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Detection and quantification of adenovirus, polyomavirus, and papillomavirus in urban sewage

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ABSTRACT

The objective of this study was to assess the occurrence and seasonal frequency of human adenovirus (HAdV), human polyomavirus (HPyV), and human papillomavirus (HPV) in urban sewage. The detection of these viruses was carried out by polymerase chain reaction (PCR), and then the viral concentrations in the positive samples were quantified by quantitative PCR (qPCR). Additionally, HAdV and HPyV genotyping was also performed by PCR. A total of 38/60 (63.3%) positive samples were found. HAdV was the most prevalent virus (26/60; 43.3%), followed by HPyV (21/60; 35%) and HPV (21/60; 35%). The viral concentrations ranged from 3.56×10^2 to 7.55×10^7 genome copies/L. The most common dual viral agents was found between HAdV and HPyV, in eight samples (8/38, 21%). HAdV types 40 and 41 as well as HPyV types JC and BK were identified, with HAdV-40 and HPyV JC being the most prevalent types. Furthermore, the detection rates of HAdV, HPyV, and HPV were higher during the winter season than the other seasons. The high prevalence of HAdV and HPyV supports their suitability as viral indicators of sewage contamination. Furthermore, this study demonstrates the advantages of environmental surveillance as a tool to elucidate the community-circulating viruses.

Key words: adenovirus, papillomavirus, polyomavirus, qPCR, sewage

HIGHLIGHTS

- This study aimed to investigate the occurrence and seasonality variation of HAdV, HPyV, and HPV in raw sewage collected regularly over 12 months from sewage pump stations.
- This study represents the first data about the occurrence of HAdV, HPyV, and HPV in Zagazig city, Al Sharqia Governorate, Egypt.
- This study supports using both HAdV and HPyV as indicators than either of them as a single fecal indicator.

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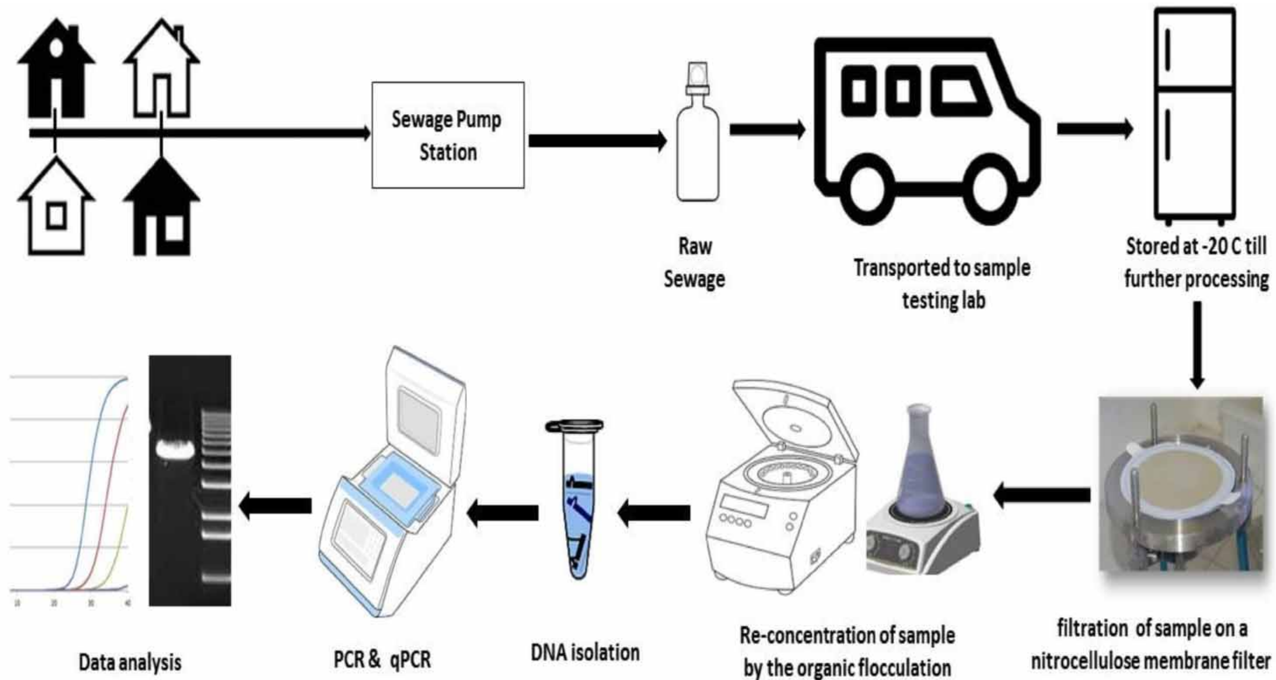
GRAPHICAL ABSTRACT



