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Human Rotavirus Genome Copies and Infectious Units in Water and Wastewater in Egypt

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Abstract

Removal of enteric viruses in water and wastewater treatment plants (WTPs and WWTPs) is an important objective to protect societies from enteric viruses' diseases. In this study, the efficiency of two concentration methods, adsorption-elution followed by organic flocculation technique and aluminum hydroxide Al $(OH)_3$ precipitation method, were compared to concentrate rotaviruses from raw sewage, treated effluents, Nile water, and drinking water. No significant difference between the two concentration methods used was observed in raw sewage, treated effluents, Nile water samples. Only in drinking water samples, there was a difference between the two concentration methods. The highest number was $9.2X10^4$ genome copies/litre in the six positive Nile water samples. Genome copies were detected once in drinking water samples but without viral infectivity. Also, the relationship between the number of genome copies of rotavirus Group A in the samples and the quality of sequencing of 155 bp from VP6 region was studied. Sequence anlaysis of 155 bp and 379 bp of VP6 rotavirus region showed successful sequencing for samples containing higher than 10^2 and 10^3 genome copies/litre, respectively. The number of viral genome copies may be a useful indicator to choose positive environmental samples by PCR/electrophoresis for sequencing.

Keywords: Rotavirus, Genome copies, Infectious units, Sequence analysis, Water, Wastewater

1. Introduction

Acute gastroenteritis is one of the most significant diseases in children, causing morbidity and mortality globally [1]. Viruses such as rotaviruses, human caliciviruses, human adenoviruses and human astroviruses (members of the families Reoviridae, Caliciviridae, Adenoviridae, and Astroviridae respectively) are recognized as a major cause of severe gastroenteritis, particularly in children. Rotaviruses are the main cause of mortality due to diarrhea in children under 5 years old [2-6].

Rotaviruses belong to the genus Rotavirus within the Reoviridae family and contain genomes consisting of eleven segments of dsRNA. These viruses are distinct in that their segmented genome undergoes reassortment during replication [7].

According to the classification system based on the gene sequence of VP6, an inner capsid protein, rotaviruses are currently categorized into ten groups (A, B, C, D, E, F, G, H, I and J) [8-10]. Rotaviruses group A is the most cause for rotavirus infections in humans. It causes severe gastroenteritis in infants and children less than 5 years of age with high mortality and morbidity rates [11]. Rotaviruses are highly contagious and very resistant to environmental conditions. A very low number (10 infectious particles) are required to cause infection. A large number, nearly 100 billion rotavirus particles, are shed per gram of fecal material [12-13].

There are a lot of barriers to protect society from infection with gastroenteritis causing viruses. The first one is the high efficient water and wastewater treatment plants in viral removal. The second one is the vaccines which give immunity against viruses and protect from viral diseases. Finally, the antiviral therapy by using drugs against viruses however, no

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drugs for rotaviruses are available in the markets yet. So, the efficiency of water and wastewater treatment plants to remove enteric viruses is a great concern.

Rotavirus is the most frequent RNA enteric viruses in Egypt and is the most resistant one to treatment processes [14-20]. Rotavirus group A is more frequent than rotavirus group C in Egyptian clinical specimens and environmental samples [21]. Moreover, El-Senousy and co-workers investigated the prevalence of rotaviruses in some well water used in drinking and irrigation in some Egyptian rural areas (Nahya and Saft Al laban) using nested RT-PCR. Through one year survey (From March 2012 to February 2013), the results showed that rotaviruses were detected in 33.3% samples of well water in Nahya village while they were detected in 25% samples of well water in Saft Al laban village [22]. In Slovenia, Steyer and co-workers detected rotavirus group A in 30.34% (27/89) and in 37.50% (27/72) of drinking water and potable groundwater samples respectively, suggesting that raw groundwater used as individual drinking water supply may be a possible source of enteric virus infections [23].

The first objective of this study is to compare the efficiency of adsorption-elution followed by organic flocculation technique and Al $(OH)_3$ to concentrate rotaviruses from raw sewage, treated effluents, Nile water, and drinking water. The second objective is to study the relationship between the number of genome copies in the concentrated samples and the quality of sequencing of a short fragment of rotavirus VP6 region.

