (Fig. 3). Rhinovirus and norovirus GI and GII genogroups were detected only in the influent samples. Furthermore, the relative amounts of enterovirus, pan-adenovirus, and adenovirus F40/41 were higher in influent compared to activated sludge ($p < 0.001$). When the Ct-values were corrected to account for the PCR inhibition, the results remained the same.

When the difference in inhibition between influent and activated sludge was further evaluated using the influent–activated sludge pairs, average PCR inhibition was shown to be stronger in influent than in activated sludge ($p < 0.001$, Fig. 4).

3.5. Effluent

A total of 84.6 % of the composite effluent samples and 80.9 % of the grab effluent samples were positive for at least one viral target (Table 2). The F40/41 adenovirus genotypes were the most frequently detected

Table 2

The viral targets and their frequency of detection in each sample type. The number of positive samples is given, and the percentage of positive samples among all samples analyzed is presented in parentheses (%). The statistical significance of the differences in the detection frequencies of the targets between different sample types is presented as p-values.

Virus	Positive samples (N = 175)	Positive grab influent (N = 20)	Positive composite influent (N = 22)	Positive grab effluent (N = 21)	Positive composite effluent (N = 78)	Positive activated sludge (N = 34)	Influent compared to effluent (p-value)	Influent compared to activated sludge (p-value)	Influent grab compared to composite (p-value)	Effluent grab compared to composite (p-value)
Positive for any of the viruses	166 (94.9 %)	20 (100 %)	22 (100 %)	17 (80.9 %)	66 (84.6 %)	34 (100 %)	0.05	–	–	0.689
Enterovirus	79 (45.1 %)	16 (80 %)	18 (81.8 %)	9 (42.9 %)	27 (34.6 %)	9 (26.5 %)	<0.001	<0.001	0.881	0.484
Rhinovirus	5 (2.9 %)	3 (13.6 %)	2 (10 %)	0 (0 %)	0 (0 %)	0 (0 %)	<0.001	0.027	0.719	–
Norovirus GI	45 (25.7 %)	6 (30 %)	11 (50 %)	12 (57.1 %)	16 (20.5 %)	0 (0 %)	0.006	<0.001	0.189	<0.001
Norovirus GII	26 (14.9 %)	4 (20 %)	6 (27.3 %)	5 (23.8 %)	11 (14.1 %)	0 (0 %)	0.147	0.001	0.582	0.285
Pan-adenovirus	122 (69.7 %)	17 (85 %)	19 (86.4 %)	12 (57.1 %)	46 (59 %)	28 (82.4 %)	0.017	0.689	0.897	0.881
Adenovirus F40/41	148 (84.6 %)	17 (85 %)	22 (100 %)	16 (76.2 %)	58 (74.4 %)	34 (100 %)	0.007	–	0.059	0.865

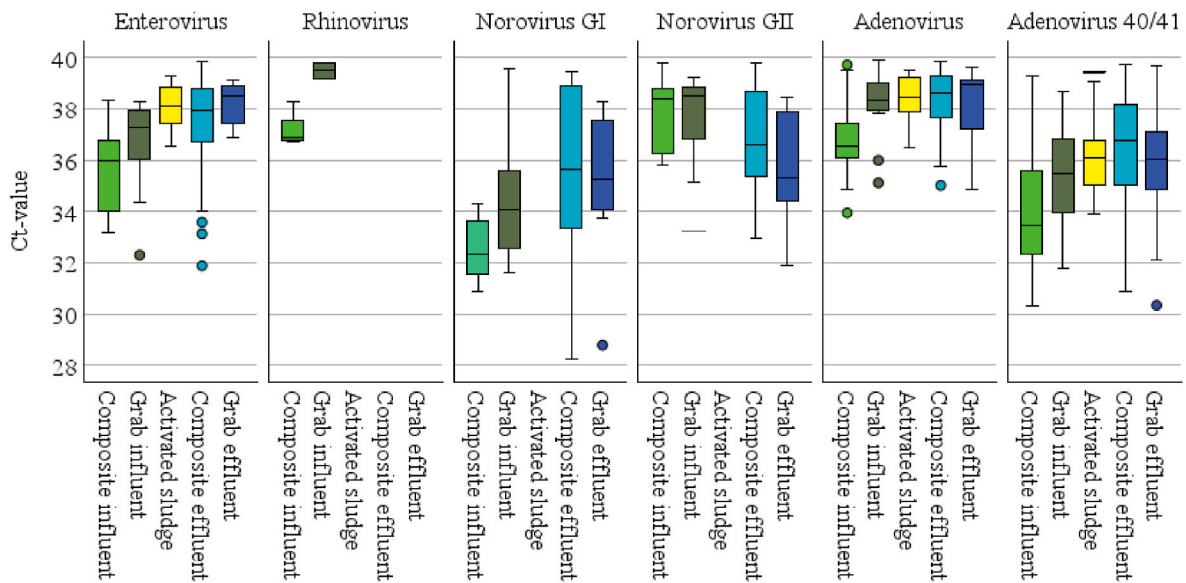


Fig. 2. Box plot of the relative amounts of viruses in all positive samples at different wastewater sampling points in the wastewater treatment train and sample collection types. The rhinovirus results included only two (composite influent) and three (grab influent) positive samples.

