Science of the Total Environment 895 (2023) 165012

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Measures against COVID-19 affected the spread of human enteric viruses in a Swedish community, as found when monitoring wastewater

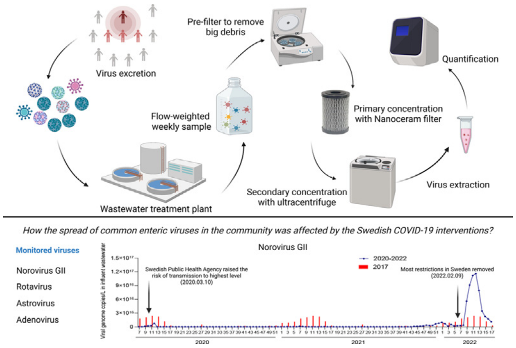
Hao Wang [a,b,*], Marianela Patzi Churqui [a], Timur Tunovic [a], Lucica Enache [c], Anette Johansson [c], Magnus Lindh [a,b], Martin Lagging [a,b], Kristina Nyström [a,b], Heléne Norder [a,b]

[a] *Institute of Biomedicine, Department of Infectious Diseases, University of Gothenburg, Gothenburg, Sweden*
[b] *Sahlgrenska University Hospital, Department of Clinical Microbiology, Region Västra Götaland, Gothenburg, Sweden*
[c] *Gryaab AB, Gothenburg, Sweden*

HIGHLIGHTS

- Changes in the amount of viruses in wastewater correspond to changes in the diagnosed patients.
- COVID-19 interventions reduced norovirus and rotavirus spread in the Swedish community.
- Astrovirus and adenovirus circulation pattern were less affected by COVID-19 interventions.
- Increase in the amount of adenovirus, norovirus GII, and rotavirus in wastewater after restrictions were removed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Warish Ahmed

Keywords:
Sewage
SARS-CoV-2
Seasonal variant
Wastewater-based epidemiology
Enteric viruses
Surveillance

ABSTRACT

The quantification of viral genomes in wastewater reflects the prevalence of viral infections within the community. Knowledge of how the spread of common enteric viruses in the community was affected by the Swedish COVID-19 interventions is limited. To investigate this, the weekly wastewater samples collected for monitoring SARS-CoV-2 throughout the COVID-19 pandemic at the Rya sewage treatment plant in Gothenburg were also analyzed for adenovirus, norovirus GII, astrovirus, and rotavirus. The amount of each viral genome was quantified by real-time-qPCR and compared with the quantity of these viral genomes in wastewater from 2017. The results showed that the winter seasonality of norovirus GII and rotavirus in wastewater observed in 2017 was interrupted shortly after the introduction of the COVID-19 interventions, and they remained at low level throughout the pandemic. The circulation pattern of astrovirus and adenovirus was less affected. When the COVID-19 restrictions were lifted in 2022, a dramatic increase was observed in the amount of norovirus GII, rotavirus, and adenovirus genomes in wastewater. The changes in abundance and seasonality of some viruses identified through wastewater monitoring were consistent with changes in the number of patients diagnosed with these viruses. These findings suggest that moderate intervention to prevent COVID-19 significantly reduced the spread of some enteric viruses in the community. The results show that wastewater monitoring is a valuable tool for detecting the spread and outbreaks of viral infections that may cause gastroenteritis also when people do not seek medical help, such as during the COVID-19 pandemic.

1. Introduction

Wastewater is a complex mixture and contains a large number of microorganisms, including bacteria, viruses, protozoa, and parasites. Some of the

---

* Corresponding author at: Sahlgrenska University Hospital, Department of Clinical Microbiology, Region Västra Götaland, Gothenburg, Sweden.
E-mail address: hao.wang@gu.se (H. Wang).

http://dx.doi.org/10.1016/j.scitotenv.2023.165012
Received 28 February 2023; Received in revised form 25 May 2023; Accepted 17 June 2023

viruses could cause human diseases and pose a health burden to society. Monitoring the occurrence and concentration of common non-enveloped enteric viruses, such as norovirus, rotavirus, enterovirus, and hepatitis A and E viruses, in wastewater enables our understanding of their spread in the communities. It also provides an early warning of potential viral outbreaks (Hellmer et al., 2014; Ndiaye et al., 2014; Pellegrinelli et al., 2019). Since the COVID-19 pandemic, many studies have shown that virus monitoring in wastewater is also a promising approach to assess the circulation of the enveloped virus SARS-CoV-2 in communities (Castiglioni et al., 2022; Lastra et al., 2022; Polo et al., 2020; Saguti et al., 2021; Wu et al., 2022). In addition, wastewater surveillance of pandemic viruses such as SARS-CoV-2, can indicate changes in the spread of an ongoing pandemic and predict the burden on the health system (Wang et al., 2022). In the post-pandemic era, this method will be particularly valuable as an epidemiological tool in understanding the spread of the virus, as less resources will be dedicated to testing and monitoring infections.

Many human enteric viruses have a seasonal circulation pattern and can cause outbreaks in the general population. For example, gastrointestinal infections caused by norovirus, rotavirus, and astrovirus are most frequently diagnosed during the winter and/or early spring (Ahmed et al., 2013; Celik et al., 2015; Wu et al., 2020), while there is no clear seasonal variation in the spread of adenovirus (Celik et al., 2015; Kim et al., 2017). Various factors such as the introduction of vaccines, dry and rainy season, climate changes, and human activities can affect the seasonal epidemiology of viral infections (Ahmed et al., 2013; Celik et al., 2015; Tate et al., 2009). These factors can lead to changes in the circulation pattern of certain viruses in different populations. As the COVID-19 pandemic hit the world in the beginning of 2020, many countries have implemented non-pharmaceutical interventions, such as social distancing, mask wearing, increased hand hygiene, country border closures, travel restrictions, isolation of the confirmed cases and their contacts, and large-scale lockdowns to reduce the transmission of SARS-CoV-2 (Flaxman et al., 2020; Perra, 2021). These interventions not only affected the spread of SARS-CoV-2, but also changed the spread of other common human viruses. Previous studies have shown that during the COVID-19 pandemic there were significantly fewer cases with acute gastroenteritis caused by common enteric viruses, including adenovirus, norovirus, astrovirus, and rotavirus, compared to the pre-pandemic period (Liu et al., 2022; Mack et al., 2021; Wang et al., 2021).

