World Applied Sciences Journal 32 (11): 2186-2205, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.11.91108

Prevalence of Noroviruses among Detected Enteric Viruses in Egyptian Aquatic Environment

[1]Waled Morsy El-Senousy, [2]Mamdouh Salem El-Gamal,
[2]Adel Abd El-Baset Mousa, [1]Shawki El-Sayed El-Hawary and [1]Mohamed Nasr Fathi

[1]Environmental Virology Laboratory, Department of Water Pollution Research,
Environmental Research Division, National Research Centre, Dokki, Cairo 12311, Egypt
[2]Department of Botany and Microbiology, Faculty of Science (Boys), Al-Azhar University, Egypt

Abstract: Two hundred and twenty clinical samples from children with ages ranging from one month to five years with acute diarrhea were collected from two hospitals. Using RT-PCR, the incidence of noroviruses (NV) was 2.2% (5/220), 32.7% (72/220) for rotaviruses (RV) and 17.7% (39/220) for astroviruses (AstV). At the same period, In 120 Nile water samples collected from inlet of three water treatment plants (WTPs) Meet Khames, Mahalet Damana and Shoha and Nile water from two sites in Salamon, the percentages of detection of RNA enteric viruses were (24.1%), (15%) and 0% for rotaviruses, astroviruses and noroviruses respectively using organic flocculation (OF) method as a secondary concentration step while the percentages were 45.8, 37.5 and 0% for rotaviruses, astroviruses and noroviruses respectively using polyethylene glycol (PEG) method as a secondary concentration step. On the other hand in 72 drinking water samples collected from the three WTPs, the percentages of detection of RNA enteric viruses were (15.2%), (5.5%) and (0%) for rotaviruses, astroviruses and noroviruses respectively using organic flocculation method as a secondary concentration step while the percentages were (22.2%), (16.6%) and (0%) for rotaviruses, astroviruses and noroviruses respectively using polyethylene glycol method as a secondary concentration step. Also, 76 sewage samples were collected from March 2008 to September 2009 from different treatment steps carried out in Meet Khames wastewater treatment plant (WWTP), in the 19 raw sewage samples the percentages of detection of RNA enteric viruses were (36.8%), (31.5%) and (0%) for rotaviruses, astroviruses and noroviruses respectively using organic flocculation method as a secondary concentration step while the percentages were (68.4%), (47.3%) and (5.2%) for rotaviruses, astroviruses and noroviruses respectively using polyethylene glycol method as a secondary concentration step. Also, in the 19 chlorinated effluent samples the percentages of detection of RNA enteric viruses were (10.5%), (15.7%) and (0%) for rotaviruses, astroviruses and noroviruses respectively using organic flocculation method as a secondary concentration step while he percentages were (21.0%), (21.0%) and (0%) for rotaviruses, astroviruses and noroviruses respectively using polyethylene glycol method as a secondary concentration step. Noroviruses are less frequent than rotaviruses and astroviruses in Egyptian clinical and environmental samples.

Key words: *Rotaviruses · Astroviruses · Noroviruses · Enteric viruses · Water treatment plant · Wastewater treatment plant*

INTRODUCTION

Enteric viruses that are important causative agents of human diseases [1] include the enteroviruses, rotaviruses, noroviruses (formerly called Norwalk-like viruses or small round structured virus), adenoviruses, reoviruses and others. They are excreted in feces of infected individuals in high numbers and are transmitted via the fecal-oral route mainly through contaminated water, food and soil. The presence of enteric viruses in water may cause a

Corresponding Author: Waled Morsy El-Senousy, Environmental Virology Laboratory, Department of Water Pollution Research, Environmental Research Division, National Research Centre, Dokki, Cairo 12311, Egypt
E-mail: waledmorsy@hotmail.com.

health risk. They are highly stable in water [2] as they are not completely eliminated by the drinking water treatment process under certain conditions [3]. A number of previous studies have examined source and treated water for enteric viruses. Enteroviruses and Adenoviruses have been frequently found [3-7]. Other enteric viruses were also found in environmental waters, such as Hepatitis A virus [8], Astroviruses [4], Noroviruses [9, 10] and Reoviruses [11, 12]. Reoviruses have often been detected either alone or associated with enteroviruses and may interfere with the propagation of the enterovirus in cell culture [13].

Noroviruses are considered as emerging pathogens due to their widespread distribution in diverse environments, in addition to their ability to cause clinically relevant infections in all age groups, numerous modes of transmission, genetic diversity and the fact that they induce only short-term immunity in humans [14]. These viruses can cause waterborne outbreaks linked either to the direct consumption of water or to its recreational use. Since infected individuals can excrete millions of viral particles (in stools and/or vomit), these viruses can be found in great numbers not only in raw sewage but also in treated waters, recreational waters and water destined for human consumption. In most countries, the absence of adequate surveillance programs results in a lack of systematic virologic research on clinical and environmental samples. Data on the distribution of the virus is thus scarce and the occurrence and scope of waterborne outbreaks are often underestimated [14].

Norovirus is considered to cause about half of all outbreaks of gastroenteritis world-wide [15]. Outbreaks of gastroenteritis in hospitals and other closed settings is a large economical problem [16]. In developing countries norovirus has been estimated to cause up to 200.000 deaths yearly in children of <5 years of age [17]. For reasons not fully understood, the number of reported Norovirus outbreaks has increased considerably since the emergence of a new virus variant in 2002 [18]. Subsequently, large epidemics caused by novel Norovirus strains have appeared world-wide approximately every other year [19].

The objectives of this study were to estimate the prevalence of noroviruses among other RNA enteric viruses in Egyptian clinical and environmental samples and to compare the efficiency of both organic flocculation and polyethylene glycol methods as secondary concentration methods for noroviruses from sewage, Nile water and drinking water samples.

MATERIALS AND METHODS

Water Samples: Twenty liters of water sample from four treatment steps in Meet khamees plant including inlet water (Nile water), after sand filtration, after sedimentation and outlet water (drinking water) were collected. Also, twenty liters of water sample from two treatment steps from the compact units water treatment plants (Shoha and Mahalet Damana) before treatment (Nile water) and after treatment (drinking water) and Nile water from two sites in Salamon from October 2007 to September 2009 were collected.

Sewage Samples: Two liters of raw sewage (before treatment) and four litters from each treatment step, after primary sedimentation, after aeration and after chlorination (chlorinated effluents) were collected from Meet Khames wastewater treatment plant from El-Mansoura city, El-Daqahlia Governorate from March 2008 to September 2009.