2. Materials and Methods

2.1. Collection of sewage and water samples

A total of 94 sewage samples (4-5 litres volume for each sample) were collected from El-Gabal El-Asfar and Zenin wastewater treatment plants (WWTPs) from January 2021 to April 2022 and from March 2021 to April 2022, respectively. They included 38 sewage samples (19 raw sewage and 19 treated effluents) from El-Gabal El-Asfar WWTP and 28 sewage samples from Zenin WWTP (14 raw sewage and 14 treated effluents). The samples were collected and transferred in clean bottles and transported to the laboratory within 3 h after collection for examination. Also, 28 water samples (10 litres volume for each sample) were collected from El-Giza drinking water treatment plant (WTP) from March 2021 to April 2022. They included 14 inlet water (Nile water) and 14 outlet water after final chlorination (drinking water).

2.2. Concentration of sewage and water samples

2.2.1. Concentration of water and wastewater by aluminum hydroxide Adsorption-Precipitation method

Sewage and water samples were concentrated according to Standard Methods for the Examination of Water and Wastewater [24]. Viruses can be concentrated from small volumes of wastewater by precipitation with aluminum hydroxide. This process probably involves both electrostatic interactions between the negatively charged virus surface and the positively charged aluminum hydroxide $[Al(OH)_3]$ surfaces and coordination of the virus surface by hydroxo-aluminum complexes. Viruses are adsorbed to an $Al(OH)_3$ precipitate that is either added to the sample or formed in the sample from a soluble aluminum salt and a base such as sodium hydroxide NaOH. Viruses are allowed to adsorb to $Al(OH)_3$ precipitate and the virus-containing precipitate is collected by centrifugation. The viruses are eluted from the precipitate with an alkaline buffer or a proteinaceous solution before virus assay.

2.2.2. Concentration of sewage and water samples using adsorption-elution technique

Sewage and water samples were concentrated by filtration through negatively charged nitrocellulose membranes (ALBET-Spain, 0.45 µm pore size, and 142 mm diameter filter series) after addition of $AlCl_3$ to a final concentration of 0.5 mM and acidification to pH 3.5 and after passing through Whatmann No. 1 filter paper. The viruses adsorbed to the membrane were eluted with 75 ml of 0.05 M glycine buffer, pH 9.5 (using HCl 5 N) containing 3% beef extract (Lab-Limco powder, OXOID, UK) [25-26]. Eluted viruses were re-concentrated by organic flocculation according to katzenelson and co-workers [27]. Samples were neutralized and kept at −70 °C until used.

2.3. Viral nucleic acid extraction

Viral RNA was extracted from 100 µl of the supernatant using BIOZOL Total RNA Extraction reagent (BIOFLUX—Japan) according to the manufacturer's instructions and to a 30 µl final volume.

2.4. RT-PCR of a fragment of the VP6-coding gene of rotaviruses group A

The primers used for RT-PCR were the forward VP6-F 5-GATGGATCNACTACATAGT-3 and the reverse VP6-R 5-GTCCGGTTCATAGGTCCTGG-3 primers (0.5 µm for each), and according to Iturriza-Gomara and co-workers using 100 U of M-MLV reverse transcriptase enzyme (Promega—USA) in a total volume of 10 µl and 1.5 U of Taq DNA polymerase (Biobasic—Canada) in a total volume of

50 µl, to amplify 379 bp [28]. Nested PCR amplification of the target rotavirus VP6 fragment was performed using the forward primer, VP6-NF 5-GCTAGTTTAAGGGATACA-3, and the reverse primer, VP6-NR 5-TCTATAGCCCGTTAATC-3 (1 µm for each), according to Gallimore and co-workers to amplify 155 bp fragment. PCR products (10 µl) were analyzed by electrophoresis on 3 % agarose gels (Panreac-Spain) [29].

2.5. Confirmation of the nested-PCR positivity by amplimer sequencing

The first (379 bp) and nested (155 bp)-PCR products of positive samples were sequenced. Fifty to one hundred µl of the PCR products were purified using a high pure PCR products purification kit (Qiagen) following the manufacturer's instructions. Sequencing was performed on 1–7 µl of the purified products with an ABI prism Big dye termination cycle sequencing ready reaction kit (Applied Biosystem) using the same primers as in the PCR and following the manufacturer's instructions. The DNA was sequenced with an ABI prism 310 automated DNA sequencer. Sequence data from both strands of the PCR products were aligned and compared using the CLUSTALW and BLAST programs (European Bioinformatics Institute).

2.6. Quantification of rotavirus group A genome copies using real-time RT-PCR method

Real-time TaqMan RT-PCR was performed for positive samples in the previous RT-PCR screening. Real-time PCR was done using rotavirus@ceeramTools™ Food & Environmental kit according to manufacturer's instructions using Rotavirus - Q Standard (Ceeram Tools).