Viruses are monitored in wastewater by determining the presence of their genomes. This reflects the number of infected individuals in a community and helps to identify seasonal patterns of virus circulation. Our previous studies from 2017 showed that the amount of norovirus GII, rotavirus, and astrovirus in wastewater reached its peak during the cold season in Gothenburg. This reflected changes in the number of patients diagnosed with gastroenteritis caused by these viruses (Hellmer et al., 2014; Wang et al., 2020).

In Sweden, a moderate strategy and limited interventions were adopted to combat the COVID-19 pandemic (Ludvigsson, 2020). However, knowledge of how the Swedish efforts to prevent the spread of SARS-CoV-2 affected the spread of other viruses is limited. To investigate this, every week for more than 2 years, we not only monitored SARS-CoV-2, but also four other viruses in wastewater from the Gothenburg area. The occurrence and concentration of norovirus GII, rotavirus, astrovirus, and adenovirus in wastewater during the pandemic were compared with the quantity of these viruses in the corresponding months of 2017 (Wang et al., 2020).

2. Materials and methods

2.1. Wastewater Samples

Weekly pooled influent wastewater samples were collected at the Rya wastewater treatment plant (WWTP), which receives wastewater from the city of Gothenburg and six nearby municipalities, as well as storm water and snow-melting water from some older parts of the city. In Rya WWTP, a fixed-site sampler was used to collect every 30 mL per 10,000 m^3 influent wastewater. The 24-h flow-weighted samples were stored at 4 °C and pooled into a weekly sample each Monday morning, and sent to the Clinical Microbiology Laboratory (CML) at the Sahlgrenska University Hospital, Gothenburg for analysis. The volume of each weekly sample varied due to changes of precipitation and was between 0.975 and 11.1 L during the period. Detailed sampling procedures have previously been described (Saguti et al., 2021).

The first confirmed case of COVID-19 infection in Sweden was reported in week 5, 2020 (Folkhälsomyndigheten, 2020), and we started the influent wastewater collection one week later from week 7, 2020. The sampling lasted until week 17, 2022. A total of 115 weekly samples were collected and analyzed in this study. Information about the sampling period, weekly wastewater flow, and the weekly volume is listed in Supplementary Table S1.

2.2. Concentration of viruses from influent wastewater samples

Viruses were concentrated from the wastewater samples the same day they were collected by our in-house developed method (Wang et al., 2018). Before virus concentration, each wastewater sample was centrifuged at 6000 $\times g$ for 15 min to remove large particles or debris. The supernatant was filtrated through a NanoCeram electropositive filter (Argonide, Florida, USA) twice. The viruses were eluted from the filter with 330 mL of 0.2 M phosphate buffer containing 3 % beef extract and 0.05 M Glycine (pH 9.5). The pH of the eluate was adjusted to 7.5 and filtered through a 0.65/0.45 μm Sartobran capsule filter (Sartorius, Göttingen, Germany) to remove small particles and bacteria. The eluate was then ultracentrifuged to further concentrate the viruses. The pellet after ultracentrifugation was suspended in 2.4 mL Tris-HCl (pH 8.0) buffer and stored at −80 °C before analysis.

2.3. Detection of norovirus GII, adenovirus, rotavirus, and astrovirus in wastewater by qPCR

The viral nucleic acids were extracted from 1 mL of dissolved pellet using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. qPCR was performed to detect rotavirus, norovirus GII, adenovirus, and astrovirus in the weekly wastewater samples.

The 20-μL qPCR reaction mixture contained 5 μL extracted nucleic acids of norovirus GII, rotavirus, or astrovirus, 4× UltraPlex 1-Step ToughMix (Quantabio, Beverly, USA), 0.75 μM of forward and reverse primer, and 0.2 μM probe. The qPCR reaction was performed with an initial reverse transcription cycle of 50 °C for 10 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 s and 60 °C for 1 min. For adenovirus, the 20-μL qPCR reaction mixture contained 2 μL extracted nucleic acids, 2× TaqMan™ Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.5 μM of forward and reverse primer, and 0.4 μM probe. The qPCR reaction was performed with an initial incubation at 50 °C for 2 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. All qPCR reactions were performed on a 7300 real-time PCR system (Applied Biosystems). Each sample was analyzed in duplicate. The primers and probes have been described previously (Wang et al., 2020). Two plasmids, one containing targeted region of adenovirus, and the other containing targeted regions of norovirus GII, astrovirus, and rotavirus, were synthesized in a pEX-A258 vector by Eurofins Genomics (Ebersberg, Germany). The plasmids were 10-fold serial diluted and five plasmid dilutions were used to generate a standard curve. Nuclease-free water (Sigma-Aldrich, St. Louis, USA) was used as a negative control. The mean Ct value of each virus were used to calculate their concentration in qPCR reaction by standard curve quantification based on the dilutions of the plasmid. Afterwards, the relative amount of viral genomes per day of the total amount of wastewater passing through Rya WWTP during the week were calculated with formula as previously described (Saguti et al., 2021). The analysis of weekly fluctuations in the levels of individual viruses in wastewater during the SARS-CoV-2 pandemic 2020–2022 and their comparison to the corresponding values during 2017 were performed using GraphPad Prism 9.5.1 software (San Diego, California, USA).