Stool Samples: Two hundred and twenty stool samples from children with ages ranging from one month to five years with acute diarrhea were collected from two hospitals, stool samples were collected from Academic children hospital in El-Mansoura city, Dakahlia governorate and 120 stool samples were collected from Abo El- Rish hospital in greater Cairo.

Concentration of Sewage and Water Samples: 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 containing 3% beef extract (Lab-Limco powder, OXOID, UK) [20, 21]. All samples were reconcentrated using both an organic flocculation method [22] and polyethylene glycol (PEG) method; briefly, 0.25 volumes of 5x PEG/NaCl solution, were mixed by shaking for 1 min and then were incubated with gentle rocking at 4°C for 60min. then centrifugation at 10000 x *g* for 30 min at 4°C was done. After discarding the supernatant, centrifugation at 10000 x *g* for 5 min at 4°C to compact pellet was done. Finally, the pellets were resuspended in PBS [23, 24].

Comparison between efficiency of polyethylene glycol and organic flocculation as reconcentration methods for noroviruses genogroups I and II (NV GGI and GGII) in the lab scale:

Inoculation with three doses from positive norovirus GGI and GGII stool samples separately for each genogroup (5×10^5, 5×10^6 and 5×10^7) in 1 L Nile water was done. The inoculated water samples were concentrated directly by filtration through an electronegatively charged filter nitrocellulose membranes (Shleicher and Schuell, 0.45 µm pore size and 142 mm diameter filter series) and adsorbed viruses were eluted with 70 ml of 0.05 M glycine buffer, pH 9.5, containing 3% beef extract (Lab-Limco powder, Oxoid) [20, 21]. The elaute distributed into two falcons and one of the two eluates was reconcentrated using organic flocculation [22] while the other was reconcentrated using polyethylene glycol [23, 24].

Concentration of Clinical Samples: Approximately 0.1 g of stool samples was weighed, diluted 1:10 in nuclease-free H_2O and vortexed for 30 sec. Samples were clarified by centrifugation at 7.000 rpm for 10 min at room temperature. Viral RNA was extracted from 140 µl of the supernatant.

Viral Nucleic Acid Extraction: Viral nucleic acid was extracted using TRIzol (BIOZOL Total RNA Extraction reagent) (Invitrogen Life Technologies, Paisley, Scotland,) according to the manufacturer's instructions.

RT-PCR for the Detection of Norovirus: Viral RNA of the capsid gene was amplified using RT-PCR method according to kageyama *et al.* [25] and kojima *et al.* [26]. Ten micro-liters from the nested-PCR were analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The lengths of the products from the nested-PCR were 330 bp for NV GGI and 344 bp for NV GGII.

RT-PCR of a Fragment of the VP6-Coding Gene of Rotaviruses (Group A): The first round RT-PCR was done. The primers used for RT-PCR were the forward VP6-F primer 5'- done according to Iturriza-Gomara *et al.* [27]. Nested PCR amplification of the target rotavirus VP6 fragment was performed according to Gallimore *et al.* [28] to amplify 155 bp fragment.

RT-PCR of a Fragment of the ORF-1a of the Astrovirus: Semi-nested RT-PCR for detection of human astrovirus (HAstV) genogroup A was done according to El-Senousy *et al.* [29] and using the primers A1 and A2 in the first round and A1 and A2 internal in the second round of the PCR. Also, Semi-nested RT-PCR for detection of human astrovirus genogroup B was done according to El-Senousy *et al.* [29] and using the primers A1 bis and A2 bis in the first round and A1 bis and A2 internal in the second round of the PCR.

Real Time RT-PCR for Quantification of Noroviruses in Environmental Samples: Real-time RT-PCR was performed using a SYBR Green Quantitative RT-PCR kit (Applied Biosystem, UK) to quantify norovirus GGI and GGII in the negative samples in the RT-PCR survey. 10 µl of mix containing primers GI-SKR for GGI and G2-SKR for GGII [25, 26], each at concentration of 1 µM, deoxynucleoside triphosphates (Fermentas, EU) at concentration of 0.2 mM, 1x RT-buffer (Fermentas, EU) and 100 U reverse transcriptase (Fermentas, EU) were added to 5 µl of each sample to give 15 µl (final volume), RT was carried out at 25°C for 10 min., 42°C for 1 hr., 99°C for 5min. and 5°C for 5 min. Real-time PCR amplification was performed using master mix containing two primers QN1F4 (FW) and NV1LCR (REV) for GGI and two primers QNIF2(FW) and COG2R (REV) for GGII [25, 26] and using SAYBR Green kit (Applied Biosystem, UK) in a total volume of 25 µl including the cDNA of the first step. The Real time PCR conditions were initial denaturation at 94°C for 5 min and 45 cycles at 95 °C for 15 sec., 60°C for 1 min. and 65°C for 1 min. Dilution endpoint standard curves were determined and compared for GI and GII noroviruses by performing real-time RT-PCR with 10-fold dilutions of positive control for Norovirus GGI and GGII (positive stool samples). The threshold (Ct) value obtained from the assay of each dilution was used to plot a standard curve by assigning a value of 1 RT-PCR unit (RT-PCRU) to the highest dilution showing a positive Ct and 10, 100 and 1,000 RT-PCRU sequentially to the lower dilutions. Data were used to plot standard curves.

CC-RT-PCR for Quantification of Infectious Rotavirus and Astroviruses Particles: Infectious rotaviruses, astrovirus genogroup A and astrovirus genogroup B were quantified using CC-RT-PCR according to El-Senousy *et al.* [29] and Ghazy *et al.* [30] using MA104 and CaCo2 cell lines for rotaviruses and astroviruses respectively.

RESULTS

Comparison Between Noroviruses among Other RNA Gastroenteritis Causing Viruses in Clinical Samples: Rotavirus was the most common viral pathogen found in the study group (72/220; 32.7%), followed by astroviruses (39/220; 17.7%) and norovirus GGI (5/220; 2.2%). Of these 39 of Astroviruses positive samples, twenty four were positive as co-infection for astrovirus group A and group B, thirteen were positive as a single infection for astrovirus group B and two were positive as a single infection for astrovirus group A (Table 1).

Molecular Detection of Human Noroviruses in Water Samples: Norovirus neither GGI nor GGII detected in Nile water or drinking water samples concentrated by polyethylene glycol or organic flocculation and collected from Meet Khames, Shoha, Mahalet Damana WTPS and Salamon Surface water.