2.7. Statistical methods

A paired Student's t test was applied to ascertain the significance at $p < 0.05$ of differences of virus recovery after ($Al(OH)_3$) precipitation method and adsorption-elution technique followed by organic flocculation method.

3. Results

3.1. Detection and quantification of rotaviruses in environmental samples

Of the Ninety-four environmental samples sewage and water samples, thirty -eight samples were positive for rotaviruses using qualitative RT-PCR (38/94, 40.43%). Using real time RT-PCR to quantify genome copies in the same samples, the number of genome copies ranged from 3.26×10^3 genome copies/litre (Ct 40.13) to 2.3×10^7 genome copies/litre (Ct 23.8) in raw sewage samples of El-Gabal El-Asfar WWTP and from 3.13×10^3 genome copies/litre (Ct 40.67) to 8.2×10^6 genome copies/litre (Ct 25.1) in raw sewage samples of Zenin WWTP, while, the number of genome copies ranged from 9.1 X 10 genome copies/litre (Ct 44.2) to 9.63×10^3 genome copies/litre (Ct 36.26) in treated effluents of El-Gabal El-Asfar WWTP and from 5.6 X 10 genome copies/litre (Ct 44.9) to 7.8×10^3 genome copies/litre (Ct 36.32) in treated effluents of Zenin WWTP (Tables 1 and 2) with 2 to 4 $\log_{10}$ reductions of rotavirus genome copies in El-Gabal El-Asfar and 2-3 $\log_{10}$ reductions of rotavirus genome copies in Zenin WWTPs. No significant difference between the two concentration methods used was observed in the results.

Six positive rotavirus samples in raw Nile water samples of El-Giza WTP with the highest number of 9.2×10^4 genome copies/litre (Ct 32.2) which rotavirus genome copies were detected in the drinking water after treatment of this sample 4.8×10^2 (Ct 43.2) genome copies/litre using adsorption-elution followed by organic flocculation technique while, 9.1X10 (Ct 44.9) genome copies/litre was recorded using Al $(OH)_3$ precipitation method (Table 3). Genome copies do not mean infectivity of the virus. Tables 4, 5, 6 showed the infectious units of rotaviruses in sewage and water samples with 1 to 3 $\log_{10}$ lower than the number of genome copies. No infectious units were detected in drinking water samples. The highest numbers of rotavirus infectious units were 8X10 and 9X10 in the treated effluents of El-Gabal El-Asfar and Zenin WWTPs, respectively.

Sequence anlaysis of the 155 bp of VP6 region of rotavirus in the positive 38 samples (Fig. 1) showed success in sequencing in 34 samples which contain higher than 10^2 genome copies/litre quantified by real time RT-PCR (Figs. 2 and 3), while, sequence analysis of the 379 bp in 29 positive samples in the first PCR showed success in sequencing in 27 samples which contain higher than 10^3 genome copies/litre.

Table 1
Rotavirus group A genome copies in sewage samples of El-Gabal El-Asfar WWTP