2.4. PCR amplification of adenovirus

Nested PCR targeting the hexon protein-coding region of adenovirus was performed on wastewater samples collected between week 38, 2021 and week 17, 2022. The primers and PCR reaction conditions were previously described (Wang et al., 2018). The amplified PCR products were purified using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instruction. The purified amplicons were sent to Eurofins Genomics (Ebersberg, Germany) for Sanger sequencing. The obtained sequences were subjected to analysis using BioEdit software version 7.2.5. All sequences were aligned with the hexon gene of 81 adenovirus sequences representing all types of human mastadenovirus downloaded from GeneBank. Phylogenetic tree was constructed using neighor-joing (NJ) method within MEGA software version 11 to type the adenovirus (Tamura et al., 2021).

2.5. Data collection of the number of notified cases of norovirus GII, astrovirus, rotavirus, and adenovirus infection in the Gothenburg region

The number of samples from patients who had sought medical help for gastroenteritis in the Gothenburg region during 2017 and 2020–2022, and sent to CML at the Sahlgrenska University Hospital for analysis were compiled. All samples had been analyzed at the routine clinical laboratory at CML by qPCR. The number of routinely analyzed patient samples with norovirus GII, astrovirus, rotavirus, and adenovirus was used in the comparison of the amount of these viruses in wastewater samples. Data from each year were imported into the Microsoft Excel database for compilation. Statistical analysis using Fisher's exact test was performed in the R programming software package (version 4.0.3). The weekly number of patients diagnosed with each enteric virus in the Gothenburg area during the pandemic 2020–2022 is provided in Supplementary Table S2.

3. Results

3.1. Weekly variation of norovirus GII, astrovirus, rotavirus, and adenovirus in wastewater compared between the COVID-19 pandemic in 2020–2022 and the non-pandemic 2017

Weekly changes of the amount of norovirus GII, astrovirus, rotavirus, and adenovirus were monitored in influent wastewater from Rya WWTP between week 7, 2020 and week 17, 2022.

Norovirus GII.

The spread of norovirus GII was clearly affected by the COVID-19 pandemic interventions. In 2017, the highest measured levels of norovirus GII in wastewater were during the winter season, with an elevated amount between weeks 1 and 15, peaking at week 11 (Fig. 1A). The amount of norovirus GII genomes during this period was about 5–176 times higher than it was before or after the peak period. In early 2020, the amount of norovirus GII genomes in influent wastewater started to rise as in 2017. But this trend was interrupted at week 12 when the first restrictions to limit SARS-CoV-2 transmission were introduced in Sweden. Since then, the amount of norovirus GII genomes was at a low level for almost 20 months. There was a small increase between week 48, 2021 and week 2, 2022, but it was still lower than the amount observed in 2017 (Fig. 1A). When the Swedish government and the Public Health Agency removed the restrictions against SARS-CoV-2 spread on February 9, 2022, there was a dramatic increase in the amount of norovirus GII genomes in the wastewater. The amount of virus peaked at week 12, when it was five times higher than in the corresponding period in 2017. This peak followed the same pattern as norovirus GII in 2017 (Fig. 1A).

Astrovirus.

Like norovirus GII, astrovirus also had a clear winter seasonality in influent wastewater in 2017 (Fig. 1B). In contrast to norovirus GII, the seasonality did not change for astrovirus during the pandemic. This is probably because it is mainly children who get infected with astrovirus, and even if the children want to adhere to the restrictions, they often forget them and can therefore spread viruses that are secreted at high levels between them.

Rotavirus.

For rotavirus, the picture is somewhat between that of norovirus GII and astrovirus. At the beginning of the pandemic in spring 2020, the amount of rotavirus was similar to that of 2017 (Fig. 1C). However, the expected spread at the beginning of 2021 did not occur, but it returned at the beginning of 2022, after the restrictions had been removed. This may be a consequence of the rotavirus vaccination, which was introduced in the national vaccination program for children in 2019 in Sweden. This means that the younger children were protected during the pandemic and that only the older children could become infected.

Adenovirus.

The spread of adenovirus did not have a clear seasonality based on the wastewater monitoring in 2017, and no larger outbreak was observed. Approximately five peaks were observed throughout 2017, and the amount of virus between each peak did not differ more than four times (Fig. 1D). The COVID-19 pandemic and the accompanying control measures did not significantly change the pattern for adenovirus. The amount of adenovirus in the wastewater was at the same level as in 2017. However, the amount of adenoviruses increased significantly from week 10, 2022, shortly after the COVID-19 pandemic interventions eased (Fig. 1D). During these weeks, the amount of adenovirus was more than six times higher than during the peaks observed during 2017 and during the COVID-19 pandemic 2020–2022. Typing the adenovirus sequences showed that there was a large outbreak of adenovirus F41, just before the norovirus GII outbreak.

3.2. Number of patient samples diagnosed with norovirus GII, astrovirus, rotavirus, and adenovirus in 2017 and during the COVID-19 pandemic in the Gothenburg region

In the Gothenburg region, the incidence of patients seeking medical treatment for gastroenteritis declined by 28–32 % during the COVID-19 pandemic period of 2020–2021 compared to the 2846 cases reported in 2017 (Table 1). However, this trend was broken in 2022 when the COVID-19 restrictions were removed in early February. During only 6 months, the total number of gastroenteritis cases reached to about 60 % of the yearly number of cases before the pandemic. Among patients with gastroenteritis, enteric viruses were significantly less often detected during the pandemic (13.6–14.9 %) than in 2017 (25.3 %; $p < 0.0001$; Table 1). This shows that the majority of those who had gastroenteritis during the pandemic and sought medical help were not infected with the enteric viruses that are diagnosed for, which was verified in the wastewater survey. The number of diagnosed cases infected with norovirus GII and rotavirus decreased significantly in 2020 and 2021 compared to the number of cases in 2017 (p < 0.0001; Table 1) and increased again in 2022. The incidence of adenovirus and astrovirus cases exhibited a decline in 2020, albeit not statistically significant when compared to 2017 (Table 1). However, in 2021, the incidence of astrovirus cases demonstrated a significant increase ($p = 0.0066$; Table 1). Both before, during and after the COVID-19 pandemic, the viruses most often associated with gastroenteritis were caused by norovirus GII, followed by adenovirus.