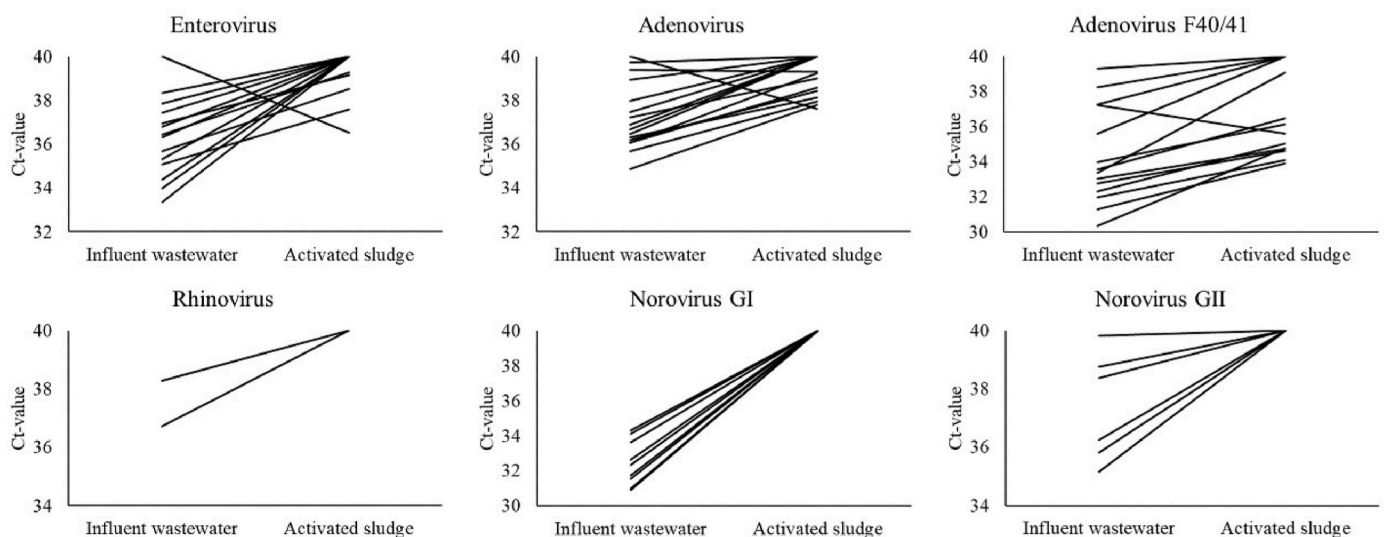


Fig. 3. Differences in the relative amounts of the viruses measured as Ct-values in influent and activated sludge. Lines represent influent and activated sludge sample pairs matched with respect to the time and location of sampling. The virus-negative samples were blotted with a Ct-value of 40 cycles. Pairs in which both samples were negative are not included.

virus in the effluent samples. Of the composite effluent and the grab effluent samples, 74.4 % and 76.2 %, respectively, were positive for adenovirus F40/41. Adenoviruses detected by pan-adenovirus assay were the second most common virus, detected in 59 % of the composite samples and 57.1 % of the grab samples. Enterovirus was detected in 34.6 % of the composite samples and 42.9 % of the grab samples. Norovirus GI genogroup was detected more often than the GII genogroup. Norovirus GI genogroup was found in 20.5 % of the composite influent samples and 57.1 % of the grab influent samples. The GII genogroup was found in 14.1 % of the composite influent samples and 23.8 % of the grab influent samples. Rhinovirus was not detected in the effluent samples.

Norovirus GI had the highest relative amount of all viruses in both composite and grab effluent samples, while pan-adenovirus had the lowest amount. The observed differences between the relative amounts of the viruses in effluent were statistically significant ($p < 0.001$).

A higher number of samples were positive among the grab effluent samples than the composite effluent samples. However, the difference was not statistically significant ($p = 0.689$), nor was there a statistically significant difference in the relative loads of the viruses between these two sampling methods ($p = 0.187$).

When PCR inhibition was evaluated for the samples, the average inhibition was found to be -0.6 cycles (SD = 1.0) for the composite effluent and -0.7 cycles (SD = 1.3) for the grab effluent.

3.6. Comparison of influent and effluent samples

To assess the prevalence and relative loads of the viruses in the wastewater treatment process, the composite influent and effluent samples taken on the same day and from the same WWTP ($N = 20$ pairs) were compared (Fig. 5). The relative amount of the viruses was higher in influent than in effluent ($p < 0.001$). The relative amounts of enterovirus

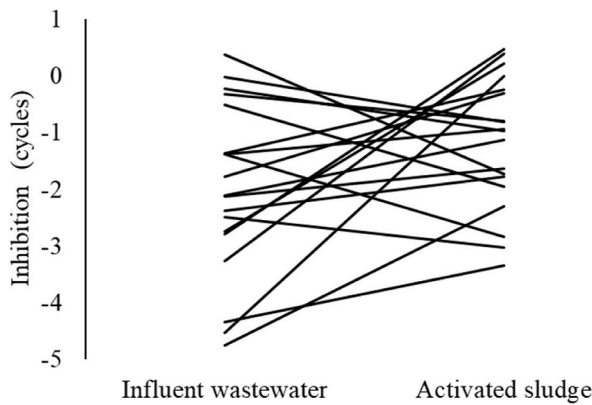


Fig. 4. Differences in the amount of inhibition between influent and activated sludge samples matched with respect to the time and location of sampling depicted with Ct-cycles. The lower the value, the higher the inhibition. Negative value means that the amount of the virus is lower due to inhibition, whereas a value ≥ 0 means that the sample was not affected by inhibition.

($p = 0.001$), pan-adenovirus ($p < 0.001$), and adenovirus F40/41 ($p = 0.002$) were higher in influent than in effluent. Rhinovirus was detected in only two composite influent samples. There was no statistically significant difference in the relative amount of norovirus GI genotype ($p = 0.422$) or norovirus GII genotype ($p = 0.470$) in influent compared to effluent. However, when the Ct-values were corrected to account for the PCR inhibition, the relative amount of norovirus GI genotype was lower in effluent compared to influent ($p = 0.008$). Otherwise, the results remained the same with PCR inhibition correction.

In addition, the extent of PCR inhibition was compared pairwise for each time point of influent and effluent (Fig. 6). The inhibition was stronger in the influent than in the effluent ($p < 0.001$).

4. Discussion

4.1. Influent

Influent from WWTPs has been used as a sample material in many wastewater virus surveillance studies (Ahmed et al., 2020; Ando et al., 2023; Hokajärvi et al., 2021; Lämsivaara et al., 2024a, 2024b; Lehto et al., 2024; Li et al., 2021; Ni et al., 2021). Still, most of these studies

have been conducted on SARS-CoV-2, influenza A, and respiratory syncytial viruses, which are all enveloped, RNA, and respiratory viruses. In this study, we included naked viruses (both RNA and DNA viruses and enteric as well as respiratory viruses) to study whether these viruses can be detected in wastewater and hence potentially be monitored using wastewater surveillance. The environmental stability of naked and enveloped viruses on surfaces has been shown to differ significantly, suggesting that their persistence in wastewater may also vary accordingly. (Firquet et al., 2015).