Molecular Detection of Human Rotavirus (HRV) in Water Samples: The frequency of human Rotavirus in water samples collected monthly from Meet Khames water treatment plant in different treatment steps using organic flocculation was 29.1% (7/24) in raw water, 25% (6/24) after sedimentation, 20.8% (5/24) after sand filtration and 16.6% (4/24) in drinking water While, the frequency of human rotavirus in different treatment steps using polyethylene glycol were 58.3 % (14/24) in raw water, 41.6%(10/24) after sedimentation, 41.6% (10/24) after sand filtration and 20.8% (5/24) in drinking water (Tables 2 and 3).

At the same time, the frequency of human rotavirus in the water samples collected from Shoha water treatment plant (First compact unit) from October 2007 until September 2009; using organic flocculation was 20.8% (5/24) in raw water and 20.8% (5/24) in drinking water, while the frequency of rotavirus in Shoha WTP, using polyethylene glycol was 41.6% (10/24) in raw water samples and 33.3% (8/24) in drinking water (Tables 4 and 5)., On other hand the frequency of rotavirus collected monthly from Mahalet Damana water treatment plant (second compact unit) from October 2007 until September 2009; using organic flocculation was 16.6% (4/24) in raw water and 8.3%(2/24) in drinking water, while the frequency of rotavirus in Mahalet Damana WTP, using polyethylene glycol was 29.1% (7/24) in raw water samples and 12.5% (3/24) in drinking water (Tables 4 and 5).

Molecular Detection of Human Astrovirus Genogroup A in Water Samples: The frequency of human astrovirus group A in water samples collected monthly from Meet Khames water treatment plant in different treatment steps using organic flocculation was 12.5% (3/24) in raw water, 4.1% (1/24) after sedimentation, 4.1% (1/24) after sand filtration, but not detected in drinking water while, the frequency of human astrovirus A in different treatment steps using polyethylene glycol was 25 % (6/24) in raw water, 12.5%(3/24) after sedimentation, 4.1% (1/24) after sand filtration and 4.1% (1/24) in drinking water (Tables 6 and 7).

At the same time, the frequency of human astrovirus A in the water samples collected from Shoha water treatment plant (First compact unit) from October 2007 until September 2009; using organic flocculation was 4.1% (1/24) in raw water and 4.1% (1/24) in drinking water, while the frequency of rotavirus in Shoha WTP, using polyethylene glycol was 16.6% (4/24) in raw water samples and 8.3% (2/24) (Tables 7and 8) On other hand the frequency of astrovirus A collected monthly from Mahalet Damana water treatment plant (second compact unit) from October 2007 until September 2009; using organic flocculation, the astrovirus group A was not detected in either raw water or drinking water of Mahalet Damana water treatment plant. While the frequency of astrovirus group A in Mahalet Damana WTP, using polyethylene glycol was 8.3% (2/24) in raw water samples but not detected in drinking water (Tables 8 and 9).

Molecular Detection of Human Astrovirus Genogroup B in Water Samples: The frequency of human astrovirus B in water samples collected monthly from Meet Khames water treatment plant in different treatment steps using organic flocculation was 12.5% (3/24) in raw water, 8.3% (2/24) after sedimentation, 0% after sand filtration and 4.1% (1/24) in drinking water while, the frequency of human astrovirus B in different treatment steps using polyethylene glycol was 37.5 % (9/24) in raw water, 29.1%(7/24) after sedimentation, 20.8% (5/24) after sand filtration and 12.5% (3/24) in drinking water (Tables 10 and11).

At the same time, the frequency of human astrovirus group B in the water samples collected from Shoha water treatment plant (first compact unit) from October 2007 until September 2009; using organic flocculation was 8.3% (2/24) in raw water and 8.3% (2/24) in drinking water, while the frequency of astrovirus B in Shoha WTP, using polyethylene glycol were 20.8% (5/24) in raw water and

Table 1: Comparison between noroviruses among other RNA gastroenteritis causing viruses in clinical samples

Type of virus	Percentage of virus in clinical samples (%)
Rotavirus	72/220 (32.7)
Astroviruses	39/220 (17.7)
Astrovirus group A	26/220 (11.8)
Astrovirus group B	37/220 (16.8)
Norovirus	5/220 (2.2)

Table 2: Frequency of rotavirus in Meet Khames water treatment plant (Oct. 2007 - Sep.2008)

	Raw Nile water		After Sedimentation		After Sand filtration		Drinking water	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2007	-	-	+	+	+	-	+	+
Nov. 2007	+	+	+	+	+	+	+	+
Dec. 2007	+	+	+	-	+	-	+	-
Jan. 2008	+	+	+	-	+	-	-	-
Feb. 2008	-	-	-	-	-	-	-	-
Mar. 2008	+	+	+	-	-	-	-	-
Apr. 2008	+	-	+	+	+	+	-	-
May 2008	-	-	-	-	-	-	-	-
Jun. 2008	+	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	-	-	-	-	-	-	-	-
Sep. 2008	+	-	-	-	-	-	-	-

Table 3: Frequency of rotavirus in Meet Khames water treatment plant (Oct. 2008- Sep.2009)

	Raw Nile water		After Sedimentation		After Sand filtration		Drinking water	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2008	-	-	+	+	+	+	+	+
Nov. 2008	+	-	-	-	-	-	-	-
Dec. 2008	-	-	-	-	-	-	-	-
Jan. 2009	+	+	-	-	+	-	-	-
Feb. 2009	+	-	-	-	-	-	-	-
Mar.2009	+	+	+	+	+	+	+	+
Apr. 2009	-	-	-	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	+	-	-	-	-	-	-	-
Jul. 2009	-	-	-	-	-	-	-	-
Aug. 2009	+	-	+	-	+	-	-	-
Sep.2009	+	+	+	+	+	+	-	-

Table 4: Frequency of rotavirus in Shoha and Mehalet Damana water treatment plants (Oct. 2007- Sep.2008).

	Raw Nile water of Shoha		Drinking water of Shoha		Raw Nile water of Mahalet Damana		Drinking water of Mahalet Damana	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2007	-	-	-	-	-	-	-	-
Nov. 2007	+	-	+	-	+	+	-	-
Dec. 2007	+	-	-	-	+	-	-	-
Jan. 2008	+	+	+	+	+	+	+	-
Feb. 2008	-	-	-	-	-	-	-	-
Mar.2008	+	+	+	+	-	-	-	-
Apr. 2008	-	-	-	-	-	-	-	-
May 2008	-	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	+	-	-	-		-	-	-
Sep.2008	+	-	-	-	+	+	-	-

Table 5: Frequency of rotavirus in Shoha and Mehalet Damana water treatment plants (Oct. 2008- Sep.2009).