Further analysis of weekly changes in these viruses showed that the implementation of COVID-19 control measures in Sweden resulted in an immediate reduction in the number of patients diagnosed with norovirus GII. This virus continued to be present at low levels in the community until the end of 2021, when the restrictions were removed and its abundance returned to pre-pandemic levels (Fig. 2A). It was also found that the COVID-19 control measures did not decrease the number of total cases of astrovirus infections or amount of viruses identified in the wastewater compared to the corresponding amount in 2017 (Table 1 and Fig. 2B). For rotavirus, there was a significant decrease in number of cases during the pandemic period of 2020–2021 (Table 1 and Fig. 2C).

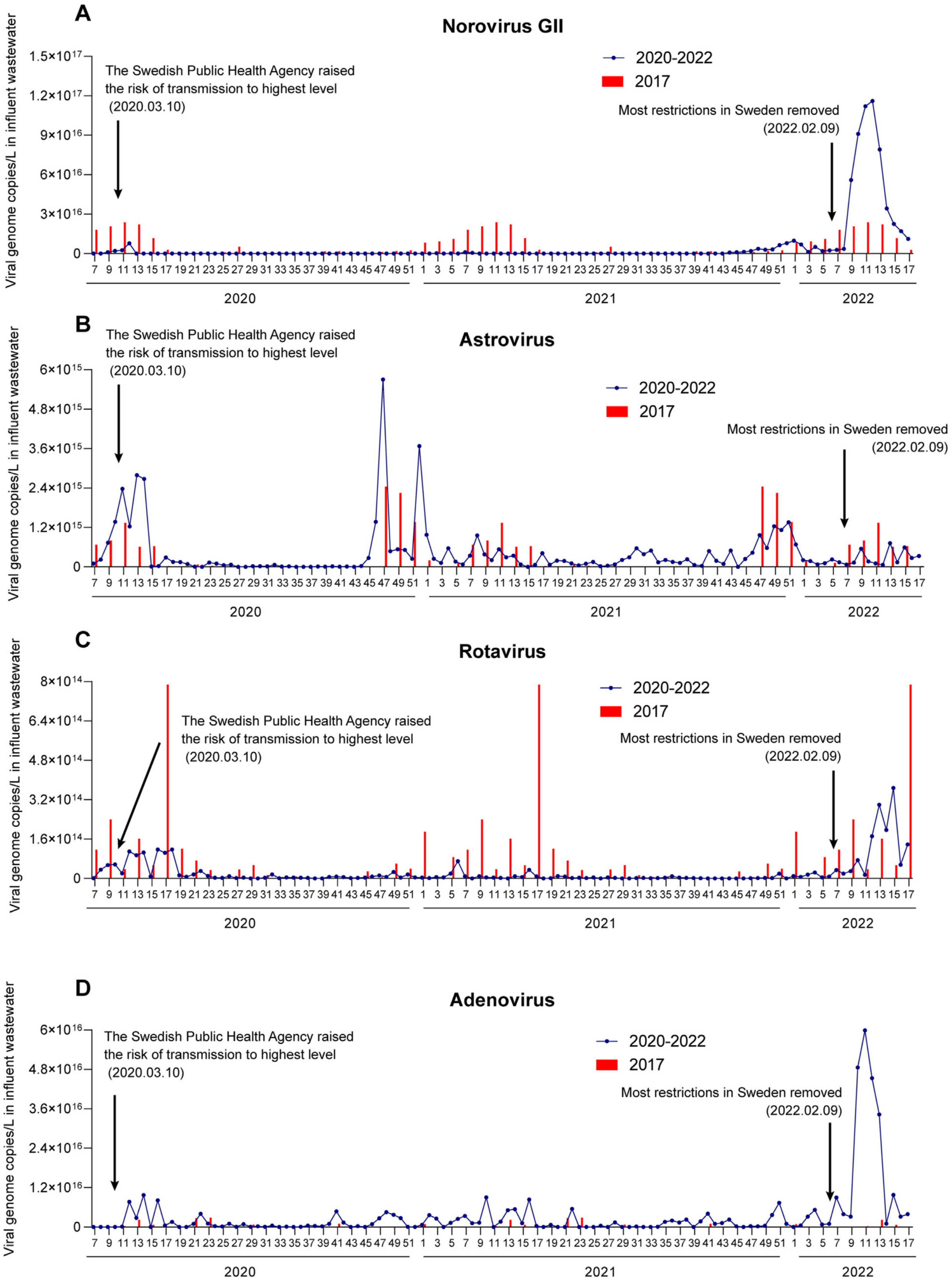


Fig. 1. Week variations in the concentrations of four different viruses, (A) norovirus GII, (B) astrovirus, (C) rotavirus, and (D) adenovirus, in influent wastewater during the SARS-CoV-2 pandemic 2020–2022 (blue line) compared to the quantification of the same viruses during the prepandemic year of 2017 (red bar).

Table 1

Number of samples sent to CML for diagnostic testing of gastroenteritis in the Gothenburg region during 2017 and 2020–2022 and number of samples tested positive for norovirus GII, astrovirus, rotavirus, and adenovirus.

Year	Total analyzed	Total positive		Norovirus GII		Rotavirus		Adenovirus		Astrovirus	
				No. (%)	*p* value	No. (%)	*p* value	No. (%)	*p* value	No. (%)	*p* value
2017	2846	721 (25.3 %)	Ref*	418 (14.69 %)	Ref	99 (3.48 %)	Ref	172 (6.04 %)	Ref	32 (1.12 %)	Ref
2020	1947	273 (13.6 %)	<0.0001	128 (6.57 %)	<0.0001	28 (1.44 %)	<0.0001	101 (5.19 %)	0.22	16 (0.82 %)	0.37
2021	2041	305 (14.9 %)	<0.0001	138 (6.76 %)	<0.0001	31 (1.52 %)	<0.0001	93 (4.56 %)	0.025	43 (2.11 %)	0.0066
2022#	1747	505 (29 %)	0.0082	247 (14.14 %)	0.63	50 (2.86 %)	0.26	147 (8.41 %)	0.0027	61 (3.49 %)	<0.0001

Until June 30, 2022.