We detected all the targets (enterovirus, rhinovirus, norovirus GI and GII, pan-adenovirus, and adenovirus F40/41) in influent. Enteric viruses were significantly more common than rhinovirus, which is a respiratory virus. All influent samples were positive for at least one of the tested viruses. Adenovirus was the most frequently detected target in influent as well as in all other sample types. Adenovirus is a DNA virus known to persist for a long time in nature and to be resistant to many different types of stressors (Okoh et al., 2010). Adenoviruses typically cause either respiratory or enteric infections, and the likely reason that F40/41 genotypes were detected more frequently in wastewater than pan-adenovirus is that F40/41 genotypes cause enteric infections (do Nascimento et al., 2022). Furthermore, in a study performed in Egypt, adenoviruses F40/41 were shown to be the most prevalent adenovirus

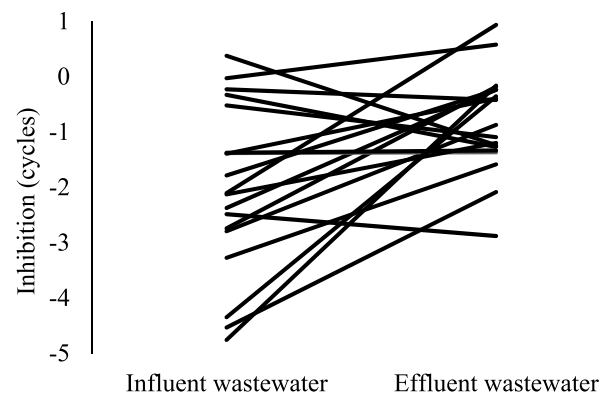


Fig. 6. Difference in the amount of inhibition between influent and effluent in samples matched with respect to the time and location of sampling depicted with Ct-cycles. The lower the value, the higher the inhibition. Negative value means that the amount of the virus is lower due to inhibition, whereas a value ≥ 0 means that the sample was not affected by inhibition.

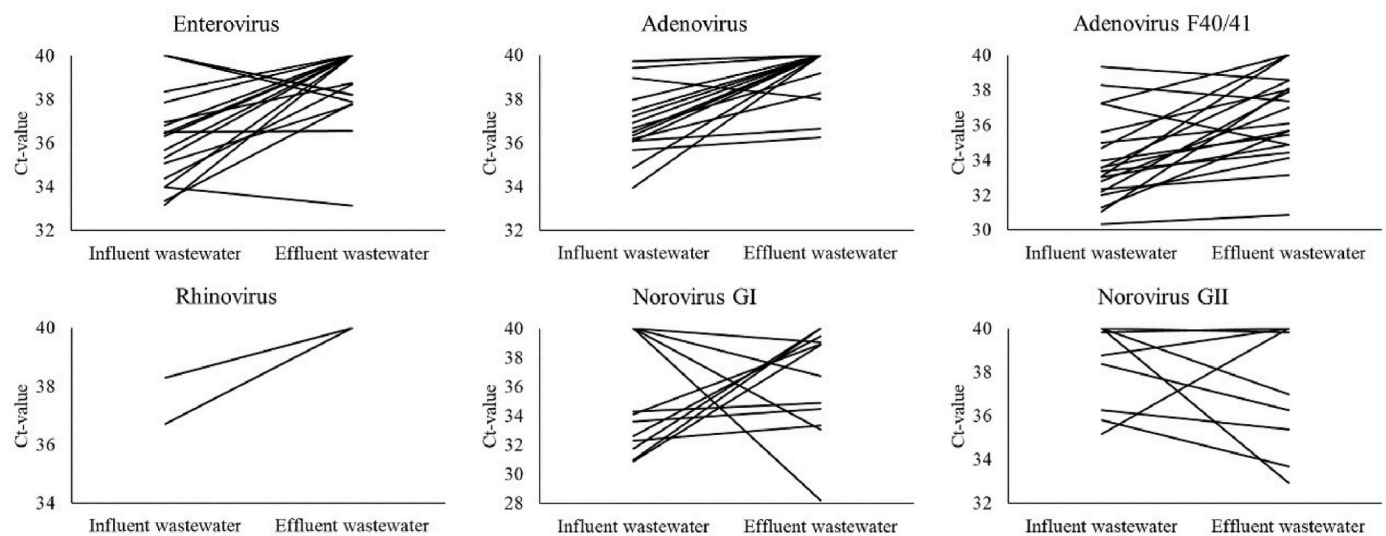


Fig. 5. Differences in the relative amounts of the viruses in the influent and effluent. Lines represent influent and effluent sample pairs matched with respect to the time and location of sampling. The virus-negative samples were blotted with a Ct-value of 40 cycles. Pairs in which both samples were negative are not included.

genotypes in wastewater (Allayeh et al., 2022). Similar to our study, which found pan-adenovirus in 86 % of the samples, high positivity rates have been observed for adenovirus in multiple studies. For example, a 100 % positivity rate was noted for adenovirus in the US (Fong et al., 2010) and Japan (Katayama et al., 2008), an 84 % positivity rate was noted in Egypt (Elmahdy et al., 2019), and a 75 % positivity rate was noted in Saudi Arabia (Maniah et al., 2023). For adenovirus F40/41, a positivity rate of 79 % was reported in Northern Ireland (Reyne et al., 2023), while in this study, all the composite influent samples were positive for the F40/41 genotype. Boehm et al. (2023) reported rhinovirus to be much more frequent compared to our study, as they detected the virus in 155 of the 216 samples examined, while in our research, rhinovirus was detected in only five of 42 influent samples. A review of 12 norovirus wastewater studies conducted by Eftim et al. (2017) showed high positivity rates for norovirus and noted that Europe and Asia had a higher norovirus occurrence than North America and New Zealand. Furthermore, in Japan, all the studied influent samples were positive for either norovirus GI or GII. In our study, the positivity rate for norovirus GI was 50 % and for norovirus GII was 27.3 % in composite influent.