	Raw Nile water of Shoha		Drinking water of Shoha		Raw Nile water of Mahalet Damana		Drinking water of Mahalet Damana	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2008	-	-	+	+	-	-	-	-
Nov. 2008	+	+	-	-	--	+	+	
Dec. 2008	-	-	-	-	+	-	-	-
Jan. 2009	+	+	-	-	-	-	-	-
Feb. 2009	-	-	+	+	-	-	-	-
Mar.2009	+	+	+	+	+	+	+	+
Apr. 2009	-	-	+	-	+	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	-	-	-	-	-	-	-	-
Aug. 2009	-	-	-	-	-	-	-	-
Sep.2009	+	-	+	-	-	-	-	-

Table 6: Frequency of astrovirus group A in Meet khames water treatment plant (Oct. 2007- Sep.2008)

	Raw Nile water		After Sedimentation		After Sand filtration		Drinking water	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2007	+	+	-	-	-	-	-	-
Nov. 2007	-	-	-	-	-	-	-	-
Dec. 2007	+	-	+	-	-	-	-	-
Jan. 2008	-	-	-	-	-	-	-	-
Feb. 2008	-	-	-	-	-	-	-	-
Mar.2008	-	-	-	-	-	-	-	-
Apr. 2008	-	-	-	-	-	-	-	-
May 2008	-	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	+	-	-	-	-	-	-	-
Sep.2008	-	-	-	-	-	-	-	-

Table 7: Frequency of astrovirus group A in Meet khames water treatment plant (Oct. 2008- Sep.2009)

	Raw Nile water		After Sedimentation		After Sand filtration		Drinking water	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2008	-	-	-	-	-	-	-	-
Nov. 2008	-	-	-	-	-	-	-	-
Dec. 2008	+	-	+	-	-	-	-	-
Jan. 2009	+	+	+	+	+	+	+	-
Feb. 2009	-	-	-	-	-	-	-	-
Mar.2009	-	-	-	-	-	-	-	-
Apr. 2009	-	-	-	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	-	-	-	-	-	-	-	-
Aug. 2009	-	-	-	-	-	-	-	-
Sep.2009	+	+	-	-	-	-	-	-

Table 8: Frequency of astrovirus group A in Shoha and Mehalet Damana water treatment plants (Oct. 2007- Sep.2008)

	Raw Nile water of Shoha		Drinking water of Shoha		Raw Nile water of Mahalet Damana		Drinking water of Mahalet Damana	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2007	-	-	-	-	-	-	-	-
Nov. 2007	-	-	-	-	-	-	-	-
Dec. 2007	+	-	+	-	-	-	-	-
Jan. 2008	-	-	-	-	+	-	-	-
Feb. 2008	-	-	-	-	-	-	-	-
Mar.2008	-	-	-	-	-	-	-	-
Apr. 2008	-	-	-	-	-	-	-	-
May 2008	-	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	-	-	-	-	-	-	-	-
Sep.2008	-	-	-	-	-	-	-	-

Table 9: Frequency of astrovirus group A in Shoha and Mehalet Damana water treatment plants (Oct. 2008- Sep.2009).

	Raw Nile water of Shoha		Drinking water of Shoha		Raw Nile water of Mahalet Damana		Drinking water of Mahalet Damana	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2008	-	-	-	-	-	-	-	-
Nov. 2008	-	-	-	-	-	-	-	-
Dec. 2008	-	-	-	-	+	-	-	-
Jan. 2009	+	-	-	-	-	-	-	-
Feb. 2009	+	+	+	+	-	-	-	-
Mar.2009	-	-	-	-	-	-	-	-
Apr. 2009	-	-	-	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	-	-	-	-	-	-	-	-
Aug. 2009	-	-	-	-	-	-	-	-
Sep.2009	+	-	-	-	-	-	-	-

Table 10: Frequency of astrovirus group B in Meet khames water treatment plant (Oct. 2007- Sep.2008)

	Raw Nile water		After Sedimentation		After Sand filtration		Drinking water	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2007	-	-	-	-	-	-	-	-
Nov. 2007	-	-	-	-	-	-	-	-
Dec. 2007	+	+	+	+	+	-	-	-
Jan. 2008	+	-	+	-	+	-	+	-
Feb. 2008	+	+	+	-	-	-	-	-
Mar.2008	-	-	-	-	-	-	-	-
Apr. 2008	-	-	-	-	-	-	-	-
May 2008	-	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	+	-	-	-	-	-	-	-
Sep.2008	-	-	-	-	-	-	-	-

Table 11: Frequency of astrovirus group B in Meet khames water treatment plant (Oct. 2008- Sep.2009).

	Raw Nile water		After Sedimentation		After Sand filtration		Drinking water	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2008	+	-	+	-	-	-	-	-
Nov. 2008	+	-	+	-	+	-	-	-
Dec. 2008	+	-	+	-	+	-	+	-
Jan. 2009	-	-	-	-	-	-	-	-
Feb. 2009	-	-	-	-	-	-	-	-
Mar.2009	+	+	+	+	+	-	+	+
Apr. 2009	-	-	-	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	+	-	-	-	-	-	-	-
Aug. 2009	-	-	-	-	-	-	-	-
Sep.2009	-	-	-	-	-	-	-	-

Table 12: Frequency of astrovirus group B in Shoha and Mehalet Damana water treatment plants (Oct. 2007- Sep.2008)

	Raw Nile water of Shoha		Drinking water of Shoha		Raw Nile water of Mahalet Damana		Drinking water of Mahalet Damana	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2007	-	-	-	-	-	-	-	-
Nov. 2007	+	+	+	+	+	-	+	-
Dec. 2007	+	-	+	-	-	-	-	-
Jan. 2008	-	-	-	-	-	-	-	-
Feb. 2008	-	-	-	-	-	-	-	-
Mar.2008	-	-	-	-	-	-	-	-
Apr. 2008	-	-	-	-	-	-	-	-
May 2008	-	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	-	-	-	-	-	-	-	-
Sep.2008	-	-	-	-	-	-	-	-

Table 13: Frequency of astrovirus group B Shoha and Mehalet Damana water treatment plants (Oct. 2008- Sep.2009).