INTRODUCTION

Environmental monitoring is an effective strategy for the control of water quality since it enables the tracing of contamination sources to avoid potential diseases caused by contaminated water. Additionally, it provides information on possible exposures linked to recreational water use (Hlavsa *et al.* 2021). Human enteric viruses are a significant cause of gastrointestinal diseases and can be found in environmental water contaminated through various modes (Gibson 2014; Desselberger 2017). The majority of human enteric viruses can spread by the fecal–oral route and remain highly stable to environmental stresses for long periods (Rzezutka & Cook 2004). Raw sewage, if discharged to the environment without prior treatment, represents one of the major sources of several pathogens associated with various illness such as gastroenteritis (Kesari *et al.* 2021).

There are more than 140 different types of enteric viruses identified in human feces and urine of which adenovirus, hepatitis A virus, rotavirus, polyomavirus, norovirus genotypes I and II, and enterovirus (EV) are most commonly detected in the environment (La Rosa *et al.* 2014; Shaheen *et al.* 2019, 2022; Stobnicka-Kupiec *et al.* 2022; Tubatsi *et al.* 2022; Anand *et al.* 2023; Kumthip *et al.* 2023). For this reason, enteric viruses play a significant role in contaminated water related to sporadic cases and outbreaks of sever gastroenteritis. Enteric virus diseases are frequently asymptomatic in healthy people or result in various diseases ranging from mild diarrhea to severe or chronic symptoms in the adults, young children, and immunocompromised persons (Desselberger & Gray 2013). There are some studies showing that processes applied in wastewater treatment do not completely eliminate enteric viruses (Lazarova *et al.* 2001; Blatchley *et al.* 2007; Shaheen *et al.* 2018), even from effluents subjected to UV treatment or adequate concentrations of chlorine (Sano *et al.* 2016).

Human papillomaviruses (HPVs), belonging to the Papilomaviridae family, and polyomaviruses (HPyVs), belonging to the family Polyomaviridae, were first discovered in the 1950s and 1970s, respectively. Both viruses are non-enveloped, small (40–60 nm in diameter), and have double-stranded DNA genomes. Recently, HPVs and HPyVs have been identified in the urine and feces of infected individuals (Brinkman *et al.* 2004; Rachmadi *et al.* 2016). They have also been detected in rivers, wastewater, sediment, and seawater, in tap water, and in swimming pools (La Rosa *et al.* 2013, 2015; Fratini *et al.* 2014; Di Bonito *et al.* 2015; Iaconelli *et al.* 2015; Rachmadi *et al.* 2016; Ahmed *et al.* 2019; Itarte *et al.* 2021). The

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transmission route of these viruses is not yet identified; however, waterborne transmission is found to be likely (Fratini *et al.* 2014).

Human adenovirus (HAdV), belonging to the genus Mastadenovirus and the family Adenoviridae, contain linear double-stranded DNA, with over 50 HAdV serotypes identified and divided into seven species (HAdV-A to HAdV-G) (8–11) (Walsh *et al.* 2010; Harrach *et al.* 2011; Liu *et al.* 2011). HAdVs cause a wide range of illnesses like enteric, ocular, and respiratory infections. HAdV-F species is commonly linked to childhood gastroenteritis (Wold & Horwitz 2007). Many HAdV types are shed for months and are excreted in high numbers (up to 10^{11} particles/g feces) (Wold & Horwitz 2007). HAdVs are more tolerant to chemical/physical agents and to UV light, particularly HAdV-F, than other enteric viruses and fecal indicator bacteria (Gerba *et al.* 2002; Nwachuku *et al.* 2005).

In the current study, a 12-month survey (June 2021–May 2022) was performed in order to evaluate the occurrence of HAdVs, HPyVs, HPVs in raw sewage samples collected from wastewater treatment plants (WWTP) located at Zagazig city, Sharqia Governorate, Egypt. This study aims to enrich the poorly available data on environmental virological studies in this area, to demonstrate the advantages of environmental surveillance to investigate the spread of viruses circulating within a given community, and to underline the importance for the design and support of long-term surveys in this area.

MATERIALS AND METHODS

Sampling area and collection of samples

A total of 60 sewage samples were collected between June 2021 and May 2022 from a sewage pump station in Zagazig city which is the largest city in Al Sharqia Governorate, Egypt with a population of 1.4 million. Samples (0.5–1.0 L) were collected weekly using the grab sampling technique, transported on ice to the laboratory and stored at 4 °C until analysis.