The lower detection rates of some of the viruses in this study than in studies performed in other countries may be explained by the fact that some viral infections, such as enterovirus infections, have been shown to be rarer in Finland than in many other countries (Oikarinen et al., 2014; Vehik et al., 2019). In addition, different wastewater surveillance studies use different methodologies, which can lead to significant differences and make comparing studies challenging (Länsivaara et al., 2024a). Furthermore, PCR inhibition was high in the influent samples, which could have resulted in lower positivity rates of the viruses in this study than in other studies. Many of the infections caused by the studied viruses are asymptomatic or cause mild symptoms, leading to cases being underreported in clinical surveillance. Because all the studied viruses were detected in wastewater, wastewater surveillance could be used to complement the data obtained from clinical surveillance.

4.2. Activated sludge

The activated sludge process uses microorganisms to degrade organic matter and remove nutrients, mainly nitrogen (Davis, 2020). The efficiency of the process is affected by factors that control the abundance, activity, and diversity of bacteria, and viruses may be one of these factors (Brown et al., 2015). Hence, the viruses in activated sludge need to be studied, and reliable methods need to be developed for their detection. Although qPCR is often used to study viruses present in influent and effluent, at the time of writing this article, only two studies were found that used qPCR to detect viruses in activated sludge (Jebri et al., 2012; Prado et al., 2014). qPCR should be used instead of cell culture-based methods in some instances, as some viruses, such as adenovirus, are challenging to grow in cell cultures (Haramoto et al., 2007).

We were able to detect adenovirus and enterovirus in activated sludge, but norovirus and rhinovirus were not detected. Moreover, in our study, the viruses were present in a higher relative amount in influent than in activated sludge. One potential explanation for more viruses being found in the influent, which is predominantly liquid, and in the activated sludge, which is more solid, is the distinct sedimentation properties of the studied viruses in these two sample types. It is hypothesized that the partitioning of viruses to the solid fraction of wastewater compared to the liquid fraction varies between viruses based on their properties, for example whether the viruses are enveloped or naked. (Ye et al., 2016). In sludge samples, viruses are subjected to prolonged exposure to the wastewater matrix compared to influent samples, which may influence their stability and persistence in these sample types. Similar to our study, which detected adenovirus in all activated sludge samples, Prado et al. found that adenovirus was abundantly present in wastewater, as it was discovered in 90 % of

activated sludge samples (Prado et al., 2014). Still, they discovered norovirus in 50 % of activated sludge samples, whereas in our study, norovirus was not found in activated sludge. In addition, Jebri et al. found enterovirus in 7.7 % of activated sludge samples, whereas our study had a higher detection rate, since 26.5 % of the activated sludge samples were positive for enterovirus (Jebri et al., 2012). In addition to detecting adenovirus, we were also able to detect enterovirus. Prado et al. were able to detect rotavirus and hepatitis A virus and Jebri et al. were able to detect enterovirus, showing that qPCR can be used to detect viruses in activated sludge in addition to influent and effluent. However, as there is limited knowledge on the topic and only a few viruses have been studied, more research on the viral contents of activated sludge is needed. As bacteriophages are known to affect bacterial ecology (Brown et al., 2015), they could be the next target to be studied using qPCR.

4.3. Effluent

One of the adverse effects of climate change is the decline of freshwater resources. One of the risk factors for freshwater is wastewater entering water bodies. Pathogens in effluent released into bodies of water can end up in drinking water treatment plants that use surface water as a source of raw water. Furthermore, effluent may contaminate groundwater sources and swimming waters. Indicator microbes, such as coliform bacteria and enterococci, are used to evaluate the microbial safety of effluent and swimming waters. However, these indicators have been shown to be insufficient, as viruses have been found in water samples shown to be pathogen-free based on indicator microbes (Ehlers et al., 2005; Fong and Lipp, 2005; Okoh et al., 2010). Thus, it is important to test the viral load of effluent and assess the prevalence of viruses in the various processes of wastewater treatment.

In this study, over 80 % of the effluent samples were positive for at least one of the viruses, and all the studied viruses, except rhinovirus, were found in the effluent. When the relative amounts of the viruses in influent and effluent samples taken on the same day and from the same WWTP were compared, in most instances, there were viruses present in effluent after treatment. For enterovirus, pan-adenovirus, and adenovirus F40/41 genotypes, a lower amount of viruses was observed along the wastewater treatment train, while no statistically significant difference in the relative amount of the studied noroviruses was found. Still, it is important to note that the PCR inhibition was stronger in the influent samples than in the effluent samples, meaning that the difference in the relative amount of the viruses is likely higher in these sample types. When the results were corrected to account for the PCR inhibition, the amount of Norovirus GI was shown to be lower in effluent compared to influent.

Wastewater treatment has been estimated to eliminate only 50–90 % of the viruses in wastewater when primary and secondary treatment is used, which leads to these viruses being released into nature in discharged effluent (Okoh et al., 2010). Enteric viruses, such as noroviruses and enteroviruses, have previously been shown to be resistant to wastewater treatment and to be present in effluent in abundance even after UV and chlorine treatment (Simhon et al., 2019). Recreational waters and foods contaminated by effluent are known to have caused enteric viral infections, as enteric viruses are persistent in nature (Okoh et al., 2010). Adenovirus and norovirus GI and GII genogroups have previously been found in effluent at concentrations 3.5–4.5 log₁₀ lower than in influent using qPCR and RT-qPCR (Wang et al., 2020). For JC polyomavirus, wastewater treatment was shown to remove the virus from 52 % of samples, and in the others, the amount of the virus decreased in most cases (Rodrigues et al., 2016). Hence, our results are in line with previous studies, as all the viruses, except rhinovirus, were detected in effluent. As effluent and natural water contaminated with effluent are known to be used for irrigation, this exposes the risk of foodborne disease outbreaks of viral infections. We also tested the presence of the viruses at drinking water treatment sampling points in the drinking water treatment train, but the presence of viruses was very

rare; only three samples out of 170 were positive. One groundwater sample and one surface water sample were positive for the norovirus GII genogroup, and one sand filtration sample was positive for enterovirus. None of the treated drinking water samples were positive (Supplementary file, Appendix C). It needs to be taken into account that the detection of viruses in effluent using molecular methods does not necessarily mean that the effluent includes infective viruses, only that the viral genome was detected. These studies were conducted using RT-qPCR and qPCR to detect the viruses and could not assess the infectivity of the viruses present in the samples. To ascertain the impact and risk related to viruses in effluent, further studies are required to determine their infectivity.