* Reference year.

Prior to the pandemic, rotavirus had a distinct winter seasonality. The prevalence and seasonal pattern of adenovirus infections were comparatively less impacted during the pandemic period of 2020–2021 (Fig. 2D). After the removal of most COVID-19 restrictions, the number of diagnosed cases for all viruses investigated in this study increased to a level that was similar to or exceeded the level observed before the pandemic period.

4. Discussion

In this study, we show that the moderate Swedish COVID-19 intervention strategies had a significant effect on seasonal transmission of human enteric viruses, specifically norovirus GII and rotavirus. This was shown as reductions of both the number of diagnosed cases and viruses in wastewater. The data reported by public health agencies during the COVID-19 pandemic might not accurately reflect the actual prevalence of SARS-CoV-2 in society, given the rapid increase in infections and limitations in laboratory testing capacities. In this scenario, the monitoring of SARS-CoV-2 in wastewater has been widely used to track the progress of COVID-19 infections in communities (Izquierdo-Lara et al., 2021; Peccia et al., 2020; Saguti et al., 2021; Wu et al., 2022). At the same time, the implemented COVID-19 interventions also affected the spread of human enteric viruses, as measured by changes in the number of diagnosed cases in the community, as reported from China, Japan and Germany (Fukuda et al., 2021; Mack et al., 2021; Wang et al., 2021). However, these changes may be underreported due to increased demands on healthcare systems and individuals with mild gastrointestinal symptoms avoiding medical attention during the pandemic. Our two-year observation of four prevalent enteric viruses in Sweden revealed a correlation between the seasonal changes in viral amount in wastewater and diagnosed cases of enteric virus infections, suggesting that wastewater monitoring is a practical and efficient means of tracking community gastroenteritis during a pandemic.

The winter seasonality of norovirus GII and rotavirus in influent wastewater observed in the non-pandemic year 2017 was abruptly interrupted by the introduction of SARS-COV-2 measures. Sweden chose a less strict intervention strategy to limit transmission as compared with many other countries. There were no general lockdowns and no enforced quarantines for infected individuals in Sweden, but other control measures, such as recommending physical distance in public spaces, limiting public gatherings, work from home if possible, banned or limited visitors to hospital inpatients or elderly care facilities, and increasing the test capacity, were introduced to mitigate the pandemic (Ludvigsson, 2020). Although most of the interventions were recommendations, not mandatory, people changed their behaviors and hygiene habits (Blom et al., 2021; Gustavsson and Beckman, 2020). These milder prevention recommendations protected people not only from SARS-CoV-2 infections, but also from other viruses. It should be noted that the rotavirus vaccine was introduced into the Swedish national vaccination program and offered to younger children from September 2019 (Schollin, 2021). The vaccine provides immunological protection against rotavirus infection to younger children born after the start of the vaccination program, while older children could still become infected, which may contribute to the low amount of rotavirus genomes in wastewater during the COVID-19 pandemic.

The amount of viral genomes for norovirus GII, rotavirus, and adenovirus had a surge in influent wastewater between week 9 and week 11, 2022. Since December 2021, the Omicron variant of SARS-CoV-2 rapidly displaced other variants, leading to the fourth wave of COVID-19 in Sweden. This resulted in a dramatic increase in both the number of confirmed cases and the amount of SARS-CoV-2 genomes in wastewater (Wang et al., 2022). Despite this, the Swedish government and the Public Health Agency of Sweden chose to remove most COVID-19 restrictions on 9 February 2022 considering a high vaccination coverage in the population and that the Omicron variant did not cause as serious illness as the previous variants. Relaxation of COVID-19 control measures immediately led to a resurgence in the amount of viral genomes in wastewater and an increase in cases of gastroenteritis. This surge could be due to people no longer keeping social distance or wearing facemasks in public areas, and did not sanitizing their hands as frequently as during the COVID-19 pandemic period.

Unlike norovirus and rotavirus, for which the COVID-19 interventions had a clear effect on their seasonal variation in wastewater, the adenovirus variation was less affected. Adenovirus was detectable in wastewater throughout the pandemic in variations that matched those we observed in 2017. Its viral genome abundance in wastewater reflected the number of samples from patients with gastroenteritis caused by adenovirus, as it did in 2017 (Wang et al., 2020). The surveillance indicated that several outbreaks of adenovirus occurred in communities. Adenoviruses are known to be relatively resistant to common disinfectants and can survive longer in the environment (Vasickova et al., 2010). Good hygiene habits in close-contact settings, such as daycare centers and schools, can decrease the spread of human enteric viruses effectively, but not for adenovirus. Adenovirus may still survive and spread on contaminated subjects, such as toys and towels, leading to sporadic cases and outbreaks. From April 2022, adenovirus was further typed in retrospective wastewater samples to determine the presence of adenovirus F41, which was suspected to be linked to cases of acute hepatitis of unknown origin in children (Gutierrez Sanchez et al., 2022). A suspected outbreak of adenovirus F41 was detected during the period of heightened adenovirus amount in wastewater. However, there was no acute hepatitis of unknown origin in children reported in Gothenburg during this period.

