


## Prevalence of enteric viruses in wastewater in Egypt after the COVID-19 pandemic

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### ABSTRACT

People's hygienic habits greatly affect the spreading rate of enteric viruses. After the COVID-19 pandemic, many people followed announced precautions and improved their hygienic status to protect themselves from SARS-CoV-2 infection. Here, we studied if this indirectly affected the prevalence of enteric viruses in Egypt. A total of 21 samples (one sample per week) were collected from the Zenin wastewater treatment plant (WWTP) through the period between August 2021 and March 2022. Detection of adenovirus, hepatitis A virus (HAV), and rotavirus showed their presence in 66, 14.3, and 9.5% of the collected samples, respectively. Comparing those percentages to previously published data concerned with the detection of the same viruses from the same WWTP or others revealed a remarkable decrease in the prevalence of the three viruses after the COVID-19 pandemic. This allows the conclusion that safety precautions against SARS-CoV-2 lead indirectly to a reduction of adenovirus, HAV, and rotavirus prevalence rates.

**Key words:** adenovirus, COVID-19 pandemic, enteric viruses, Hepatitis A virus, rotavirus, wastewater

### HIGHLIGHTS

- Safety precautions against SARS-CoV-2 lead indirectly to a remarkable reduction in the prevalence rates of enteric viruses.
- Prevalence of adenovirus decreased to 66% after the pandemic.
- Hepatitis A virus showed 14.3% positivity of tested samples.
- Rotavirus prevalence became 9.5% after the pandemic.
- All those prevalence percentages were remarkably less than detection percentage before the COVID-19 pandemic.

### INTRODUCTION

Prevalence of enteric viruses is affected by hygienic status, therefore, their infection rates increase in countries with low hygienic levels (Payment 2001). According to the WHO, enteric viruses such as HAV, HEV, rotavirus, adenovirus, norovirus, and others are classified as pathogens having moderate to high harmful effects on human health (WHO 2011). Reports detected those viruses in Egypt (Shaheen *et al.* 2018; Elmahdy *et al.* 2020).

Usage of wastewater in crops irrigation makes them a source of enteric viral infection (Gerba & Choi 2006) and this was shown in different reports in Egypt with the presence of human adenovirus and rotavirus in green onion and lettuce (Shaheen *et al.* 2019), presence of HAV and human norovirus in watercress, strawberry, and coriander (Elmahdy *et al.* 2022).

Following hygienic habits such as carefully washing fruits and vegetables before eating, washing hands with soap and water, cleaning surfaces from dust, and using water filters in houses can help protect humans from enteric virus infections.

After the outbreak of the COVID-19 pandemic, many people tended to follow announced hygienic precautions to protect themselves from SARS-CoV-2 infection including not only the above-mentioned precautions but in addition many people soak fruits and vegetables in water containing vinegar before eating, give up buying street food and boil drinking water, which can all protect from enteric viruses transmission.

Thus, here we aimed to study if safety precautions against SARS-CoV-2 indirectly affected the prevalence of enteric viruses among Egyptians.

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## MATERIALS AND METHODS

### Wastewater samples

One litre of wastewater was collected weekly through the period between August 2021 and March 2022 (21 samples in total) from the Zenin wastewater treatment plant (WWTP) which is one of the largest WWTPs in the Giza government, Egypt. It receives 275,000–550,000 m<sup>3</sup>/day with an average of 385,000 m<sup>3</sup>/day of wastewater flow (Latif 2022).

### Adsorption-elution water concentration method

50 mL of 1 M aluminium chloride was added to each wastewater sample to give a final concentration of 0.05 M followed by adjusting pH to 3.5 with 1 N HCl. Samples were filtered through 0.45 µm nitrocellulose membrane then 200 mL 0.5 mM H<sub>2</sub>SO<sub>4</sub>, pH 3.0 passed after to ensure viral particle retention and eliminate all bio-solids. The membrane was removed from the holder and soaked in a Petri dish containing 11 mL of 1 mM NaOH; pH 10.5 for 10 min. Later, the upper surface of the membrane was scraped to elute the viruses. Volumes of 50 µL 50 mM H<sub>2</sub>SO<sub>4</sub> and 50 µL 100X 1 nM Tris-EDTA, pH 8.0 were added to neutralize the elute and samples were stored at –20 °C until use (Katayama *et al.* 2002).

### Enteric virus detection in concentrated wastewater samples

A volume of 140 µL of concentrated sample was subjected to viral RNA/DNA extraction (Qiagen, Germany) followed by reverse transcription (SuperScript III Reverse transcriptase, Invitrogen, USA) for RNA viruses. Quantitative RT-PCR was performed using SYBER green master mix (Luna, New England) and in-house designed primers (Table 1). Reaction mixture was subjected to the following temperature conditions, initial denaturation: 95 °C for 60 s, denaturation at 95 °C for 15 s, annealing: 55 °C for adenovirus and 57 °C for both HAV and rotavirus for 10 s, extension at 60 °C for 15 s for 40 cycles (Buston *et al.* 2009).