4.4. Comparison of composite and grab sampling

Wastewater can be sampled by taking a grab sample or by using a wastewater sampler that collects a composite sample of subsamples taken at regular intervals over a period of time, usually 24 h. Both methods have been applied in the wastewater surveillance of viruses. The findings of this study indicated that the positivity rate of viruses in composite influent samples was higher than in grab influent samples; however, the difference was not statistically significant. Additionally, the composite samples had a higher relative amount of viruses compared to the grab samples. The difference between composite and grab sampling in influent samples may be greater than reported, as the composite samples had a higher RT-qPCR inhibition rate compared to grab samples. Notably, one study conducted on SARS-CoV-2 did not find a statistically significant difference between grab and composite sampling (Augusto et al., 2022). In effluent, there was no statistically significant difference in the positivity rates or relative amount of the viruses between grab effluent samples and composite effluent samples. The findings of this study indicate that composite sampling, particularly of influent samples, is a more representative method for viral wastewater surveillance than the grab sampling method. The method of collection can be of particular importance for viruses that exhibit fluctuations in amounts over the course of a day (Wilson et al., 2022).

4.5. Limitations of the study

In this study, frozen samples were used. It is important to note that freezing can potentially degrade RNA. Significant degradation of adenovirus, enterovirus and norovirus GI and GII has been noted, when samples were stored for several years before analysis (Crank et al., 2025). However, Hokajärvi et al. (2021) demonstrated that no RNA degradation was observed for SARS-CoV-2 RNA, whereas a 1-log₁₀ reduction in norovirus GII RNA was observed within 84 days at either -20 °C or -75 °C. We have tested the degradation of SARS-CoV-2 RNA, and noted no degradation during 5 cycles of freezing and thawing of samples. Consequently, it can be assumed that the degradation of the viral RNA may not have been significant. However, this applies as we determined only the relative amounts of viruses present in the samples by evaluating the Ct values of the RT-qPCR and qPCR reactions to mitigate the potential issue of nucleic acid degradation. It was possible to use relative quantification in this study, as the aim was to compare different sampling methods and sample materials as well as to see whether the viruses could be detected in wastewater, not to analyze the absolute number of viruses in wastewater. If the exact concentration of viruses is determined, the effect of the degradation of viral nucleic acids needs to be taken into account.

One challenge in virus detection in wastewater is the presence of PCR inhibitors, which may influence the estimation of viruses in different sample types. Wastewater includes multiple chemical and biological compounds that can act as PCR inhibitors, which can cause issues in the detection of viruses in wastewater (Kumbalathan et al., 2022). To address this issue, we conducted a PCR inhibition test on all samples. Our findings revealed that the inhibition rates differed across the different

sampling points in the wastewater treatment train. In the composite influent samples, which had the highest inhibition of all the sample types, the inhibition resulted in the relative amount of the viruses being approximately 3.7 times lower than it would be in the absence of inhibition. Inhibitor removal was not employed because the objective was to investigate the differences in inhibition between the various sample types. Consequently, the comparison of the relative virus amounts in various sample types and sample collection methods was interpreted with caution. Also, we accounted for the inhibition by correcting the Ct-values of the samples with the inhibition detected in the sample. This correction did not alter the comparison of the viral amounts between influent and activated sludge. However, it did affect the comparison of the viral amounts between influent and effluent. Without the inhibition correction, there was no statistically significant difference in the relative amount of norovirus GI between influent and effluent. When the inhibition correction was used, the relative amount of norovirus GI was lower in effluent compared to influent. Still, it is acknowledged that samples with a low viral load might be false negative due to potential PCR inhibition. As the detection of viruses in wastewater was shown to be affected by inhibition, it should be monitored when PCR-based methods are used.

5. Conclusion

The objective of this study was to assess the viral contents of different sampling points in wastewater treatment trains and wastewater sampling methods for reliable virus monitoring and the viral load of effluent and activated sludge. We detected enterovirus, rhinovirus, norovirus, and adenovirus in influent, activated sludge, and effluent using RT-qPCR. We were able to detect all the studied viruses in influent, showing a possibility for wastewater surveillance of these viruses. The implementation of an effective and well-designed wastewater-based viral surveillance system can supplement the data obtained from clinical surveillance. The composite sampling of influent was found to be a more representative sampling method than the grab sampling, as the relative amount of viruses was higher in composite samples, showing that composite sampling should be preferred when studying viruses in wastewater. Effluent was shown to include all the studied enteric viruses, but not respiratory rhinovirus, which is in line with previous research, as enteric viruses have often been found in effluent. This study indicated the potential risk of viruses being an environmental pollutant in effluent. However, the true risk should be further investigated using methods that can assess the infectivity of these viruses. Adenovirus and enterovirus were found in activated sludge. However, more research on the viruses in activated sludge is needed, as the viruses in activated sludge are much less studied than those in influent and effluent, and their presence can impact the function of the activated sludge process. Furthermore, RT-PCR inhibition was identified in the studied wastewater sample types, indicating the necessity for an evaluation of detection methods and the potential benefit of using an inhibitor removal method. In addition to viruses, targets such as other pathogens, organic micropollutants and heavy metals should be studied from wastewater to evaluate the effects of wastewater on human health.

CRedit authorship contribution statement

Annika Lämsivaara: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Marja Palmroth:** Writing – review & editing, Funding acquisition, Conceptualization. **Outi Kaarela:** Writing – review & editing, Conceptualization. **Heikki Hyöty:** Writing – review & editing, Funding acquisition, Conceptualization. **Sami Oikarinen:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Kirsi-Maarit Lehto:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition,

Formal analysis, Conceptualization.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2025.121776>.

Data availability

Data will be made available on request.