Sample	Date of sampling	Number of rotavirus genome copies/litre using adsorption-elution technique	Ct	Number of rotavirus genome copies/litre using $Al(OH)_3$	Ct
Raw sewage (Inlet)	10/1/2021	3.65×10^4	36.37	3.82×10^4	36.29
Treated effluent (Outlet)	10/1/2021	0		0	
Raw sewage (Inlet)	24/1/2021	9.7×10^5	32.3	9.58×10^5	32.7
Treated effluent (Outlet)	24/1/2021	0		0	
Raw sewage (Inlet)	7/2/2021	1.5×10^4	36.43	1.44×10^4	36.8
Treated effluent (Outlet)	7/2/2021	0		0	
Raw sewage (Inlet)	16/2/2021	1.17×10^4	37.17	1.1×10^4	37.21
Treated effluent (Outlet)	16/2/2021	0		0	
Raw sewage (Inlet)	8/3/2021	6.87×10^3	37.84	6.95×10^3	37.4
Treated effluent (Outlet)	8/3/2021	0		0	
Raw sewage (Inlet)	17/3/2021	0		0	
Treated effluent (Outlet)	17/3/2021	0		0	
Raw sewage (Inlet)	13/4/2021	0		0	
Treated effluent (Outlet)	13/4/2021	0		0	
Raw sewage (Inlet)	4/5/2021	0		0	
Treated effluent (Outlet)	4/5/2021	0		0	
Raw sewage (Inlet)	15/6/2021	0		0	
Treated effluent (Outlet)	15/6/2021	0		0	
Raw sewage (Inlet)	4/7/2021	0		0	
Treated effluent (Outlet)	4/7/2021	0		0	
Raw sewage (Inlet)	17/8/2021	0		0	
Treated effluent (Outlet)	17/8/2021	0		0	
Raw sewage (Inlet)	28/9/2021	3.51×10^3	40.05	3.26×10^3	40.13
Treated effluent (Outlet)	28/9/2021	0		0	
Raw sewage (Inlet)	26/10/2021	4.18×10^4	36.12	4.43×10^4	36.04
Treated effluent (Outlet)	26/10/2021	0		0	
Raw sewage (Inlet)	24/11/2021	4.3×10^5	33	4.11×10^5	33.8
Treated effluent (Outlet)	24/11/2021	3.2×10^3	41.26	3.12×10^3	41.34
Raw sewage (Inlet)	13/12/2021	1.2×10^6	27.82	1.1×10^6	27.9
Treated effluent (Outlet)	13/12/2021	2.4×10^3	39.76	2.26×10^3	39.8
Raw sewage (Inlet)	10/1/2022	2.3×10^7	23.8	2.18×10^7	23.84
Treated effluent (Outlet)	10/1/2022	9.5×10^3	36.3	9.63×10^3	36.26
Raw sewage (Inlet)	14/2/2022	3.4×10^6	26.82	3.22×10^6	26.9
Treated effluent (Outlet)	14/2/2022	7.2×10^2	41.7	7.28×10^2	41.66
Raw sewage (Inlet)	14/3/2022	8.5×10^4	32.62	8.26×10^4	32.7
Treated effluent (Outlet)	14/3/2022	9.3×10	44.12	9.1×10	44.2
Raw sewage (Inlet)	11/4/2022	7.3×10^3	37.72	7.1×10^3	37.8
Treated effluent (Outlet)	11/4/2022	0		0	

Table 2

Rotavirus group A genome copies in sewage samples of Zenin WWTP

Sample	Date of sampling	Number of rotavirus genome copies/litre using adsorption-elution technique	Ct	Number of rotavirus genome copies/litre using $Al(OH)_3$	Ct
Raw sewage (Inlet)	29/3/2021	0		0	
Treated effluent (Outlet)	29/3/2021	0		0	
Raw sewage (Inlet)	21/4/2021	0		0	
Treated effluent (Outlet)	21/4/2021	0		0	
Raw sewage (Inlet)	23/5/2021	0		0	
Treated effluent (Outlet)	23/5/2021	0		0	
Raw sewage (Inlet)	22/6/2021	0		0	
Treated effluent (Outlet)	22/6/2021	0		0	
Raw sewage (Inlet)	12/7/2021	0		0	
Treated effluent (Outlet)	12/7/2021	0		0	
Raw sewage (Inlet)	30/8/2021	0		0	
Treated effluent (Outlet)	30/8/2021	0		0	
Raw sewage (Inlet)	28/9/2021	$3.13 X 10^3$	40.67	$3.24 X 10^3$	40.63
Treated effluent (Outlet)	28/9/2021	0		0	
Raw sewage (Inlet)	31/10/2021	$3.55 X 10^3$	40.22	$3.27 X 10^3$	40.34
Treated effluent (Outlet)	31/10/2021	0		0	
Raw sewage (Inlet)	22/11/2021	$1.3 X 10^4$	36.3	$1.48 X 10^4$	36.22
Treated effluent (Outlet)	22/11/2021	$8.4 X 10^2$	39.77	$8.52 X 10^2$	39.73
Raw sewage (Inlet)	20/12/2021	$3.2 X 10^5$	31.7	$3.37 X 10^5$	31.62
Treated effluent (Outlet)	20/12/2021	$6.1 X 10^3$	37.69	$6.18 X 10^3$	37.65
Raw sewage (Inlet)	17/1/2022	$8.2 X 10^6$	25.1	$8.11 X 10^6$	25.14
Treated effluent (Outlet)	17/1/2022	$7.8 X 10^3$	36.32	$7.42 X 10^3$	36.48
Raw sewage (Inlet)	21/2/2022	$8.2 X 10^5$	28.78	$8.15 X 10^5$	28.8
Treated effluent (Outlet)	21/2/2022	$7.8 X 10^2$	41.22	$7.64 X 10^2$	41.3
Raw sewage (Inlet)	21/3/2022	$6.1 X 10^3$	37.66	$6 X 10^3$	37.7
Treated effluent (Outlet)	21/3/2022	5.8 X 10	44.88	5.6 X 10	44.9
Raw sewage (Inlet)	18/4/2022	$5.4 X 10^2$	41.72	$5.2 X 10^2$	41.8
Treated effluent (Outlet)	18/4/2022	0	0	0	0