Viral concentration in wastewater samples

Viruses from each sample of volume 500–1,000 mL were concentrated by filtration on a nitrocellulose membrane filter as described by USEPA (2001). In brief, the pH of sample was adjusted to 3.5 by 1-N HCl, and then the sample was concentrated by filtration on a nitrocellulose membrane filter (0.2 μm pore size, and 142 mm diameter). Afterward, viruses were eluted from the membrane filter using 100 mL of 3% beef extract-0.05-M glycine solution (pH 9.5). Eluates were further concentrated by the organic flocculation method (Katzenelson *et al.* 1976). The pellet was suspended in 500 μL of phosphate buffer saline (PBS) (pH 7.2) and the suspension was stored at −20 °C until used.

DNA extraction

Viral nucleic acids from 250 μL of concentrate were extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's guidelines, resulting in 60 μL of extracted DNA. To examine possible cross-contamination, nuclease-free water as a negative control of isolation was used with each viral DNA extraction set. To monitor PCR inhibition, a representative sample was inoculated with 7.2×10^7 GC/mL of human adenovirus type 2 (Hernroth *et al.* 2002) and 7×10^6 GC/mL of murine norovirus (MNV-1) and as a sample process control virus, previously tested negative for MNV-1 by qPCR (Lee *et al.* 2015), and no inhibitory effects could be observed (data are not shown).

HAdV genomic detection

We performed nested PCR for the analysis of the presence of human adenovirus F40/41 by targeting hexon genes of adenovirus F40/41 (Allard *et al.* 1992; Pring-Akerblom & Adrian 1994; Puig *et al.* 1994) In the first round, to a total of 25 μL of reaction, we used 5 μL of DNA extract, 12.5 μL of GoTaq® Green Master Mix (Promega, USA), 0.5 μL of forward primer (hexAA1885: 5′-GCCGCAGTGGTC TTACATGCACATC-3′), 0.5 μL of reverse primer (hexAA1913: 5′-CAGCACGCCGCG GATGTCAAAGT-3′), and 9 μL of nuclease-free water. To genotype the positive samples, we used 5 μL of DNA extract from first PCR-positive samples as a template and the amplification was carried out as the pervious reaction except using a group-specific primer (H1: 5′-TTGACATCCGCGGCGTGCTG-3′) and type-specific primers (H40: 5′-TATTCTGAGACCAGTTAGTT-3′) for Ad40 type or (H41: 5′ CTGCAGTCCAGGTTTGGCCA-3′) for Ad41 type. The cycling conditions for both first and nested PCR were as follows: 95 °C for 15 min followed by 35 cycles that consisted of 95 °C for 30 s, 57 °C

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for 30 s, 72 °C for 30 s, and finally 72 °C for 5 min. All PCR assays included negative controls. The expected size band was 301 bp for first PCR, 939 bp for Ad40 type, and 942 bp for Ad41 type.

HPV genomic detection

Samples were analyzed by nested PCR assay and were able to target the L1 coding region of a broad spectrum of cutaneous and mucosal HPV genotypes (Manos *et al.* 1989; de Roda *et al.* 1995). The first round used forward primer (MY09: 5′GCA CAG GGA CAT AAC AAT GG3′) and reverse primer (MY11: 5′ CGT CCA AAA GGA AAC TGA TC 3′), providing an approximately 452-bp amplicon. The primers used in the second round were GP5+ (5′TTTGTTACTGTGGTAGATACTAC3′) and GP6+ (5′ GAAAAATAAACTGTAAATCATATTC3′) with a 150 bp product. Amplification conditions for the first and second round were as follows: 94 °C for 5 min, followed by 40 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s, and one cycle: 72 °C for 5 min.

HPyV genomic detection

A semi-nested PCR using two outer primer pairs (FW: 5′-AAGTCT TTA GGG TCT TCT AC-3′) and (Rev: 5′-GTG CCA ACCTAT GGA ACA GA-3′) was used to amplify a part (176-bp) common to both viruses. To discriminate JCPyV and BKPyV, the common primer (5′-AAG TCTTTAGGGTCTTCTAC-3′) was combined with an inner primer (BKV: 5′-GAGTCCTGG TGGAGTTCC-3′) to amplify a BKV fragment of 149 bp or with (JCV: 5′-GAATCCTGGTGG AATACA-3′) producing a JCV fragment of 146 bp. Reactions for the first and second rounds were carried out under the following thermal cycler conditions: 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min, and one cycle: 72 °C for 5 min (Haghighi *et al.* 2019).