References

- Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tschärke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholz, P., Thomas, K.V., Mueller, J.F., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. *Sci. Total Environ.* 728, 138764. <https://doi.org/10.1016/j.scitotenv.2020.138764>.
- Allayeh, A.K., Al-Daim, S.A., Ahmed, N., El-Gayar, M., Mostafa, A., 2022. Isolation and genotyping of adenoviruses from wastewater and diarrheal samples in Egypt from 2016 to 2020. *Viruses* 14, 2192. <https://doi.org/10.3390/v14102192>.
- Ando, H., Ahmed, W., Iwamoto, R., Ando, Y., Okabe, S., Kitajima, M., 2023. Impact of the COVID-19 pandemic on the prevalence of influenza A and respiratory syncytial viruses elucidated by wastewater-based epidemiology. *Sci. Total Environ.* 880, 162694. <https://doi.org/10.1016/j.scitotenv.2023.162694>.
- Arraj, A., Bohatier, J., Laveran, H., Traore, O., 2005. Comparison of bacteriophage and enteric virus removal in pilot scale activated sludge plants. *J. Appl. Microbiol.* 98, 516–524. <https://doi.org/10.1111/j.1365-2672.2004.02485.x>.
- Augusto, M.R., Claro, I.C.M., Siqueira, A.K., Sousa, G.S., Caldeireiro, C.R., Duran, A.F.A., de Miranda, T.B., Bomediano Camillo, L. de M., Cabral, A.D., de Freitas Bueno, R., 2022. Sampling strategies for wastewater surveillance: evaluating the variability of SARS-CoV-2 RNA concentration in composite and grab samples. *J. Environ. Chem. Eng.* 10, 107478. <https://doi.org/10.1016/j.jece.2022.107478>.
- Boehm, A.B., Hughes, B., Duong, D., Chan-Herur, V., Buchman, A., Wolfe, M.K., White, B. J., 2023. Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. *Lancet Microbe* 4 (5), e340–e348. [https://doi.org/10.1016/S2666-5247\(22\)00386-X](https://doi.org/10.1016/S2666-5247(22)00386-X).
- Brown, M.R., Camézuli, S., Davenport, R.J., Petelenz-Kurdiel, E., Øvreås, L., Curtis, T.P., 2015. Flow cytometric quantification of viruses in activated sludge. *Water Res.* 68, 414–422. <https://doi.org/10.1016/j.watres.2014.10.018>.
- Cinek, O., Witsø, E., Jeansson, S., Rasmussen, T., Drevinek, P., Wetlesen, T., Vavrinc, J., Grinde, B., Ronningen, K.S., 2006. Longitudinal observation of enterovirus and adenovirus in stool samples from Norwegian infants with the highest genetic risk of type 1 diabetes. *J. Clin. Virol.* 35 (1), 33–40. <https://doi.org/10.1016/j.jcv.2005.03.007>.
- Corpus, M.V.A., Buonerba, A., Vigliotta, G., Zarra, T., Ballesteros, F., Campiglia, P., Belgiorno, V., Korshin, G., Naddeo, V., 2020. Viruses in wastewater: occurrence, abundance and detection methods. *Sci. Total Environ.* 745, 140910. <https://doi.org/10.1016/j.scitotenv.2020.140910>.
- Crank, K., Papp, K., Barber, C., Chung, K., Clements, E., Frehner, W., Hannoun, D., Lane, T., Morrison, C., Mull, B., Oh, E., Wang, P., Gerrity, D., 2025. Pathogen and indicator trends in southern Nevada wastewater during and after the COVID-19 pandemic. *Environ. Sci. Water Res. Tech.* 11 (2), 262–280. <https://doi.org/10.1039/D4EW00620H>.
- Davis, M.L., 2020. *Water and wastewater engineering: design principles and practice*. In: McGraw-Hill Education, second ed.
- do Nascimento, L.G., Fialho, A.M., de Andrade, J. da S.R., de Assis, R.M.S., Fumian, T.M., 2022. Human enteric adenovirus F40/41 as a major cause of acute gastroenteritis in children in Brazil, 2018 to 2020. *Sci. Rep.* 12, 11220. <https://doi.org/10.1038/s41598-022-15413-1>.
- Eftim, S.E., Hong, T., Soller, J., Boehm, A., Warren, I., Ichida, A., Nappier, S.P., 2017. Occurrence of norovirus in raw sewage – a systematic literature review and meta-analysis. *Water Res.* 111, 366–374. <https://doi.org/10.1016/j.watres.2017.01.017>.
- Ehlers, M.M., Grabow, W.O.K., Pavlov, D.N., 2005. Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. *Water Res.* 39, 2253–2258. <https://doi.org/10.1016/j.watres.2005.04.014>.
- Elmahdy, E.M., Ahmed, N.I., Shaheen, M.N.F., Mohamed, E.-C.B., Loutfy, S.A., 2019. Molecular detection of human adenovirus in urban wastewater in Egypt and among children suffering from acute gastroenteritis. *J. Water Health* 17, 287–294. <https://doi.org/10.2166/wh.2019.303>.
- European Parliament, 2024. Revision of the Urban Wastewater Treatment Directive | Legislative Train Schedule. European Parliament. <https://www.europarl.europa.eu/legislative-train/theme-a-european-green-deal/file-revision-of-the-urban-wastewater-treatment-directive-refit>.