The COVID-19 measures also had little effect on the weekly variantion of astrovirus, and its genome in wastewater even increased in 2020–2021 compared to the pre-pandemic levels. This discrepancy was also reflected in the number of confirmed cases of astrovirus infection, which increased by 34 % in 2021 compared to 2017. A recent study from Spain demonstrated that astrovirus reduction due to pandemic non-pharmaceutical measures was least pronounced among several tested gastrointestinal viruses (Maldonado-Barrueco et al., 2022). Similar finding was reported from South Korea, where the reduction rate for astrovirus is 7.0 %, far less than reduction rate for norovirus (40.2 %) and rotavirus A (31.8 %) (Ahn et al., 2021). This is probably because astrovirus mostly infects young children, and it is difficult for them to adhere to good hygiene habits, like conscientious hand and food washing, let alone those COVID-19 restrictions. Moreover, Sweden's policy of keeping day care and primary schools operational throughout the pandemic likely facilitated the circulation of astrovirus in young children. It was noted that a respiratory syncytial virus (RSV) outbreak was reported in winter 2021–2022 during the pandemic (Folkhälsomyndigheten, 2022). RSV also

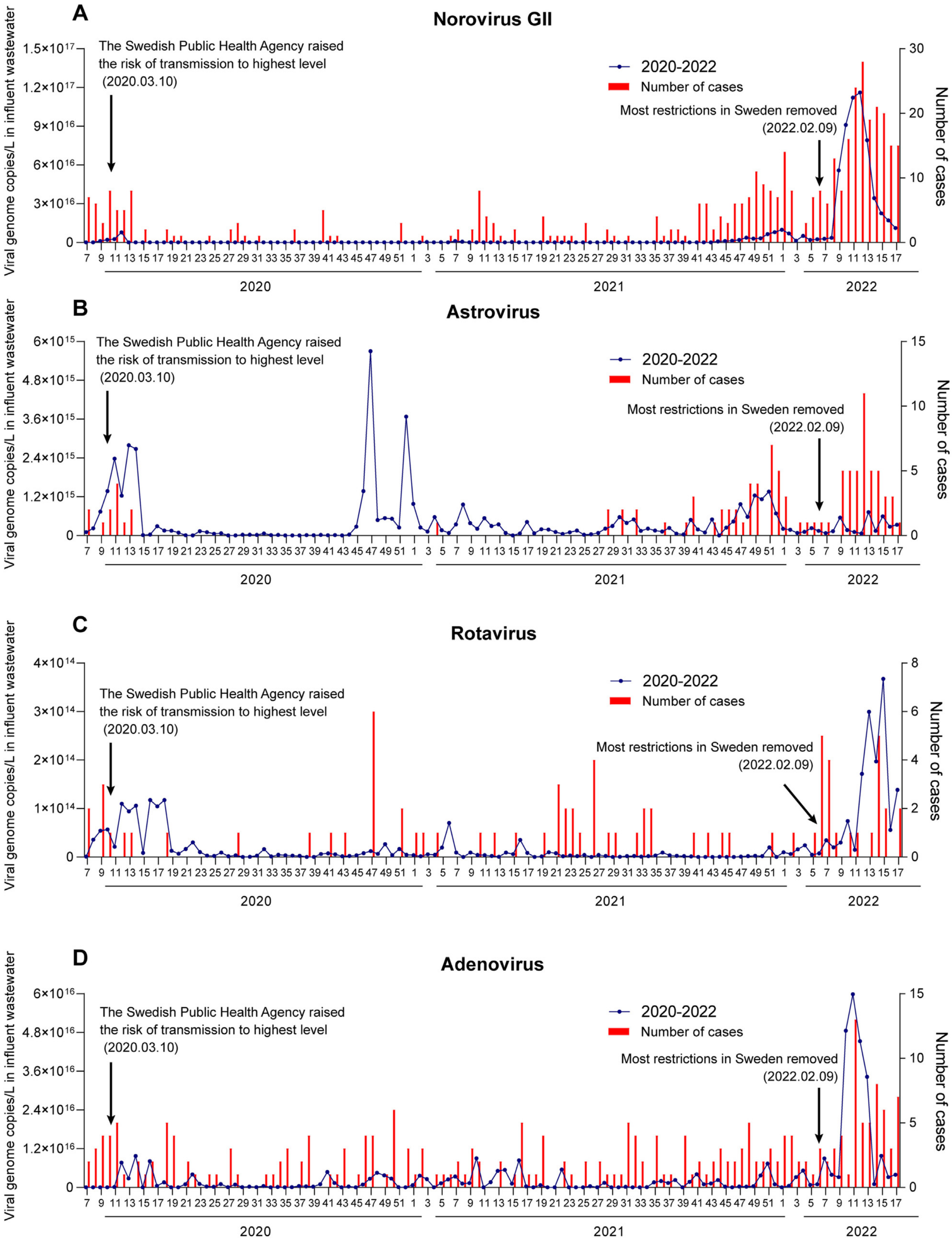


Fig. 2. Week variations in the concentrations of four different viruses, (A) norovirus GII, (B) astrovirus, (C) rotavirus, and (D) adenovirus, in influent wastewater during the SARS-CoV-2 pandemic 2020–2022 (blue line) compared to the number of patients diagnosed with same viruses in Gothenburg area (red bar).

infects children who could not follow the COVID-19 restrictions, and a similar trend has been observed in many regions (Garg et al., 2022; Liu et al., 2022). Furthermore, RSV genomes have been detected in wastewater (Hughes et al., 2022), highlighting the need for additional research to evaluate the feasibility of using wastewater surveillance as a tool for the early warning system of respiratory viruses.

This study contributes to our comprehension of how the Swedish COVID-19 measures affect the spread of other common enteric viruses in the community. This knowledge will be valuable in enhancing our preparedness for future pandemics and epidemics. However, this study has several limitations. Only the wastewater and clinical data from 2017 were utilized as a representative baseline year. Despite the absence of wastewater monitoring data from other years, the monitoring of several calicivirus by the Swedish Public Health Agency revealed that the spread of norovirus in society in 2017 was about the same extent as the previous seasons (Folkhälsomyndigheten, 2018). As a result, we have assumed that 2017 represents an average year prior to the pandemic and have employed it as a reference for comparisons in this study. Nevertheless, the inclusion of wastewater data from other years would have been beneficial in supporting our conclusions. In addition, most of the COVID-19 control measures implemented in Sweden are only recommendations, which were periodically evaluated and updated by the Swedish Public Health Agency to align with the dynamic nature of the pandemic situation. These vague and constantly updated recommendations present a challenge in quantifying the impacts of various COVID-19 interventions on the levels of viruses present in wastewater. Further studies are needed to investigate the efficacy of implementing specific restrictions or recommendations in effectively reducing the transmission of viruses, both within the community and in wastewater, which would be beneficial for managing future pandemics.