Table 3

Rotavirus group A genome copies in water samples of Giza WTP

Sample	Date of sampling	Number of rotavirus genome copies/litre using adsorption-elution technique	Ct	Number of rotavirus genome copies/litre using $Al(OH)_3$	Ct
Nile water	29/3/2021	0		0	
Drinking water	29/3/2021	0		0	
Nile water	26/4/2021	0		0	
Drinking water	26/4/2021	0		0	
Nile water	23/5/2021	0		0	
Drinking water	23/5/2021	0		0	
Nile water	22/6/2021	0		0	
Drinking water	22/6/2021	0		0	
Nile water	12/7/2021	0		0	
Drinking water	12/7/2021	0		0	
Nile water	30/8/2021	0		0	
Drinking water	30/8/2021	0		0	
Nile water	28/9/2021	0		0	
Drinking water	28/9/2021	0		0	
Nile water	31/10/2021	0		0	
Drinking water	31/10/2021	0		0	
Nile water	22/11/2021	9.1×10^2	39.9	9.24×10^2	39.8
Drinking water	22/11/2021	0		0	
Nile water	20/12/2021	9.8×10^2	40.16	9.68×10^2	40.2
Drinking water	20/12/2021	0		0	
Nile water	17/1/2022	9.2×10^4	32.2	8.8×10^4	32.36
Drinking water	17/1/2022	4.8×10^2	43.2	9.1X10	44.9
Nile water	21/2/2022	9.2×10^3	36.8	9.33×10^3	36.75
Drinking water	21/2/2022	0		0	
Nile water	21/3/2022	8.4 X 10	44.6	8.5 X 10	44.56
Drinking water	21/3/2022	0		0	
Nile water	18/4/2022	6.3 X 10	44.82	6.1 X 10	44.85
Drinking water	18/4/2022	0		0	

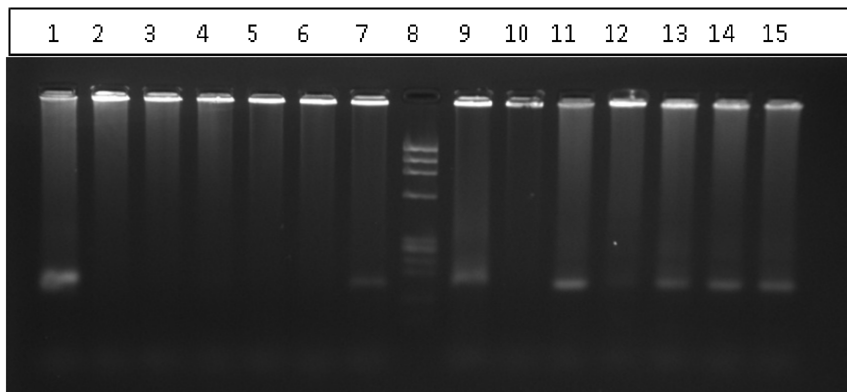

Figure 1: Frequency of rotaviruses in some environmental samples. Lanes 1 and 2, 4 to 7 and 9 to 14, lane 3 negative control, lane 15 positive control, and lane 8 phiX174 ladder

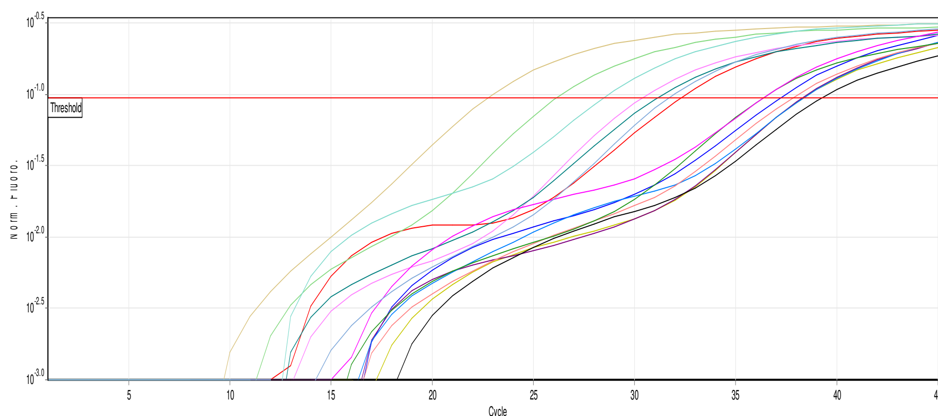

Figure 2: Ct values for number of human rotavirus genome copies in some environmental samples