- Firquet, S., Beaujard, S., Lobert, P.-E., Sané, F., Caloone, D., Izard, D., Hober, D., 2015. Survival of enveloped and non-enveloped viruses on inanimate surfaces. *Microb. Environ.* 30 (2), 140–144. <https://doi.org/10.1264/jsm2.ME14145>.
- Fong, T.-T., Lipp, E.K., 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiol. Mol. Biol. Rev.* 69, 357–371. <https://doi.org/10.1128/MMBR.69.2.357-371.2005>.
- Fong, T.-T., Phanikumar, M.S., Xagorarakis, I., Rose, J.B., 2010. Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river. *Appl. Environ. Microbiol.* 76, 715–723. <https://doi.org/10.1128/AEM.01316-09>.
- Haramoto, E., Katayama, H., Oguma, K., Ohgaki, S., 2007. Quantitative analysis of human enteric adenoviruses in aquatic environments. *J. Appl. Microbiol.* 103, 2153–2159. <https://doi.org/10.1111/j.1365-2672.2007.03453.x>.
- Hellmér, M., Paxéus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T., Norder, H., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. *Appl. Environ. Microbiol.* 80, 6771–6781. <https://doi.org/10.1128/AEM.01981-14>.
- Hokajärvi, A.M., Rytönen, A., Tiwari, A., Kauppinen, A., Oikarinen, S., Lehto, K.M., Kankaanpää, A., Gunnar, T., Al-Hello, H., Blomqvist, S., Miettinen, I.T., Savolainen-Kopra, C., Pitkänen, T., 2021. The detection and stability of the SARS-CoV-2 RNA biomarkers in wastewater influent in helsinki, Finland. *Sci. Total Environ.* 770, 145274. <https://doi.org/10.1016/j.scitotenv.2021.145274>.
- Honkanen, H., Oikarinen, S., Pakkanen, O., Ruokoranta, T., Pulkki, M.M., Laitinen, O.H., Tauriainen, S., Korpela, S., Lappalainen, M., Vuorinen, T., Haapala, A.-M., Veijola, R., Simell, O., Ilonen, J., Knip, M., Hyöty, H., 2013a. Human enterovirus 71 strains in the background population and in hospital patients in Finland. *J. Clin. Virol.* 56 (4), 348–353. <https://doi.org/10.1016/j.jcv.2012.11.018>.
- Honkanen, H., Oikarinen, S., Peltonen, P., Simell, O., Ilonen, J., Veijola, R., Knip, M., Hyöty, H., 2013b. Human rhinoviruses including group C are common in stool samples of young Finnish children. *J. Clin. Virol.* 56 (3), 334–338. <https://doi.org/10.1016/j.jcv.2012.11.020>.
- Hughes, B., Duong, D., White, B.J., Wigginton, K.R., Chan, E.M.G., Wolfe, M.K., Boehm, A.B., 2022. Respiratory syncytial virus (RSV) RNA in wastewater settled solids reflects RSV clinical positivity rates. *Environ. Sci. Technol. Lett.* 9, 173–178. <https://doi.org/10.1021/acs.estlett.1c00963>.
- Jebri, S., Jofre, J., Barkallah, I., Saidi, M., Hmaied, F., 2012. Presence and fate of coliphages and enteric viruses in three wastewater treatment plants effluents and activated sludge from Tunisia. *Environ. Sci. Pollut. Res.* 19, 2195–2201. <https://doi.org/10.1007/s11356-011-0722-y>.
- Katayama, H., Haramoto, E., Oguma, K., Yamashita, H., Tajima, A., Nakajima, H., Ohgaki, S., 2008. One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. *Water Res.* 42, 1441–1448. <https://doi.org/10.1016/j.watres.2007.10.029>.
- Keshaviah, A., Hu, X.C., Henry, M., 2021. Developing a flexible national wastewater surveillance system for COVID-19 and beyond. *Environ. Health Perspect.* 129, 45002. <https://doi.org/10.1289/EHP8572>.
- Krogvold, L., Edwin, B., Buanes, T., Frisk, G., Skog, O., Anagandula, M., Korsgren, O., Undlien, D., Eike, M.C., Richardson, S.J., Leete, P., Morgan, N.G., Oikarinen, S., Oikarinen, M., Laiho, J.E., Hyöty, H., Ludvigsson, J., Hanssen, K.F., Dahl-Jørgensen, K., 2014. Detection of a low-grade enteroviral infection in the islets of Langerhans of living patients newly diagnosed with type 1 diabetes. *Diabetes* 64 (5), 1682–1687. <https://doi.org/10.2337/db14-1370>.
- Kumblathar, T., Piroddi, N., Hrudey, S.E., Li, X.-F., 2022. Wastewater based surveillance of SARS-CoV-2: challenges and perspective from a Canadian inter-laboratory study. *J. Environ. Sci. China* 116, 229–232. <https://doi.org/10.1016/j.jes.2022.01.039>.
- Lämsivaara, A., Lehto, K.-M., Hyder, R., Luomala, O., Lipponen, A., Hokajärvi, A.-M., Heikinheimo, A., Pitkänen, T., Oikarinen, S., 2024a. Wastewater-based surveillance of respiratory syncytial virus epidemic at the national level in Finland. *ACS EST Water*. <https://doi.org/10.1021/acsestwater.3c00752>.
- Lämsivaara, A., Lehto, K.-M., Hyder, R., Janhonen, E.S., Lipponen, A., Heikinheimo, A., Pitkänen, T., Oikarinen, S., Group, W.S., 2024b. Comparison of different reverse