SYBR green quantitative real-time PCR

Quantification of HAdV, HPV, and HPyV DNA in positive samples was carried out using Maxima SYBR Green/Rox qPCR master mix (2×) (Fermentas, California, USA). For molecular detection of HAdV, HPyV, and HPV the qPCR was carried out according to Dong *et al.* (2010), de Araujo *et al.* (2009) and Biel *et al.* (2000), respectively. Primers used in this assay were JTVFF (Adv40–41: AAC TTT CTC TCT TAA TAG ACG CC) and JTVFR (Adv40–41: AGG GGG CTA GAA AAC AAA A) for HAdV; GP5 + /GP6 + primers for HPV; and PV-TMFOR (TCTATTACTAAACA-CAGCTTGACT) and PV-BACK (GGTGCCAACCTATGGAACAG) for HPyV. Standard curves were prepared by 10-fold serial dilutions (10^1–10^8 copies/mL) of positive control plasmids (pBR22 for HAdV and pCR2.1-TOPO for HPV and HPyV). The reaction mixture (25 μL) contained 6.5 μL (100 ng/μL) of extracted DNA, 12.5 μL of Maxima SYBR Green/Rox qPCR master mix (2×) (Fermentas, California, USA), 3 μL (30 pmol/μL) of each forward and reverse primer, and nuclease-free water up to 25 μL. This qPCR mixture was transferred into the Rotor-Gene Q system. Each run used ultra-pure water as a non-template control to ensure that the assay was free of contamination. For each sample, the fluorescence signal data were collected at the end of each extension step and the sample was considered positive if its fluorescence exceeded the threshold.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 5.0 (USA) technology. The critical *P*-value for the test was set at 0.05. A one-way variance analysis was used to test the associations between viral concentrations in the positive samples.

RESULTS

Detection of HAdV, HPyV, and HPV

From June 2021 to May 2022, a total of 60 sewage samples were tested for the presence of HAdV, HPyV, and HPV. In total, viruses were detected in 63.3% of samples. HAdV, HPyV, and HPV were detected in 43.3% (26/60), 35% (21/60), 15% (9/60) of sewage samples, respectively. HAdV genome was detected in all months, HPyVs were also found in all months, except for the samples of August 2021 and November 2021 that were negative for the HPyV genome while the HPV genome was absent in some months (September 2021, December 2021, and April 2022). The results are summarized in Table 1.

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Table 1 | PCR detection of HAdV, HPyV, and HPV in sewage samples

Data	Sewage samples														
	HAdV					HPyV					HPV				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
June 2021					■							■			
July 2021		■					■								
Aug 2021				■						■					
Sept 2021	■							■	■						■
Oct 2021		■	■			■					■				
Nov. 2021					■		■		■	■					
Dec 2021	■		■	■		■	■	■	■					■	
Jan 2022	■	■	■	■	■		■		■			■	■		
Feb 2022	■			■	■	■		■		■	■				
March 2022		■		■										■	
April 2022	■		■		■		■	■							
May 2022	■	■	■			■			■				■		
Total	26 (43.3%)					21 (35%)					9 (15%)				

HAdV and HPyV genotypes by PCR

PCRs with nested primers were used to monitor the variability of the types of human adenovirus and polyomavirus. Of 26 wastewater samples that were positive for adenovirus, 38.5% (10/26) and 61.5% (16/26) were identified as positive for HAdV-40 and HAdV-41 types, respectively. Of 21 wastewater samples that were positive for polyomavirus, JC and BK DNAs were found in 61.9% (13/21) and 38.1% (8/21), respectively (Figure 1).

Seasonality of viruses

As for seasonality, increased detection rates of HAdVs, HPyV, and HPV were generally observed in winter (December–February) than the other seasons. In contrast, the lowest detection rates for viruses were found in the hot months (June–August). The detection rate of HAdV in spring, summer, autumn, and winter was 30.8% (8/26), 11.5% (3/26), 15.4% (4/26), and 42.3% (11/26), respectively. HPyV detection rates in spring, summer, autumn, and winter seasons were 19% (4/21), 9.5% (2/21), 28.6% (6/21), 42.8% (9/21), respectively. The detection rates of HPV were 22.2% (2/9), 11% (1/9), 22.2% (2/9), and 44.4% (4/9) in spring, summer, autumn, and winter, respectively (Figure 2).

Distribution of single, double, and multiple viral genomes in the positive samples

Only one targeted virus was found in 60.5% (23/38) of the positive samples, with HAdV being the most frequently detected virus in samples containing a single viral agent, followed by HPyV and HPV. Two viral agents were detected in 12/38 (31.6%) positive samples. Only three positive samples (7.9%) were co-contaminated with the three viruses, as shown in Table 2.