	Raw Nile water of Shoha		Drinking water of Shoha		Raw Nile water of Mahalet Damana		Drinking water of Mahalet Damana	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Oct. 2008	+	+	-	-	-	-	-	-
Nov. 2008	-	-	-	-	-	-	-	-
Dec. 2008	+	-	+	-	-	-	-	-
Jan. 2009	-	-	-	-	-	-	-	-
Feb. 2009	-	-	-	-	-	-	-	-
Mar.2009	-	-	+	+	+	+	+	-
Apr. 2009	-	-	-	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	-	-	-	-	-	-	-	-
Aug. 2009	-	-	-	-	-	-	-	-
Sep.2009	+	-	+	-	+	-	-	-

20% (5/24) in drinking water (Tables 11and 12). On other hand the frequency of astrovirus group B collected monthly from Mahalet Damana water treatment plant (second compact unit) from October 2007 until September 2009; using organic flocculation was 4.1% (1/24) in raw water, but astrovirus group B was not detected in drinking water of Mahalet Damana WTP. While the frequency of Astrovirus group B in Mahalet Damana WTP in water samples using polyethylene glycol was 12.5% (3/24) in raw water samples and 8.3% (2/24) in drinking water (Tables 12 and 13).

Molecular Detection for Human Noroviruses in Sewage Samples: One raw sewage sample from nineteen samples (5.2 %) was concentrated by PEG and collected in September month 2009 was positive for norovirus GGI but norovirus GGII was not detected at all samples. On the other hand noroviruses was not detected in chlorinated effluents.

Molecular Detection for Human Rotavirus in Sewage Samples: The frequency of human rotavirus in sewage samples collected monthly from Meet Khames wastewater treatment plant in different treatment steps from March 2008 to September 2009; using organic flocculation was 36.8%(7/19) in raw sewage, 21%(4/19) after primary treatment, 15.7%(3/19) after Aeration and 10.5%(2/19) in chlorinated effluents. while the frequency of rotavirus in different treatment steps, using polyethylene glycol was 68.4%(13/19) in raw sewage, 52.6%(10/19) after primary treatment, 31.5%(6/19) after aeration and 21%(4/19) in chlorinated effluents (Tables 14 and 15).

Molecular Detection for Human Astrovirus Genogroup A in Sewage Samples: The frequency of human astrovirus genogroup A in sewage samples collected monthly from Meet Khames wastewater treatment plant in different treatment steps from March 2008 to September 2009; using organic flocculation was 15.7% (3/19) in raw sewage, 10.5%(2/19) after primary treatment, 5.2%(1/19) after aeration and 5.2%(1/19) in chlorinated effluents while the frequency of astrovirus group A in different treatment steps, using polyethylene glycol was 31.5% (6/19) in raw sewage, 21% (4/19) after primary treatment, 5.2% (1/19) after aeration and 10.5% (2/19) in chlorinated effluents (Tables 16 and 17).

Molecular Detection for Human Astrovirus Genogroup B in Sewage Samples: The frequency of human astrovirus genogroup B in sewage samples collected monthly from Meet Khames wastewater treatment plant in different treatment steps from March 2008 to September 2009; using organic flocculation was 26.3% (5/19) in raw sewage, 15.7% (3/19) after primary treatment, 5.2% (1/19) after aeration and 10.5%(2/19) in chlorinated effluents. While the frequency of astrovirus group B in different treatment steps, using polyethylene glycol was 42.1% (8/19) in raw sewage, 36.8% (7/19) after primary treatment, 10.5% (2/19) after Aeration and 15.7% (3/19) in chlorinated effluents (Tables 18 and 19).

Comparison Between Efficiency of Polyethylene Glycol and Organic Flocculation as Reconcentration Methods for Noroviruses: The results indicated that polyethylene glycol method is one $\log_{10}$ more sensitive than organic flocculation method in re-concentration of noroviruses Genogroup I and II (Tables 20 and 21).

Real Time RT-PCR for Quantification of Norovirus GGI and GGII Genome Copies in Water Samples: All negative samples of Nile water, reconcentrated with organic flocculation and polyethylene glycol were estimated by real time RT-PCR for norovirus GGI and GGII. After estimation using real time RT-PCR, six samples were positive for norovirus GGI; two samples in the raw water of Meet Khames WTP, two samples in the raw of Shoha WTP and two samples in raw water of Salomon. The results of RNA copies/l of norovirus GGI after estimation ranged from 4.1 X $10^1$to 7.2 X 10^2 in Meet Khamees WTP, 1.2 X 10^1 to 4.1 X 10^2 in Shoha WTP and 3.2 X 10^1 to 6.2 X10^2 in Salamon (Table 22) and 1x10 ^{1}to 1x10 ^{2}in raw sewage of Meet Khames wastewater treatment plant. On the other hand only one sample was positive for norovirus GGII in raw water of Meet Khames in Jan 2009 and the result RNA copies/l of norovirus GGII after estimation ranged from 0 to 1 x10^1 (Table 23).

Quantification of Rotavirus Infectious Units Using CC-RT-PCR in the Three WTPs: The results of the number of infectious units of rotavirus (CC-RT-PCR u/l) in Meet khames WTP using organic flocculation ranged from 0.5x10 to 0.5 x10^3 while using polyethylene glycol ranged from 0.5x 10^1 to 0.5x10^4. Also, in Shoha WTP the infectious units using organic flocculation were 0.5x10 while using polyethylene glycol ranged from 0.5x10^1 to 0.5x10^2. Also, in Mahalet Damana WTP, the infectious units using organic flocculation were 0.5x10 while using polyethylene glycol were 0.5 x 10^2. But in Salamon site one, the infectious units using organic flocculation were 0.5 x 10^1 while using polyethylene glycol were 0.5 x10^2. on the other hand in raw sewage, the infectious units using organic flocculation ranged from 0.5 x 10^2 to 0.5 x 10^3 while using polyethylene glycol ranged from 0.5 x 10^3 to 0.5 x 10^4 (Table 24).

Quantification of Astrovirus Genogroup A Infectious Units Using CC-RT-PCR in the Three WTPs: The results of the number of infectious units of astrovirus genogroup A are shown in Table (25). CC-RT-PCR u/l in

Table 14: Frequency of rotavirus in Meet Khames wastewater treatment plant (Mar. 2008- Feb..2009).