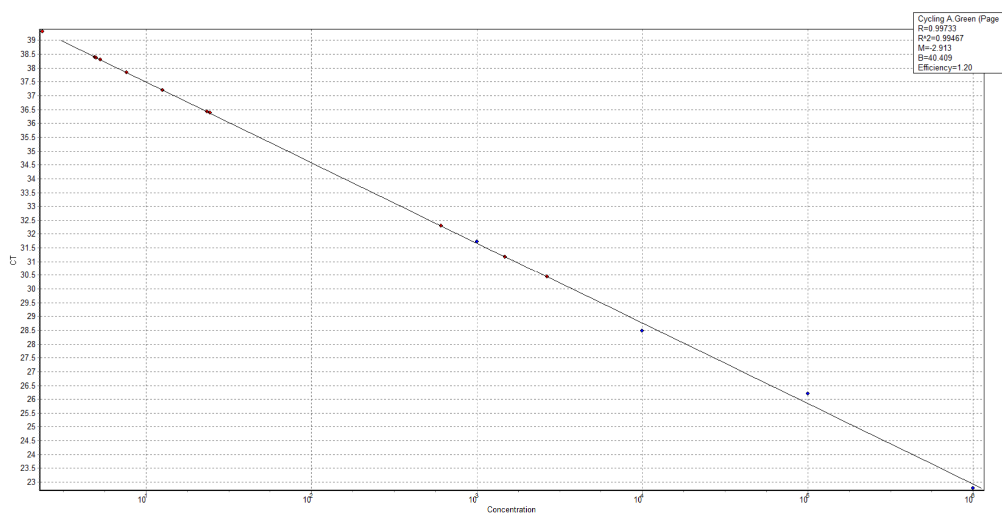

Figure 3: Standard curve for number of human rotavirus genome copies in some environmental samples

Table 4

Rotavirus group A infectious units in sewage samples of El-Gabal El-Asfar WWTP

Sample	Date of sampling	Number of rotavirus infectious units/litre using adsorption-elution technique	Number of rotavirus infectious units/litre using $Al(OH)_3$
Raw sewage (Inlet)	10/1/2021	$1X\ 10^2$	$1\ X\ 10^2$
Treated effluent (Outlet)	10/1/2021	0	0
Raw sewage (Inlet)	24/1/2021	$1X\ 10^3$	$1\ X\ 10^3$
Treated effluent (Outlet)	24/1/2021	0	0
Raw sewage (Inlet)	7/2/2021	$5X\ 10^2$	$4\ X\ 10^2$
Treated effluent (Outlet)	7/2/2021	0	0
Raw sewage (Inlet)	16/2/2021	$6X\ 10^2$	$5\ X\ 10^2$
Treated effluent (Outlet)	16/2/2021	0	0
Raw sewage (Inlet)	8/3/2021	9X10	9X10
Treated effluent (Outlet)	8/3/2021	0	0
Raw sewage (Inlet)	17/3/2021	0	0
Treated effluent (Outlet)	17/3/2021	0	0
Raw sewage (Inlet)	13/4/2021	0	0
Treated effluent (Outlet)	13/4/2021	0	0
Raw sewage (Inlet)	4/5/2021	0	0
Treated effluent (Outlet)	4/5/2021	0	0
Raw sewage (Inlet)	15/6/2021	0	0
Treated effluent (Outlet)	15/6/2021	0	0
Raw sewage (Inlet)	4/7/2021	0	0
Treated effluent (Outlet)	4/7/2021	0	0
Raw sewage (Inlet)	17/8/2021	0	0
Treated effluent (Outlet)	17/8/2021	0	0
Raw sewage (Inlet)	28/9/2021	9 X 10	8 X 10
Treated effluent (Outlet)	28/9/2021	0	0
Raw sewage (Inlet)	26/10/2021	$7\ X\ 10^2$	$5\ X\ 10^2$
Treated effluent (Outlet)	26/10/2021	0	0
Raw sewage (Inlet)	24/11/2021	$1\ X\ 10^2$	$1\ X\ 10^2$
Treated effluent (Outlet)	24/11/2021	8 X 10	8 X 10
Raw sewage (Inlet)	13/12/2021	$5\ X\ 10^4$	$3\ X\ 10^4$
Treated effluent (Outlet)	13/12/2021	8 X 10	8 X 10
Raw sewage (Inlet)	10/1/2022	$3\ X\ 10^3$	$1\ X\ 10^3$
Treated effluent (Outlet)	10/1/2022	0	0
Raw sewage (Inlet)	14/2/2022	$4\ X\ 10^4$	$6\ X\ 10^4$
Treated effluent (Outlet)	14/2/2022	8 X 10	8 X 10
Raw sewage (Inlet)	14/3/2022	$1\ X\ 10^3$	$2\ X\ 10^3$
Treated effluent (Outlet)	14/3/2022	0	0
Raw sewage (Inlet)	11/4/2022	$2\ X\ 10^2$	$1\ X\ 10^2$
Treated effluent (Outlet)	11/4/2022	0	0