Quantification of HAdV, HPyV, and HPV in sewage samples

Concentration of enteric HAdV in positive samples ranged between 1.72×10^4 and 7.55×10^7 GC/L with a median of 8.54×10^6 GC/L. The concentrations of HAdV-40 ranged between 2.31×10^4 and 3.15×10^7 while HAdV-41 ranged between 1.72×10^4 and 7.55×10^7 GC/L. The viral load of the HPyV genomes in sewage ranged from 1.81×10^3 to 5.52×10^6

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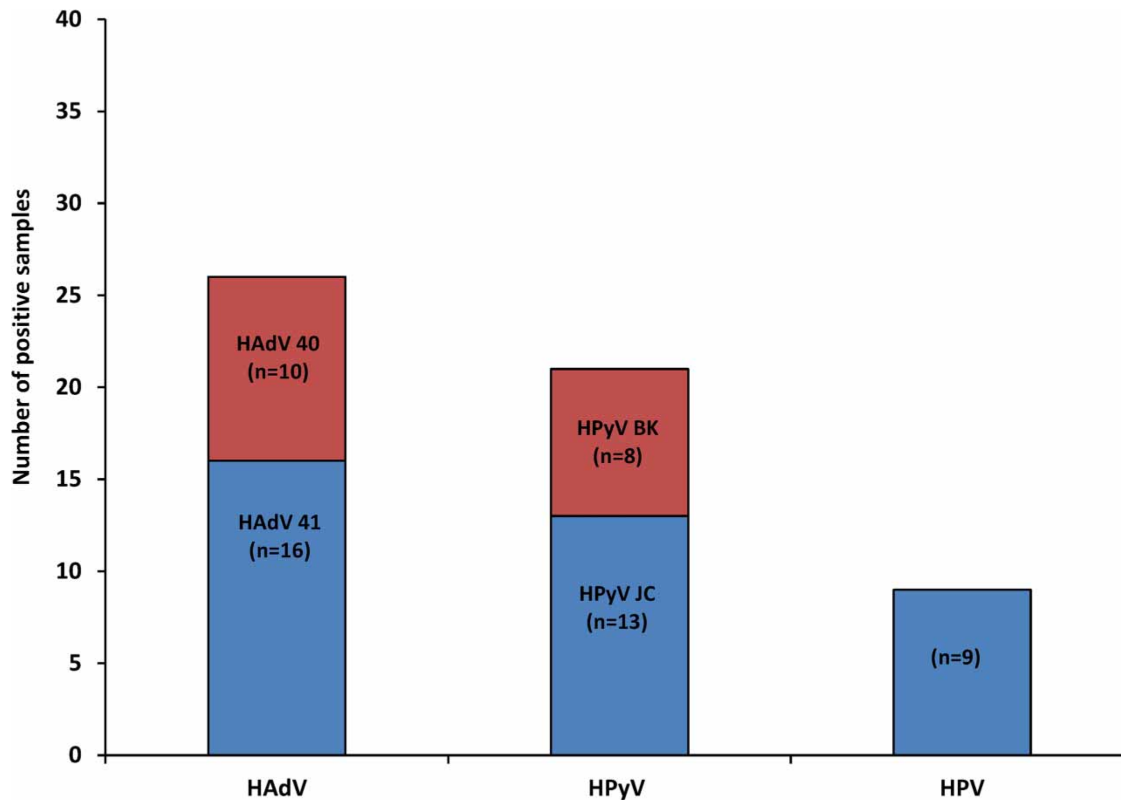

Figure 1 | Detection frequency of HAdV, HPyV, and HPV in sewage samples.

GC/L with a mean viral load of 6.63×10^5 GC/L. The concentration of HPyV BK genome ranged from 1.81×10^3 to 3.15×10^6 GC/L while HPyV JC ranged from 3.81×10^3 to 5.52×10^6 GC/L. The HPV concentration ranged from 3.56×10^2 to 7.41×10^4 GC/L with a mean viral load of 2.10×10^4 (Figure 3). The lowest concentrations of HAdV, HPyV, and HPV were detected in July 2021, September 2021, and May 2022, respectively, while the highest concentrations of the three viruses were recorded in January 2022. There were statistically significant differences between the concentrations of HAdV, HPyV, and HPV in sewage samples ($P < 0.0001$).

DISCUSSION

Human enteric viruses are shed at high concentrations in feces of infected individuals and are primarily transmitted by consumption of contaminated water or food exposed to contaminated water. Wastewater-based epidemiology is an important strategy to better understand the viral contamination sources and the virus epidemiology. The aim of this study was to investigate the prevalence of HAdV, HPyV, and HPV in sewage samples collected from a sewage pump station in Zagazig city, Al Sharqia Governorate, Egypt. This study represents the first data about the occurrence of HAdV, HPyV, and HPV in this region.