- transcriptase–polymerase chain reaction–based methods for wastewater surveillance of SARS-CoV-2: Exploratory study. *JMIR Public Health Surveill* 10, e53175. <https://doi.org/10.2196/53175>.
- Lehto, K.-M., Lämsivaara, A., Hyder, R., Luomala, O., Lipponen, A., Hokajärvi, A.-M., Heikinheimo, A., Pitkänen, T., Oikarinen, S., 2024. Wastewater-based surveillance is an efficient monitoring tool for tracking influenza A in the community. *Water Res.* 257, 121650. <https://doi.org/10.1016/j.watres.2024.121650>.
- Li, B., Di, D.Y.W., Saingam, P., Jeon, M.K., Yan, T., 2021. Fine-scale temporal dynamics of SARS-CoV-2 RNA abundance in wastewater during A COVID-19 lockdown. *Water Res.* 197, 117093. <https://doi.org/10.1016/j.watres.2021.117093>.
- Maniah, K., Nour, I., Hanif, A., Yassin, M.T., Alkathiri, A., Al-Ashkar, I., Eifan, S., 2023. Molecular identification of human adenovirus isolated from different wastewater treatment plants in Riyadh, Saudi Arabia: surveillance and meteorological impacts. *Water* 15, 1367. <https://doi.org/10.3390/w15071367>.
- Mao, K., Zhang, K., Du, W., Ali, W., Feng, X., Zhang, H., 2020. The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks. *Curr. Opin. Environ. Sci. Health, Environmental Health: COVID-19* 17, 1–7. <https://doi.org/10.1016/j.coesh.2020.04.006>.
- Mercier, E., D'Aoust, P.M., Thakali, O., Hegazy, N., Jia, J.-J., Zhang, Z., Eid, W., Plaza-Diaz, J., Kabir, M.P., Fang, W., Cowan, A., Stephenson, S.E., Pisharody, L., MacKenzie, A.E., Graber, T.E., Wan, S., Delatolla, R., 2022. Municipal and neighbourhood level wastewater surveillance and subtyping of an influenza virus outbreak. *Sci. Rep.* 12, 15777. <https://doi.org/10.1038/s41598-022-20076-z>.
- Ni, G., Lu, J., Maulani, N., Tian, W., Yang, L., Harliwong, I., Wang, Z., Mueller, J., Yang, B., Yuan, Z., Hu, S., Guo, J., 2021. Novel multiplexed amplicon-based sequencing to quantify SARS-CoV-2 RNA from wastewater. *Environ. Sci. Technol. Lett.* 8, 683–690. <https://doi.org/10.1021/acs.estlett.1c00408>.
- Oikarinen, S., Tauriainen, S., Hober, D., Lucas, B., Vazeou, A., Sioofy-Khojine, A., Bozas, E., Muir, P., Honkanen, H., Ilonen, J., Knip, M., Keskinen, P., Saha, M.-T., Huhtala, H., Stanway, G., Bartsocas, C., Ludvigsson, J., Taylor, K., Hyöty, H., the VirDiab Study Group, 2014. Virus antibody survey in different European populations indicates risk association between coxsackievirus B1 and type 1 diabetes. *Diabetes* 63, 655–662. <https://doi.org/10.2337/db13-0620>.
- Okoh, A.I., Sibanda, T., Gusha, S.S., 2010. Inadequately treated wastewater as a source of human enteric viruses in the environment. *Int. J. Environ. Res. Public Health* 7, 2620–2637. <https://doi.org/10.3390/ijerph7062620>.
- Prado, T., Gaspar, A.M.C., Miagostovich, M.P., 2014. Detection of enteric viruses in activated sludge by feasible concentration methods. *Braz. J. Microbiol.* 45, 343–349. <https://doi.org/10.1590/S1517-83822014000100049>.
- Reyne, M.I., Allen, D.M., Levickas, A., Allingham, P., Lock, J., Fitzgerald, A., McSparron, C., Nejad, B.F., McKinley, J., Lee, A., Bell, S.H., Quick, J., Houldcroft, C. J., Bamford, C.G.G., Gilpin, D.F., McGrath, J.W., 2023. Detection of human adenovirus F41 in wastewater and its relationship to clinical cases of acute hepatitis of unknown aetiology. *Sci. Total Environ.* 857, 159579. <https://doi.org/10.1016/j.scitotenv.2022.159579>.
- Rodrigues, F., Gonçalves, D., Luxo, C., Matos, A.M., 2016. High frequency of JC human polyomavirus in Portuguese wastewaters: a possible source for human infection. In: *J. Clin. Virol., Abstracts of the 19th Annual Meeting of the European Society for Clinical Virology*, vol. 82, S140. <https://doi.org/10.1016/j.jcv.2016.08.282>, 14th–17th September 2016, Lisbon, Portugal.
- Simhon, A., Pileggi, V., Flemming, C.A., Bicudo, J.R., Lai, G., Manoharan, M., 2019. Enteric viruses in municipal wastewater effluent before and after disinfection with chlorine and ultraviolet light. *J. Water Health* 17, 670–682. <https://doi.org/10.2166/wh.2019.111>.
- Vehik, K., Lynch, K.F., Wong, M.C., Tian, X., Ross, M.C., Gibbs, R.A., Ajami, N.J., Petrosino, J.F., Rewers, M., Toppari, J., Ziegler, A.G., She, J.-X., Lernmark, A., Akolkar, B., Hagopian, W.A., Schatz, D.A., Krischer, J.P., Hyöty, H., Richard, E.L., 2019. Prospective virome analyses in young children at increased genetic risk for type 1 diabetes. *Nat. Med.* 25, 1865–1872. <https://doi.org/10.1038/s41591-019-0667-0>.
- Wang, H., Neyvaldt, J., Enache, L., Sikora, P., Mattsson, A., Johansson, A., Lindh, M., Bergstedt, O., Nordner, H., 2020. Variations among viruses in influent water and effluent water at a wastewater plant over one year as assessed by quantitative PCR and metagenomics. *Appl. Environ. Microbiol.* 86, e02073-20. <https://doi.org/10.1128/AEM.02073-20>.
- Wilson, M., Qiu, Y., Yu, J., Lee, B.E., McCarthy, D.T., Pang, X., 2022. Comparison of auto sampling and passive sampling methods for SARS-CoV-2 detection in wastewater. *Pathogens* 11, 359. <https://doi.org/10.3390/pathogens11030359>.
- Wolfe, M.K., Duong, D., Bakker, K.M., Ammerman, M., Mortenson, L., Hughes, B., Arts, P., Laurant, A.S., Fitzsimmons, W.J., Bendall, E., Hwang, C.E., Martin, E.T., White, B.J., Boehm, A.B., Wigginton, K.R., 2022. Wastewater-based detection of two influenza outbreaks. *Environ. Sci. Technol. Lett.* 9, 687–692. <https://doi.org/10.1021/acs.estlett.2c00350>.
- Wu, F., Zhang, J., Xiao, A., Gu, X., Lee, W.L., Armas, F., Kauffman, K., Hanage, W., Matus, M., Ghaeli, N., Endo, N., Duvallet, C., Poyet, M., Moniz, K., Washburne, A.D., Erickson, T.B., Chai, P.R., Thompson, J., Alm, E.J., 2020. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *mSystems* 5, e00614-20. <https://doi.org/10.1128/mSystems.00614-20>.
- Ye, Y., Ellenberg, R.M., Graham, K.E., Wigginton, K.R., 2016. Survivability, partitioning, and recovery of enveloped viruses in untreated municipal wastewater. *Environmental Science & Technology* 50 (10), 5077–5085. <https://doi.org/10.1021/acs.est.6b00876>.