	Raw sewage		After primary sedimentation		After aeration		Chlorinated effluents	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Mar.2008	+	-	+	-	-	-	-	-
Apr. 2008	+	-	-	-	-	-	-	-
May 2008	+	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	+	+	+	-	+	+	+	-
Aug. 2008	-	-	-	-	-	-	-	-
Sep.2008	+	-	-	-	-	-	-	-
Oct. 2008	+	-	-	-	-	-	-	-
Nov. 2008	+	+	+	-	+	-		-
Dec. 2008	+	+	+	+	+	+	+	+
Jan. 2009	+	+	+	+	+	+	+	+
Feb. 2009	-	-	-	-	-	-	-	-

Table 15: Frequency of rotavirus in Meet Khames wastewater treatment plant (Feb. 2009- Sep.2009).

	Raw sewage		After primary sedimentation		After aeration		Chlorinated effluents	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Mar.2009	+	+	+	+	-	-	-	-
Apr. 2009	+	+	+	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	+	-	+	-	-	-	-	-
Aug. 2009	-	-	+	-	+	-	-	-
Sep.2009	+	+	+	+	+	-	+	-

Table 16: Frequency of astrovirus genogroup A in Meet khames wastewater treatment plant (March 2008- Feb. 2009).

	Raw sewage		After primary sedimentation		After aeration		Chlorinated effluents	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Mar.2008	+	+	+	+	-	-	-	-
Apr. 2008	+	-	-	-	-	-	+	-
May 2008	-	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	-	-	-	-	-	-	-	-
Sep.2008	+	-	-	-	-	-	-	-
Oct. 2008	-	-	-	-	-	-	-	-
Nov. 2008	+	-	+	-	-	-	-	-
Dec. 2008	-	-	-	-	-	-	-	-
Jan. 2009	+	+	+	+	+	+	+	+
Feb. 2009	-	-	-	-	-	-	-	-

Table 17: Frequency of astrovirus genogroup A in Meet khames wastewater treatment plant (March 2009- Sep.2009)

	Raw sewage		After primary sedimentation		After aeration		Chlorinated effluents	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Mar.2009	-	-	-	-	-	-	-	-
Apr. 2009	-	-	-	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	-	-	-	-	-	-	-	-
Aug. 2009	-	-	-	-	-	-	-	-
Sep.2009	+	+	+	-	-	-	-	-

Table 18: Frequency of astrovirus genogroup B in Meet Khames wastewater treatment plant (March 2008- Feb. 2009)

	Raw sewage		After primary sedimentation		After aeration		Chlorinated effluents	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Mar.2008	-	-	-	-	-	-	-	-
Apr. 2008	+	+	+	+	-	-	+	-
May 2008	+	-	-	-	-	-	-	-
Jun. 2008	-	-	-	-	-	-	-	-
Jul. 2008	-	-	-	-	-	-	-	-
Aug. 2008	-	-	-	-	-	-	-	-
Sep.2008	+	-	-	-	-	-	-	-
Oct. 2008	-	-	+	+	+	-	+	+
Nov. 2008	+	+	+	-	-	-	-	-
Dec. 2008	-	-	-	-	-	-	-	-
Jan. 2009	+	+	+	-	-	-	-	-
Feb. 2009	-	-	-	-	-	-	-	-

Table 19: Frequency of astrovirus genogroup B in Meet khames wastewater treatment plant (March 2008- Sep.2009).

	Raw sewage		After primary treatment		After Aeration		Chlorinated effluents	
Sampling date	PEG	OF	PEG	OF	PEG	OF	PEG	OF
Mar.2009	+	+	+	-	-	-	-	-
Apr. 2009	-	-	-	-	-	-	-	-
May 2009	-	-	-	-	-	-	-	-
Jun. 2009	-	-	-	-	-	-	-	-
Jul. 2009	-	-	-	-	-	-	-	-
Aug. 2009	+	-	+	-	-	-	-	-
Sep.2009	+	+	+	+	+	+	+	+

Table 20: Quantification of RNA copies of noroviruses GGI in seeded samples using real time RT-PCR

Initial dose of norovirus GGI RNA copies / l	RNA copies/l of norovirus GGI estimated after polyethylene glycol	% efficiency of PEG method	RNA copies/l of norovirus GGI estimated after organic flocculation	% efficiency of OF method
5 x 10^4	1.2x 10^4	24%	5.5x 10^3	11%
5 x 10^5	2.1X10^5	42%	7.2 X10^4	14.4%
5 x 10^6	1.3X10^6	26%	6.3X 10^5	12.6%
5 x 10^7	2.2X10^7	44%	8.3 X10^6	16.6%
5 x 10^8	1.1 x 10^8	22%	6.4 x 10^7	12.8%
		Average 31.6%		Average 13.48%

Slope:-3.266, Rsq: 0.993, Ct ranged from 19.55 to 38.95

Table 21: Quantification of RNA copies of noroviruses GGII in seeded samples using real time RT-PCR

Initial dose of norovirus GGII RNA copies / l	RNA copies/l of norovirus GGII estimated after polyethylene glycol	% efficiency of PEG method	RNA copies/l of norovirus GGII estimated after organic flocculation	% efficiency of OF method
5 x 10^4	1X10^4	20%	6.7X10^3	13.4%
5 x 10^5	1.9X10^5	38%	7.4X10^4	14.8%
5 x 10^6	1.1X10^6	22%	7.5X10^5	15%
5 x 10^7	1.9X10^7	38%	7.8X10^6	15.6%
5 x 10^8	1.1X10^5	22%	6.5X10^7	13%
		28%		14.36%

Slope:-3.266, Rsq: 0.993, Ct ranged from 19.55 to 38.95

Table 22: Real time RT-PCR for quantification of norovirus GGI in water samples

Sample	Date	RNA copies/l of Norovirus GGI estimated after polyethylene glycol	RNA copies/l of Norovirus GGI estimated after organic flocculation
Raw Shoha	Dec. 2008	4.1 X 10^2	1.2 X 10^1
Raw Salamon	Dec. 2008	3.9 X 10^2	3.2 X 10^1
Raw Salamon	July 2008	6.2 X10^2	5.3 X 10^1
Raw Meet Khames	Dec. 2008	3.6 X 10^2	4.1 X 10^1
Raw Meet Khames	June 2009	7.2 X 10^2	4.6 X 10^1
Raw Shoha	April 2009	3.8 X 10^2	4.4 X 10^1