In the current study, we used the traditional PCR technique in order to monitor the presence or absence of HAdV, HPyV, and HPV genomes in the collected samples. This technique is an enzymatic reaction so it is susceptible to inhibitors (e.g. organic and inorganic substances, fats, humic and fulvic acids, proteins, etc.) that could be concentrated during the virus concentration process, forming complexes with nucleic acids and resulting in less positive samples. A recommended method to eliminate inhibitory substances is using silica columns for viral nucleic acid extraction (Hale *et al.* 1996). Therefore, in this study, we used this column in the nucleic acid extraction which has successfully removed inhibitors in a previous study (Baggi & Peduzzi 2000). Furthermore, to avoid any false-positive results, to ensure specificity of detection, and to enhance amplification signals, we applied a nested PCR for the detection of HPV genomes and semi-nested for the detection

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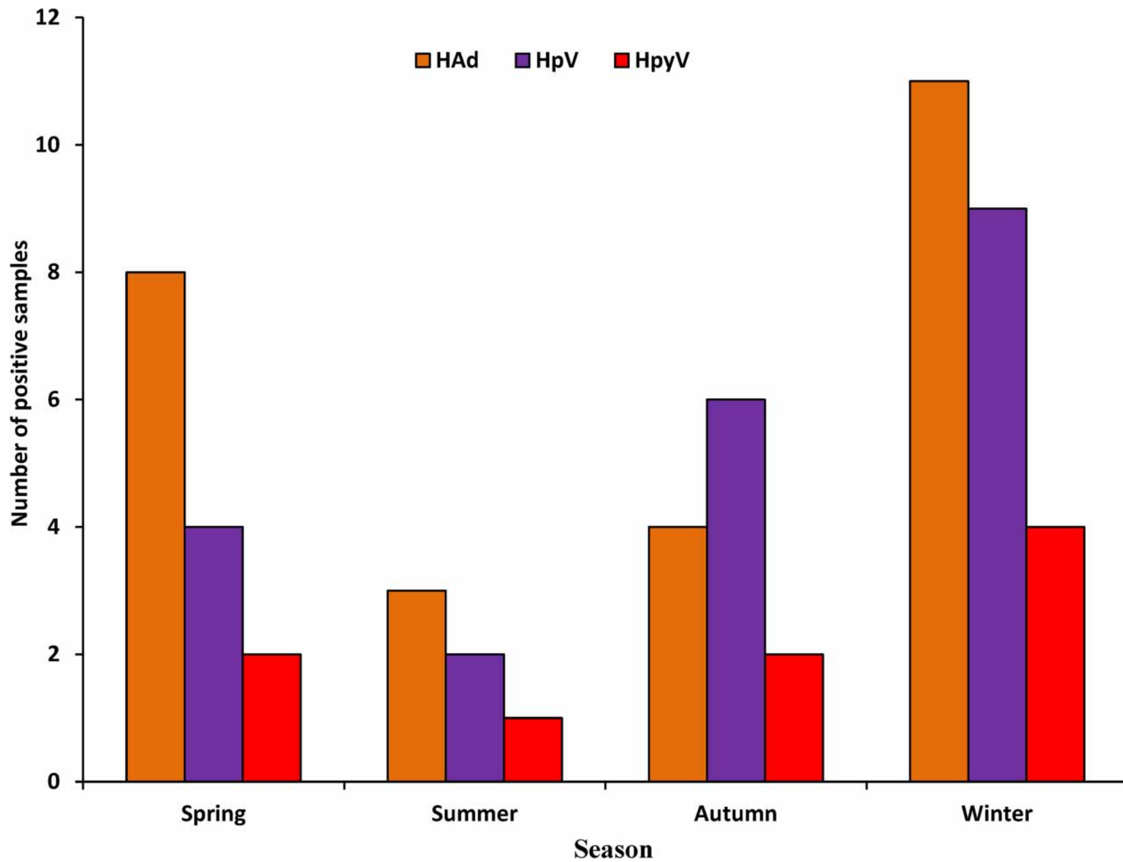

Figure 2 | Seasonal variation of HAdV, HPyV, and HPV in sewage samples ($n = 16$ per season).

of HPyV genomes in all samples. This strategy enhances PCR efficiency to detect a small number of viral genomes in wastewater (Schlindwein *et al.* 2010).