In conclusion, this study showed that the number of confirmed cases and the amount of viral genomes in wastewater for norovirus GII and rotavirus decreased significantly during the COVID-19 pandemic 2020–2021, and rapidly rebounded in 2022. These changes corresponded to the changes in the Swedish COVID-19 interventions. Measures, such as keeping social distance and having good hygiene habits, could effectively limit the transmission of some enteric viruses in society, and ultimately protect the risk groups. Monitoring of human enteric viruses in wastewater has proved to be a useful epidemiological tool for understanding their emergence and spread in society during and after the pandemic period.

CRediT authorship contribution statement

Hao Wang: Methodology, Investigation, Data curation, Visualization, Formal analysis, Writing – original draft. **Marianela Patzi Churqui:** Investigation, Formal analysis. **Timur Tunovic:** Investigation. **Lucica Enache:** Resources. **Anette Johansson:** Resources. **Magnus Lindh:** Resources, Writing – review & editing. **Martin Lagging:** Resources, Supervision. **Kristina Nyström:** Project administration, Writing – review & editing. **Heléne Norder:** Conceptualization, Data curation, Funding acquisition, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

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

Acknowledgments

The authors are grateful to staff in the Clinical Microbiology Laboratory at Sahlgrenska University Hospital, Gothenburg for the routine test of common enteric viruses in patients with gastroenteritis. This study was financially supported by the Swedish Research Council for Sustainable Development, Formas, grant number 2020-02710.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165012.

References

Ahmed, S.M., Lopman, B.A., Levy, K., 2013. A systematic review and meta-analysis of the global seasonality of norovirus. PLoS One 8, e75922. https://doi.org/10.1371/journal.pone.0075922.

Ahn, S.Y., Park, J.Y., Lim, I.S., Chae, S.A., Yun, S.W., Lee, N.M., et al., 2021. Changes in the occurrence of gastrointestinal infections after COVID-19 in Korea. J. Korean Med. Sci. 36, e180. https://doi.org/10.3346/jkms.2021.36.e180.

Blom, V., Lonn, A., Ekblom, B., Kallings, L.V., Vaisanen, D., Hemmingsson, E., et al., 2021. Lifestyle habits and mental health in light of the two COVID-19 pandemic waves in Sweden, 2020. Int. J. Environ. Res. Public Health, 18 https://doi.org/10.3390/ijerph18063313.

Castiglioni, S., Schiarea, S., Pellegrinelli, L., Primache, V., Galli, C., Bubba, L., et al., 2022. SARS-CoV-2 RNA in urban wastewater samples to monitor the COVID-19 pandemic in Lombardy, Italy (March–June 2020). Sci. Total Environ. 806, 150816. https://doi.org/10.1016/j.scitotenv.2021.150816.

Celik, C., Gozel, M.G., Turkay, H., Bakici, M.Z., Guven, A.S., Elaldi, N., 2015. Rotavirus and adenovirus gastroenteritis: time series analysis. Pediatr. Int. 57, 590–596. https://doi.org/10.1111/ped.12592.

Flaxman, S., Mishra, S., Gandy, A., Unwin, H.J.T., Mellan, T.A., Coupland, H., et al., 2020. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. Nature 584, 257–261. https://doi.org/10.1038/s41586-020-2405-7.

Folkhälsomyndigheten. Calicivirus – sjukdomsstatistik, 2018.

Folkhälsomyndigheten. Bekräftat fall i Jönköping av nya coronaviruset (2019-nCoV) Folkhälsomyndigheten, 2020.

Folkhälsomyndigheten. RSV-rapport vecka 4, säsong 2021/2022, 2022.

Fukuda, Y., Tsugawa, T., Nagaoka, Y., Ishii, A., Nawa, T., Togashi, A., et al., 2021. Surveillance in hospitalized children with infectious diseases in Japan: pre- and post-coronavirus disease 2019. J. Infect. Chemother. 27, 1639–1647. https://doi.org/10.1016/j.jiac.2021.07.024.

Garg, I., Shekhar, R., Sheikh, A.B., Pal, S., 2022. Impact of COVID-19 on the changing patterns of respiratory syncytial virus infections. Infect Dis Rep 14, 558–568. https://doi.org/10.3390/idr14040059.

Gustavsson, J., Beckman, L., 2020. Compliance to recommendations and mental health consequences among elderly in Sweden during the initial phase of the COVID-19 pandemic-a cross sectional online survey. Int. J. Environ. Res. Public Health, 17 https://doi.org/10.3390/ijerph17155380.

Gutierrez Sanchez, L.H., Shiau, H., Baker, J.M., Saaybi, S., Buchfellner, M., Britt, W., et al., 2022. A case series of children with acute hepatitis and human adenovirus infection. N. Engl. J. Med. 387, 620–630. https://doi.org/10.1056/NEJMoa2206294.

Hellmer, M., Paxeus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., et al., 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.

Hughes, B., Duong, D., White, B.J., Wigginton, K.R., Chan, E.M.G., Wolfe, M.K., et al., 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.

Izquierdo-Lara, R., Elsinga, G., Heijnen, L., Munnink, B.B.O., Schapendonk, C.M.E., Nieuwenhuijse, D., et al., 2021. Monitoring SARS-CoV-2 circulation and diversity through community wastewater sequencing, the Netherlands and Belgium. Emerg. Infect. Dis. 27, 1405–1415. https://doi.org/10.3201/eid2705.204410.

Kim, J.S., Lee, S.K., Ko, D.H., Hyun, J., Kim, H.S., Song, W., et al., 2017. Associations of adenovirus genotypes in Korean acute gastroenteritis patients with respiratory symptoms and intussusception. Biomed. Res. Int. 2017, 1602054. https://doi.org/10.1155/2017/1602054.