Slope:-3.266, Rsq: 0.993, Ct ranged from 19.55 to 38.95

Table 23: Real time RT-PCR for quantification of norovirus GGII in water samples

Sample	Date	RNA copies/l of Norovirus GGI estimated after polyethylene glycol	RNA copies/l of Norovirus GGI estimated after organic flocculation
Raw Meet Khames	Jan 2009	0	1 x10^1

Slope:-3.266, Rsq: 0.993, Ct ranged from 19.55 to 38.95

Table 24: CC-RT-PCR for quantification of infectious units of rotavirus in the environmental positive samples using organic flocculation and polyethylene glycol

Sample	Date	Number of infectious units CC-RT-PCR U/L using polyethylene glycol	Number of infectious units CC-RT-PCR U/L using organic flocculation
Raw Meet Khames	Sep. 2009	0.5 x 10^1	0
Raw Meet Khames	Nov. 2007	0.5 x10^4	0.5 x10^3
After sedimentation in Meet Khames	Nov. 2007	0.5 x10^3	0.5 x10^2
After sand filtration in Meet Khames	April 2008	0.5 x10^2	0.5 x10
Raw Shoha	Sep. 2009	0.5 x 10^1	0
Raw Shoha	March 2008	0.5 x10^2	0.5 x10
Raw Mahalet Damana	Nov. 2007	0.5 x10^2	0.5 x10
Raw Mahalet Damana	March 2009	0.5 x10^2	0.5 x10
Salamon site one	Jan 2009	0.5 x 10^2	0.5 x 10^1
Raw sewage	Sep. 2009	0.5 x 10^3	0.5 x 10^2
Raw sewage	Mar. 2009	0.5 x 10^4	0.5 x 10^3

Table 25: CC-RT-PCR for quantification of infectious units of astrovirus group A in the environmental positive samples using organic flocculation and polyethylene glycol

Sample	Date	Number of infectious units CC-RT-PCR U/L using polyethylene glycol	Number of infectious units CC-RT-PCR U/L using organic flocculation
Raw Meet Khames	Jan. 2009	0.5 x 10^1	0

Table 26: CC-RT-PCR for quantification of infectious units of astrovirus group B in the environmental positive samples using organic floculation and polyethylene glycol

Sample	Date	Number of infectious units CC-RT-PCR U/L using polyethylene glycol	Number of infectious units CC-RT-PCR U/L using organic flocculation
Raw Meet Khames	Mar. 2009	0.5 x 10^2	0.5 x 10^1
After sedimentation in Meet Khames	Mar. 2009	0.5 x 10^2	0.5 x 10^1
After sedimentation in Meet Khames	Dec. 2007	0.5 x 10^2	0.5 x 10^1
Raw Shoha	Dec. 2008	0.5 x 10^1	0
Raw Salamon site two	Nov. 2007	0.5 x 10^2	0.5 x 10^1
Raw Salamon site one	March. 2008	0.5 x 10^1	0

the infectious units of raw water of Meet Khames WTP were not detected using organic flocculation while using polyethylene glycol the infectious units were 0.5×10^1.

Quantification of Astrovirus Genogroup B Infectious Units Using CC-RT-PCR in the Three WTPs: The results of the number of infectious units of astrovirus genogroup B are shown in Table (26). CC-RT-PCR u/l in the infectious samples of Meet Khames WTP were 0.5×10^1 using organic flocculation while using polyethylene glycol were 0.5×10^2. Also in Shoha WTP, the infectious units were not detected using organic flocculation while using polyethylene glycol were 0.5×10^1. Also the infectious units of Salamon Nile water using organic flocculation was 0.5×10^1 while ranged from 0.5×10^1 to 0.5×10^2 using polyethylene glycol.

DISCUSSION

The question in our study was why noroviruses are less frequent than other RNA enteric viruses in Egyptian sewage and water samples? So that there were two possibilities to study that, the first possibility is that, the noroviruses are actually less frequent in the community while the second possibility is that the noroviruses are more sensitive to concentration methods than other enteric viruses.

To study the first possibility, the prevalence of noroviruses in stool samples in realtion to rotaviruses and astroviruses was investigated. The incidence of noroviruses was 2.2 % (5/220). All of them were norovirus GGI but norovirus GGII was not detected in all stool samples. The prevalence of noroviruses was lower than the prevalence of rotaviruses 32.7% (72/220) and the prevalence of astroviruses (genogroups A and B) 17.7% (39/220). Our results for noroviruses are greatly lower than Dominguez *et al.* [31] in Catalonia (Spain) in 2004–2005, who recorded that the incidence of noroviruses in a total of 534 stool samples analyzed, of which 278 (52%) were positive for norovirus. and this high incidence might be due to the collected samples were taken from outbreaks of gastroenteritis. In Central Australia, Roger *et al.* [32], in 1995, 235 stool samples were collected, the incidence of norovirus was 1.3% (3/235), which is lower than our result, in the same study 158 stool samples were collected, the incidence of norovirus was 3,2% (5/158).and this results is close to our results. In Paraguay, Coluchi *et al.* [33] who recorded that the incidence of rotavirus was 31.8% (70/220) in stool samples, this result is nearly similar to our results. Our results are higher than that recorded by Rosa e Silva *et al.* [34] in Brazil who detected rotaviruses in 8.9% (94/1056) stool samples obtained during January 1998 to December 1999. Our results are greatly lower than that of Antunes *et al*. [35] in Portugal who recorded that the incidence of rotavirus in stool samples collected during January to march 2007 was 55.2% (the season of the peak incidence of rotavirus). In Egypt, Kamel *et al.* [36] recorded that the incidence of rotaviruses in stool samples collected during March 2006 through February 2007 was 57.4%(66/115) and this high incidence might be due to collection of 73% of the samples during the cold season, September 2006 to February 2007. In the study of Young Kenyan, Nicholas *et al.* [37] reported that astrovirus prevalence using enzyme immunoassay method for detection was found to be 5.5% (26/476) in clinical samples and this result is lower than our result and this may be due to the less sensitivity of enzyme immunoassay than RT-PCR. In Australia, Mustafa *et al.* [38] reported that the incidences of astrovirus infection each year were 4.4% (1995), 2.2% (1996), 3.9% (1997) and 1.4% (1998). This lower percentage might be due to that Mustafa and co-workers have used another less sensitive technique (Northern hybridization). On contrast, in day care centers, recorded by Douglas *et al.* [39], astrovirus incidence was detected in 32% of specimens by RT-PCR, this result is greater than our results and this may be due to that collected samples were taken from an outbreak.