Table 5

Rotavirus group A infectious units in sewage samples of Zenin WWTP

Sample	Date of sampling	Number of rotavirus infectious units/litre using adsorption-elution technique	Number of rotavirus infectious units/litre using $Al(OH)_3$
Raw sewage (Inlet)	29/3/2021	0	0
Treated effluent (Outlet)	29/3/2021	0	0
Raw sewage (Inlet)	21/4/2021	0	0
Treated effluent (Outlet)	21/4/2021	0	0
Raw sewage (Inlet)	23/5/2021	0	0
Treated effluent (Outlet)	23/5/2021	0	0
Raw sewage (Inlet)	22/6/2021	0	0
Treated effluent (Outlet)	22/6/2021	0	0
Raw sewage (Inlet)	12/7/2021	0	0
Treated effluent (Outlet)	12/7/2021	0	0
Raw sewage (Inlet)	30/8/2021	0	0
Treated effluent (Outlet)	30/8/2021	0	0
Raw sewage (Inlet)	28/9/2021	8 X 10	8 X 10
Treated effluent (Outlet)	28/9/2021	0	0
Raw sewage (Inlet)	31/10/2021	$9 X 10^3$	$9 X 10^3$
Treated effluent (Outlet)	31/10/2021	0	0
Raw sewage (Inlet)	22/11/2021	9 X 10	8 X 10
Treated effluent (Outlet)	22/11/2021	0	0
Raw sewage (Inlet)	20/12/2021	$6 X 10^2$	$8 X 10^2$
Treated effluent (Outlet)	20/12/2021	9 X 10	8 X 10
Raw sewage (Inlet)	17/1/2022	$9 X 10^4$	$7 X 10^4$
Treated effluent (Outlet)	17/1/2022	9 X 10	8 X 10
Raw sewage (Inlet)	21/2/2022	$2 X 10^3$	$1 X 10^3$
Treated effluent (Outlet)	21/2/2022	0	0
Raw sewage (Inlet)	21/3/2022	$4 X 10^2$	$2 X 10^2$
Treated effluent (Outlet)	21/3/2022	0	0
Raw sewage (Inlet)	18/4/2022	7 X 10	8 X 10
Treated effluent (Outlet)	18/4/2022	0	0

Table 6

Rotavirus group A infectious units in water samples of Giza WTP

Sample	Date of sampling	Number of rotavirus infectious units/litre using adsorption-elution technique	Number of rotavirus infectious units/litre using $Al(OH)_3$
Nile water	29/3/2021	0	0
Drinking water	29/3/2021	0	0
Nile water	26/4/2021	0	0
Drinking water	26/4/2021	0	0
Nile water	23/5/2021	0	0
Drinking water	23/5/2021	0	0
Nile water	22/6/2021	0	0
Drinking water	22/6/2021	0	0
Nile water	12/7/2021	0	0
Drinking water	12/7/2021	0	0
Nile water	30/8/2021	0	0
Drinking water	30/8/2021	0	0
Nile water	28/9/2021	0	0
Drinking water	28/9/2021	0	0
Nile water	31/10/2021	0	0
Drinking water	31/10/2021	0	0
Nile water	22/11/2021	0	0
Drinking water	22/11/2021	0	0
Nile water	20/12/2021	7 X 10	7 X 10
Drinking water	20/12/2021	0	0
Nile water	17/1/2022	6×10^2	8×10^2
Drinking water	17/1/2022	0	0
Nile water	21/2/2022	8 X 10	7 X 10
Drinking water	21/2/2022	0	0
Nile water	21/3/2022	0	0
Drinking water	21/3/2022	0	0
Nile water	18/4/2022	0	0
Drinking water	18/4/2022	0	0