Lastra, A., Botello, J., Pinilla, A., Urrutia, J.I., Canora, J., Sanchez, J., et al., 2022. SARS-CoV-2 detection in wastewater as an early warning indicator for COVID-19 pandemic. Madrid region case study. Environ. Res. 203, 111852. https://doi.org/10.1016/j.envres.2021.111852.

Liu, P., Xu, M., Lu, L., Ma, A., Cao, L., Su, L., et al., 2022. The changing pattern of common respiratory and enteric viruses among outpatient children in Shanghai, China: two years of the COVID-19 pandemic. J. Med. Virol. 94, 4696–4703. https://doi.org/10.1002/jmv.27896.

Ludvigsson, J.F., 2020. The first eight months of Sweden's COVID-19 strategy and the key actions and actors that were involved. Acta Paediatr. 109, 2459–2471. https://doi.org/10.1111/apa.15582.

Mack, D., Gohl, P., Kolbert, M., Schui, D., Kusters, U., Harzer, O., et al., 2021. Where have the enteric viruses gone? - differential effects on frequent causes of infectious diarrhoea by SARS-CoV-2 pandemic lockdown measures. Infect Prev Pract 3, 100184. https://doi.org/10.1016/j.infpip.2021.100184.

Maldonado-Barrueco, A., Garcia-Rodriguez, J., Yaniz-Ramirez, J., Serrano-Vaquero, I., Parra-Alonso, J.C., Vega-Nieto, C., et al., 2022. Impact of the SARS-CoV-2 pandemic on the prevalence and incidence of gastrointestinal viruses in children up to five years old: a retrospective cohort study. Microbiol Spectr 10, e0266921. https://doi.org/10.1128/spectrum.02669-21.

Ndiaye, A.K., Diop, P.A., Diop, O.M., 2014. Environmental surveillance of poliovirus and non-polio enterovirus in urban sewage in Dakar, Senegal (2007-2013). Pan Afr. Med. J. 19, 243. https://doi.org/10.11604/pamj.2014.19.243.3538.

Peccia, J., Zulli, A., Brackney, D.E., Grubaugh, N.D., Kaplan, E.H., Casanovas-Massana, A., et al., 2020. Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. Nat. Biotechnol. 38, 1164–1167. https://doi.org/10.1038/s41587-020-0684-z.

Pellegrinelli, L., Galli, C., Binda, S., Primache, V., Tagliacarne, C., Pizza, F., et al., 2019. Molecular characterization and phylogenetic analysis of enteroviruses and hepatitis a viruses in sewage samples, northern Italy, 2016. Food Environ Virol 11, 393–399. https://doi.org/10.1007/s12560-019-09401-4.

Perra, N., 2021. Non-pharmaceutical interventions during the COVID-19 pandemic: a review. Phys. Rep. 913, 1–52. https://doi.org/10.1016/j.physrep.2021.02.001.

Polo, D., Quintela-Baluja, M., Corbishley, A., Jones, D.L., Singer, A.C., Graham, D.W., et al., 2020. Making waves: wastewater-based epidemiology for COVID-19 - approaches and challenges for surveillance and prediction. Water Res. 186, 116404. https://doi.org/10.1016/j.watres.2020.116404.

Saguti, F., Magnil, E., Enache, L., Churqui, M.P., Johansson, A., Lumley, D., et al., 2021. Surveillance of wastewater revealed peaks of SARS-CoV-2 preceding those of hospitalized patients with COVID-19. Water Res. 189, 116620. https://doi.org/10.1016/j.watres.2020.116620.

Schollin, Ask L., 2021. Global and Swedish review of rotavirus vaccines showed considerable reductions in morbidity and mortality. Acta Paediatr. 110, 3161–3169. https://doi.org/10.1111/apa.16046.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Tate, J.E., Panozzo, C.A., Payne, D.C., Patel, M.M., Cortese, M.M., Fowlkes, A.L., et al., 2009. Decline and change in seasonality of US rotavirus activity after the introduction of rotavirus vaccine. Pediatrics 124, 465–471. https://doi.org/10.1542/peds.2008-3528.

Vasickova, P., Pavlik, I., Verani, M., Carducci, A., 2010. Issues concerning survival of viruses on surfaces. Food and Environmental Virology 2, 24–34. https://doi.org/10.1007/s12560-010-9025-6.

Wang, H., Sikora, P., Rutgersson, C., Lindh, M., Brodin, T., Bjorlenius, B., et al., 2018. Differential removal of human pathogenic viruses from sewage by conventional and ozone treatments. Int. J. Hyg. Environ. Health 221, 479–488. https://doi.org/10.1016/j.ijheh.2018.01.012.

Wang, H., Neyvaldt, J., Enache, L., Sikora, P., Mattsson, A., Johansson, A., et al., 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 https://doi.org/10.1128/AEM.02073-20.

Wang, H., Churqui, M.P., Tunovic, T., Enache, L., Johansson, A., Karmander, A., et al., 2022. The amount of SARS-CoV-2 RNA in wastewater relates to the development of the pandemic and its burden on the health system. iScience 25, 105000. https://doi.org/10.1016/j.isci.2022.105000.

Wang, L.P., Han, J.Y., Zhou, S.X., Yu, L.J., Lu, Q.B., Zhang, X.A., et al., 2021. The changing pattern of enteric pathogen infections in China during the COVID-19 pandemic: a nation-wide observational study. Lancet Reg Health West Pac 16, 100268. https://doi.org/10.1016/j.lanwpc.2021.100268.

Wu, F., Xiao, A., Zhang, J., Moniz, K., Endo, N., Armas, F., et al., 2022. SARS-CoV-2 RNA concentrations in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases. Sci. Total Environ. 805, 150121. https://doi.org/10.1016/j.scitotenv.2021.150121.

Wu, L., Teng, Z., Lin, Q., Liu, J., Wu, H., Kuang, X., et al., 2020. Epidemiology and genetic characterization of classical human Astrovirus infection in Shanghai, 2015-2016. Front. Microbiol. 11, 570541. https://doi.org/10.3389/fmicb.2020.570541.