In this study, HAdV, HPyV, and HPV were detected in 43.3% (26/60), 35% (21/60), and 15% (9/60) of sewage samples. Previous studies from Egypt documented higher detection rates in raw sewage, ranging from 53.3 and 100% for HAdV, between 0 and 100% for HPyV, and between 28.3 and 30.5% for HPV (Kamel *et al.* 2009; Hamza *et al.* 2011; Hamza & Hamza 2018; Ahmed *et al.* 2019; Elmahdy *et al.* 2019). A similar detection rate for adenovirus in untreated sewage was reported in two studies from Greece (45.8%) and Iran (44.4%) (Kokkinos *et al.* 2011; Mokhtary-Irani *et al.* 2020). Higher HAdV, HPyV, and HPV positivity were also reported in Uruguay, Greece, New Zealand, Argentina, Norway, and USA

Table 2 | Single, double, and multiple viral agents identified in sewage samples

Virus	No. of positive samples	(%)
Only HAdV	12	31.6
Only HpyV	9	23.7
Only HPV	2	5.3
HAdV + HPyV	8	21
HAdV + HPV	3	7.9
HPyV + HPV	1	2.6
HAdV + HPyV + HPV	3	7.9
Total (%)	38/60 (63.3%)	100

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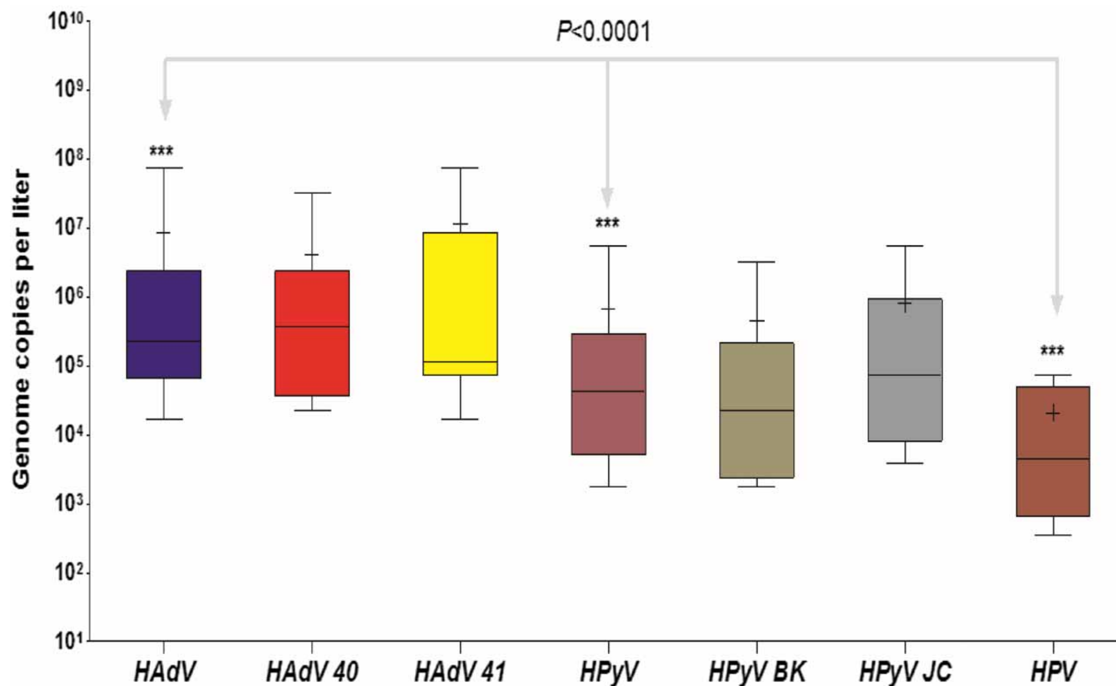

Figure 3 | Viral concentrations in sewage samples, measured in log GC/l.

(Kokkinos *et al.* 2011; Hewitt *et al.* 2013; Grøndahl-Rosado *et al.* 2014; Kitajima *et al.* 2014; Ferreyra *et al.* 2015; Iaconelli *et al.* 2017; Barrios *et al.* 2018; Elmahdy *et al.* 2020; Victoria *et al.* 2022). However, a prevalence rate lower than our study for adenovirus has been found in a study from Italy (Petrinca *et al.* 2009). Overall, variation in the surveillance results from one country to another can be attributed to differences in geographical area and climate, methodology, sample volume, concentration method, type of PCR assay (traditional PCR, semi- or nested PCR, multiplex PCR, real-time PCR), primers used, genomic region, and/or PCR condition.

PCR data indicated that HAdVs type 41 was the predominant species of human HAdV present in sewage (20/26, 76.9%). This finding agrees with previous studies from several countries, including Egypt (Santos *et al.* 2004; Sdiri-Loulizi *et al.* 2009; Fong *et al.* 2010; Khoshdel *et al.* 2015; Zhang *et al.* 2016; Gad *et al.* 2019). This suggests that HAdVs type 41 may have greater persistence and stability in aquatic environments than other serotypes of adenovirus. Enriquez *et al.* (1995) showed that the predicted time for 99% inactivation (T_{99}) of HAdV-41 was longer than the T_{99} value of HAdV-40 when they were incubated in tap water at 23 °C (84 vs. 60 days), secondary sewage effluent at 15 °C (45 vs. 43 days), primary sewage effluent at 15 °C (43 vs. 40 days), and in sea water at 15 °C (85 vs. 77 days). Furthermore, our findings support a previous report from Egypt conducted on sewage and clinical samples that HPV JC was more prevalent than HPV BK in the Egyptian environment (Ahmed *et al.* 2019). Additionally, seasonal distribution of HAdV, HPyV, and HPV was observed throughout the study period. However, the higher detection rates of these viruses were observed in the winter months. This may be due to lower temperature in winter months that increase viral survival for a prolonged period (Lipp *et al.* 2001). Higher incidence of HAdV, HPyV, and HPV in winter seasons has also been reported in previous studies from Egypt and other countries (Adefisoye *et al.* 2016; Fernandez-Cassi *et al.* 2018; Ahmed *et al.* 2019; Elmahdy *et al.* 2019).