## RESULTS AND DISCUSSION

Since the beginning of the COVID-19 pandemic, many people cared more about the hygienic status of their children, family, and houses and stopped many habits such as eating food from shops or street food, eating fruits and vegetables washed only with water and carefully washed their hands with soap and water and used disinfectants to sterilize many products before taking them inside their houses to protect themselves from SARS-CoV-2 infection. As the prevalence of enteric viruses in a given population depends greatly on hygienic status, we believe that protective precautions against SARS-CoV-2 must have an indirect effect on decreasing their infection rate.

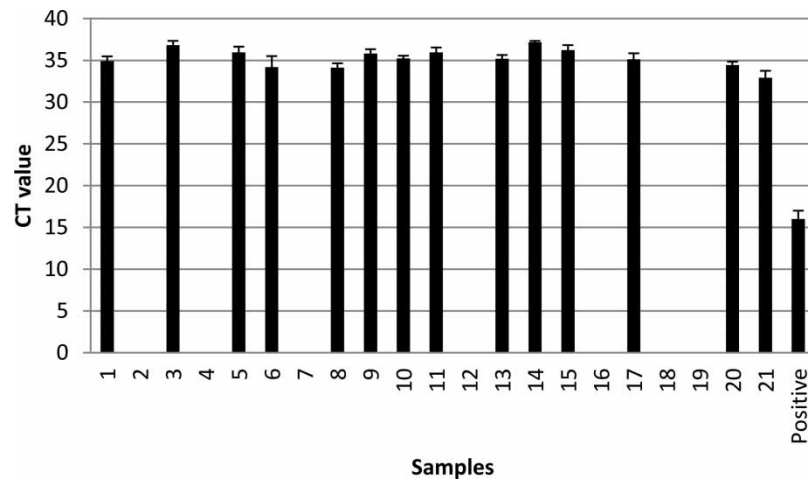
To prove that, 21 samples were collected weekly through the period from August 2021 to March 2022 covering periods of increasing infection rates among Egyptians during the summer months and school season.

Samples were collected from Zenin WWTP, which is one of the largest WWTP in Egypt and receives a huge amount of wastewater per day, and thus detection rate of viruses in it can represent a wide sector of other WWTPs.

Adenovirus is one of the enteric viruses spreading in Egypt. Results in Figure 1 and Table 2 showed that 66% of samples were positive. Although this is a high prevalence, comparing it to pre-corona pandemic reports, it is lower than the recorded prevalence in the same WWTP in 2017 in untreated and treated wastewater which was 84 and 50%, respectively (Elmahdy *et al.* 2019). Another study also concerned with the same WWTP showed 91.7% positivity of adenovirus (El-Senousy *et al.*

**Table 1** | Sets of in-house primers used for viral detection

Virus	Primer sequence	Annealing temperature
Adenovirus	F: CCCACGGTGGCGCCTAC R: AACCGCAGCGTCAAACGCT	55
HAV	F: TGATTAGCATGGAGCTGTAGG R: CAAAGCATCTTTCATAGAAGTA	57
Rotavirus	F: TCAGTCTATTTTAAAGAGTACTCA R: TTTGATTCTCCCGATTGTTGATA	57



**Figure 1** | CT values for adenovirus detection in wastewater samples showing 14 positive samples with prevalence percentage of 66%.

**Table 2** | Number of adenovirus, HAV and rotavirus positive wastewater samples

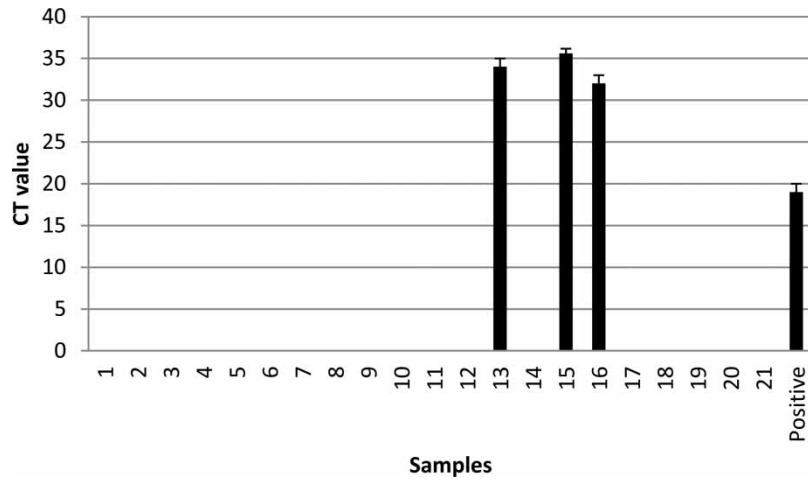
Months/number of samples	Viruses		
	Adenovirus	HAV	rotavirus
August/1	1	0	0
September/3	2	1	0
October/3	2	2	0
November/2	1	0	0
December/2	1	0	0
January/4	3	0	1
February/4	2	0	0
March/2	2	0	1
Positive/total	14/21	3/21	2/21
Prevalence percentages	66%	14.3%	9.5%

2013). On the contrary, another study reporting on a different WWTP called Abou-Rawash showed the presence of adenovirus in 50% of wastewater samples (Elmahdy *et al.* 2020).