4. Discussion

Rotavirus is the most frequent RNA enteric viruses in Egypt [14, 20, 30] and other developing countries [31-33] and also some developed countries with higher mortality rate in the developing countries [32, 34-36]. The efficiency of water and wastewater treatment plants to remove rotavirus Group A is the first line of defense to protect the societies from rotavirus infections. Results showed 2 to 5 $\log_{10}$ reductions of rotavirus genome copies in El-Gabal El-Asfar and 2 to 3 $\log_{10}$ reductions in Zenin WWTPs. El-Gabal El-Asfar WWTP serves Cairo Governorate, while Zenin WWTP serves Giza Governorate representing the highest population density in Egypt. The higher sensitivity in El-Gabal El-Asfar WWTP than Zenin WWTP may be due to the operation instructions such as time of sedimentation in each treatment basins however, no significant difference in the viral load in the raw sewage of both treatment plants was observed. The infectious units of rotaviruses in sewage samples were with 1 to 3 $\log_{10}$ lower than the number of genome copies. The highest numbers of rotavirus infectious units were 8X10 and 9X10 in the treated effluents of El-Gabal El-Asfar and Zenin WWTPs, respectively. This difference between number of genome copies and number of infectious units was supported by other reports [15, 21, 30, 37]. This may return to the longer persistence and higher resistance of genome copies than infectious units to environmental conditions and treatment processes.

Six positive rotavirus samples in raw Nile water samples of El-Giza WTP with the highest number of $9.2X10^4$ genome copies/litre which rotavirus genome copies were detected in the drinking water after treatment of this sample. The higher the number of genome copies in the raw water, the higher chance to have virus genome copies in the drinking water even if the treatment processes go in proper way. No infectious units were detected in drinking water samples. Genome copies do not always mean infectivity of the virus. Sometimes, genome copies could be detected with complete absence of viral infectivity due to longer persistence and higher resistance to treatment processes of genome copies. So, loss of infectivity could be happened, while, genome copies could be still detected [15, 21, 37].

No significant difference was observed between the two methods used to concentrate rotaviruses from all types of water except for drinking water samples which 1 $\log_{10}$ higher in the number of genome copies in drinking water sample of Giza WTP in January 2022 was observed in adsorption-elution followed by organic flocculation technique. This may be due to the higher number of litres concentrated in the drinking water samples (20 litres) using adsorption-elution followed by organic flocculation technique in relation to 1 litre concentrated using $Al(OH)_3$ precipitation method. Although, there was difference in number of litres concentrated with the other water types between the two methods (3 litres for raw sewage and 5 litres for both treated effluents and Nile water samples for adsorption-elution technique and 1 litre for all water types with $Al(OH)_3$ precipitation method), there was not significant difference between the results of the two concentration methods. This may return to the higher viral load/litre in these water types in relation to the viral load/litre in drinking water samples. This result is supported by the results reported by Rashed and co-workers, who reported the same results for adenoviruses and polyomaviruses (double stranded DNA viruses), Pepper mild motle virus PMMoV (single stranded RNA plant virus), and bacteriophage phi X174 virus (single stranded DNA bacterial virus) [38]. In our study, rotavirus Group A (double stranded RNA viruses) was used as a model for the comparison between the two concentration methods. Also, a lot of previous studies showed higher viral frequency in treated and untreated sewage and river water than its frequency in the drinking water samples [15, 21, 39-50].

Sequence analysis is one of the most important methods which confirm the amplified genome positivity in addition to determination of genotypes [51-55]. Sequence anlaysis of the 155 bp of VP6 region of rotavirus in the 38 positive samples showed successful sequencing in 34 samples which contain higher than 10^2 genome copies/litre, while, failure in sequencing was observed for four samples with 10^1 genome copies/litre. This may indicate the higher quality of sequencing when the number of genome copies is higher than a specific value. This specific value may be varied according to the amplified fragment length and position and if the amplified product resulted from first, semi-nested, or nested RT-PCR. Sequence analysis of the 379 bp in 29 positive samples in the first PCR indicated failure in sequencing for samples less than 10^3 genome copies/litre. Choosing the samples for sequencing depends on the number of genome copies may avoid researchers the loss of money resulting from failure of sequencing which sometimes happens when the choosing of samples depends on the intensity of the bands by gel electrophoresis.

5. Conclusions

No significant difference between adsorption-elution followed by organic flocculation technique and $Al(OH)_3$ precipitation methods to concentrate rotaviruses from raw sewage, treated effluents, and Nile water samples. Only in drinking water samples, adsorption-elution followed by organic flocculation technique is more sensitive than $Al(OH)_3$ precipitation. Also, the number of viral genome copies is better to be used as an indicator when

choosing samples for sequencing than choosing according to the intensity of the bands in the agarose gel after electrophoresis. Each PCR may have its own specific or cut-off value for viral genome copies depending on fragment length and position in the genome, PCR conditions, and if the PCR is first or nested PCR.

6. Conflicts of interest

All authors declare that there are no conflicts of interest.

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