Moreover, molecular techniques are capable of identifying only viral genomes and do not provide information on virus infectivity, which is a limitation of the current study, and thus the presence of virus in sewage samples does not necessarily indicate a public health threat. In previous studies from New Zealand, HAdV concentrations in raw sewage ranged from 1.00 to 4.08 $\log_{10}$ infectious units/L (Hewitt *et al.* 2011). Despite the important role of wastewater treatment in pathogens' removal from wastewater prior to disposal or discharge to reduce public health risks, conventional WWTP does not sufficiently eliminate and/or inactivate these viruses. Therefore, the levels of viral contamination detected in the samples should induce

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precautions for the wastewater treatment efficiency before its discharge. Quantitative wastewater data can offer an additional perspective to understand the infectious disease transmission and aid public health decision-making for pandemic and epidemic responses. Furthermore, integration of wastewater and clinical monitoring will be cost-effective for disease management, interventions, and mass surveillance for endemic infectious diseases and any future pandemics (Wu *et al.* 2022; Gitter *et al.* 2023).

A second limitation of this study was that clinical samples are not available. However, a previous report on clinical samples from Egypt supports our finding that the occurrence of HPyV was higher than HPV among Egyptian patients (Ahmed *et al.* 2019). Another study from Egypt showed that the prevalence rate of HAdV-41 was higher than HAdV-40 (453.3% vs. 46.7%) among hospitalized children with acute gastroenteritis (Montasser *et al.* 2022), suggesting that wastewater can reflect the circulated viruses among the human population. High prevalence of HAdV and HPyV in the population and environment provides confidence that they could serve as potential indicators for fecal contamination of water (Albinana-Gimenez *et al.* 2009; Silva *et al.* 2011; Hewitt *et al.* 2013; Elmahdy *et al.* 2019). Our results, however, demonstrated that HAdV was more abundant than HPyV and detected throughout the year in sewage samples. The quantification and stability evaluation of adenovirus and polyomavirus in WWTP in Barcelona was performed by Bofill-Mas *et al.* (2006), who reported that both viruses had high levels of stability in urban sewage and were abundant in influent, effluent, sludge, and biosolid samples. Hewitt *et al.* (2013) suggested that using both HAdV and HPyV as indicators will be more valuable than either of them as a single fecal indicator.

Although this study is only a 1-year monitoring, the seasonality pattern was obvious. The detection peaks for HAdV, HPyV, and HPV were notably higher in winter than those detected in other seasons. This finding is consistent with previous reports from Egypt conducted on wastewater and stool specimens (Ahmed *et al.* 2019; Elmahdy *et al.* 2019; Gad *et al.* 2019), and supporting other studies from South Africa (Adefisoye *et al.* 2016). However, there was no evident seasonality for polyomavirus in urban wastewaters in Italy and Brazil (Di Bonito *et al.* 2015; Urbano *et al.* 2019), peak prevalence of adenovirus in wastewater was in summer season in USA and Japan ((Haramoto *et al.* 2007; Kitajima *et al.* 2014), and this may be due to change of humidity, temperature, and difference in geographical area.

CONCLUSION

We have assessed the occurrence and seasonality variation of HAdV, HPyV, and HPV in raw sewage collected regularly over 12 months from the sewage pump station located at Zagazig city, Al Sharqia governorate, Egypt. We have detected HAdV, HPyV, and HPV by PCR, with HAdV being the most detected in the analyzed samples. Although it is unknown if these viral DNAs correlate to infectious viruses, it is important to address virus contamination risk resulting from treated wastewater usage. Further study is needed to interpret the findings of PCR testing in correlation to the occurrence of infectious virus particles using a cell culture system. Also, additional studies using samples from wastewater and clinical samples are needed to improve surveillance of seasonal diseases and to understand the implications of the presence of these viruses in wastewater. Finally, wastewater-based epidemiology would be a promising tool to monitor emerging threats and to present data on entire communities' health.

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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