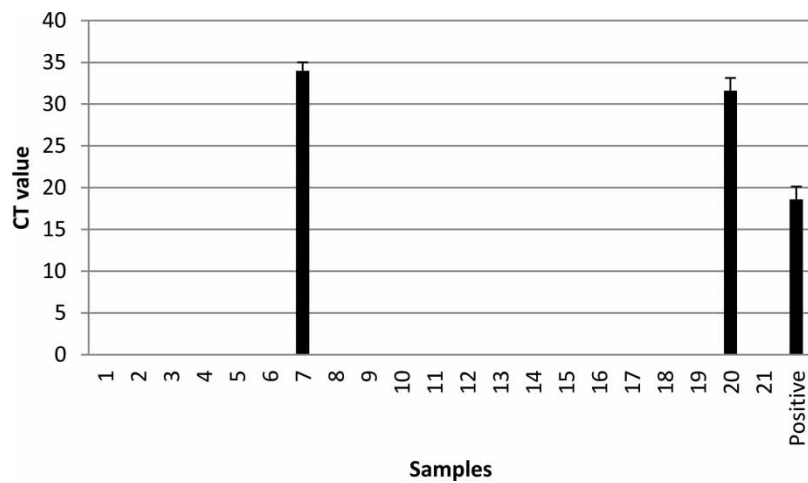
After a number of reported HAV outbreaks among European travellers, Egypt was considered to be an endemic country (Frank *et al.* 2007; MacDonald *et al.* 2013) due to the high prevalence rate of the virus (Divizia *et al.* 1999). Reports showed the presence of HAV in 83.3% of wastewater samples collected from Zenin (Hamza *et al.* 2017). Results in Figure 2 and Table 2 showed that only 14.3% of the samples were positive, indicating a sharp decrease in HAV spreading.

Finally, our results showed a prevalence rate of 9.5% to rotavirus (Figure 3; Table 2), which when compared with the pre-corona period indicates a remarkable decrease. The virus was detected in Egyptian wastewater with different percentages starting from 50% in Abou-Rawash WWTP (Elmahdy *et al.* 2020) and 25% in the same WWTP (Shaheen *et al.* 2018) to 21.9% in Zenin (Shaheen & Elmahdy 2019).

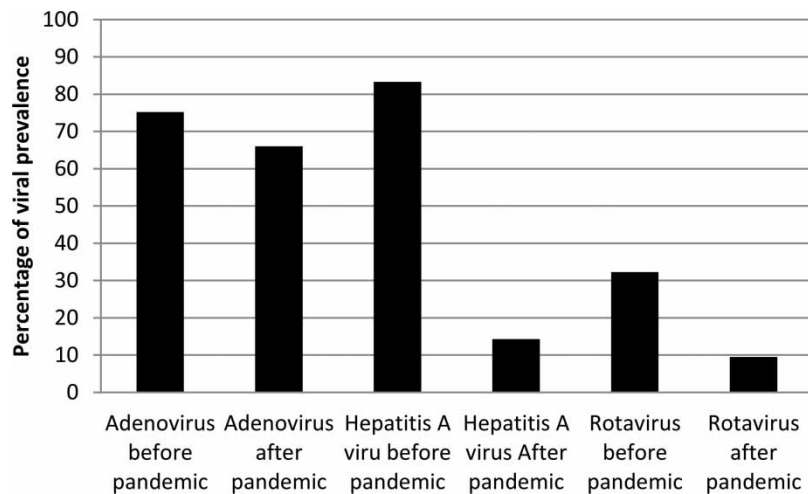
Figure 4 compared prevalence rates of the three detected viruses before and after the COVID-19 pandemic. Results showed that before the pandemic the mean percentages of detection of adenovirus, hepatitis A virus, and rotavirus were 75.2, 83.3, and 32.2%, respectively, while after the pandemic it was 66, 14.3, and 9.5%, respectively, confirming a real decrease in the prevalence of the three tested enteric viruses.



**Figure 2** | CT value of hepatitis A virus detection in wastewater samples showing three positive samples with prevalence percentage of 14.3%.



**Figure 3** | CT value of rotavirus detection in wastewater samples showing two positive samples with prevalence percentage of 9.5%.



**Figure 4** | Comparing prevalence rates of adenovirus, hepatitis A virus and rotavirus before and after the COVID-19 pandemic.

## CONCLUSION

Safety precautions against SARS-CoV-2 lead indirectly to a remarkable reduction in the prevalence rates of adenovirus, HAV, and rotavirus prevalence in Egypt.

## FUNDING

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## DATA AVAILABILITY STATEMENT

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

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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