To study the second possibility, is that the noroviruses were more sensitive to concentration methods than other enteric viruses. Two hundred and fourty water samples from El-Dakahlia Governorate were collected monthly from October 2007 to September 2009, 120 Nile water, 72 drinking water of three WTPs and 48 after sand and sedimentation of Meet Khames WTP. All collected water samples were concentrated using polyethylene glycol and organic flocculation. conventional RT-PCR was used for investigation of the prevalence of noroviruses GGI and GGII in water samples reconcentrated with two methods (polyethylene glycol and organic flocculation methods). Neither norovirus GGI genome nor norovirus GGII genome could be detected in Nile water or drinking water. The prevalence of noroviruses was less than the prevalence of rotaviruses and astroviruses (genogroups A and B) in Nile water and drinking water of both traditional water treatment plants and the two compact units. This result was confirmed by using either organic flocculation or polyethylene glycol as reconcentration methods. The frequency of rotavirus, astroviruses genogroups A and B was higher in raw water

of Meet Khames than the other two compact units (Shoha and Mahalet Damana), rotavirus detection were fourteen times positive in RT-PCR screening/24 total inlet water samples in Meet Khames WTP, ten times positive in RT-PCR screening/24 total inlet water samples in Shoha WTP, followed by seven times positive in RT-PCR screening/24 total inlet water samples in Mahalet Damana WTP. On the other hand astrovirus group A detection were six times positive in RT-PCR screening/24 total inlet water samples in Meet Khames WTP, four times positive in RT-PCR screening/24 total inlet water samples in Shoha WTP, two times positive in RT-PCR screening/24 total inlet water samples in Mahalet Damana WTP. Also astrovirus group B detection were nine times positive in RT-PCR screening/24 total inlet water samples in Meet Khames WTP, five times positive in RT-PCR screening/24 total inlet water samples in Shoha WTP and three times positive in RT-PCR screening/24 total inlet water samples in Mahalet Damana WTP. This variation in the prevalence might be attributed to additional viral load entered Meet Khames WTP, it serves about 500000 persons in El-Mansoura city while other two compact units serve about 10000-15000 persons; as the number of people who need drinking water increases, the capacity of the WTPs should be done in parallel (design criteria of WTP can't be operate correctly because it needs to supply drinking water to large population). In the fact the presence of viruses in the chlorinated effluents depends on the resistance of the virus to the treatment processes of the treatment plants and the high load of viral particles in the raw water and this might be the causative agents that the occurrence of rotavirus-positive, astroviruses positive samples in drinking water of Meet Khames WTP higher than then two compact units (Shoha and Mahalet Damana WTPs), however Meet Khames WTP as traditional plant was more efficient in the viral removal than Shoha and Mehalet Damana as two compact units, where rotavirus RNA removal in three WTPs was 64.2%(9/14), 20% (2/10) and 57% (4/7) in Meet Kames, Shoha and Mahalet Damana WTPs respectively. On the other hand the efficiency of three WTPs in astrovirus RNA group A removal was 83.3 % (5/6), 50% (2/4), 100% (2/2), in Meet Kames, Shoha and Mahalet Damana WTPs respectively. Also the efficiency of three WTPs in astrovirus RNA group B removal was 66.6% (6/9), 0% (5/5), 33.3% (1/3) in Meet Kames, Shoha and Mahalet Damana WTPs respectively. From above our results, Shoha WTP has low efficient in the rotavirus RNA and astroviruses RNA removal than Meet Khames and Mahalet Damana WTPs.

Raw sewage could contain enteric viruses shed from affected people and therefore, detectable viruses in raw sewage would reflect the actual state of the circulating viruses in the area. In present study 76 sewage samples collected from March 2008 to September 2009 from different treatment steps carried out in Meet Khames wastewater treatment plant (raw, after primary treatment, after aeration and chlorinated effluent). The sewage samples were concentrated by polyethylene glycol and organic flocculation. Using RT-PCR, The noroviruses have not been detected at all sewage samples with organic flocculation but using polyethylene glycol, noroviruses have been detected in one raw sewage sample from nineteen raw sewage 5.2 % (1/19) was collected in September month 2009 and the genotype was norovirus GGI. Norovirus GGII was not detected at all samples. The prevalence of noroviruses was less than the prevalence of rotaviruses and astroviruses (genogroups A and B) in raw sewage and after all the treatment steps of meet Khames wastewater treatment plant. This result was confirmed by using either organic flocculation or polyethylene glycol as re concentration methods. From our result, noroviruse GGI was more frequent than norovirus GGII in Egypt which it was detected in both clinical and environmental samples as result for the circulating virus in the area in contrast norovirus GG II was not detected neither in clinical nor in environmental samples. Also, the low incidence of noroviruses in clinical samples in addition to environmental samples although of the improvement of the efficiency of concentration method and this might be attributed to the low viremia of noroviruses in clinical Egyptian samples.

From our results, the polyethylene glycol was more efficient than organic flocculation as reconcentration methods for virus recovery from raw sewage and Nile water samples where in one hundred and twenty Nile water samples, rotavirus RNA using polyethylene glycol was detected in fifty five samples while only in 29 samples using organic flocculation. Also using polyethylene glycol astroviruses were found in forty five samples while it was found only in eighteen samples using organic flocculation. On the other hand in nineteen raw sewage samples norovirus has been detected only with polyethylene glycol but has not been detected with organic flocculation, rotavirus RNA with polyethylene glycol method has been detected in thirteen samples while only in seven samples using organic flocculation and astroviruses have been detected in nine samples using polyethylene glycol while only in six samples using organic flocculation. These results agree with the study

of Kittigul *et al.* [40] in Thailand (2001), 38 water samples from different water sources (river, domestic sewage and drinking water) were collected and concentrated with two methods, organic flocculation and polyethylene glycol. The presence of rotavirus antigen in the concentrated eluate by ELISA, less rotavirus was recovered using organic flocculation (23.3%) than with PEG precipitation (45.7%). In Colombia, from two hundred and twenty-six water samples collected in three different years (69 samples in 2000, 69 samples in 2002 and 88 samples in 2005) from different water sources (river, within the plant and fresh water), using RT-PCR, in the samples of the year 2000, 8/69 (11.6%) samples were positive for rotavirus while, norovirus and astrovirus have been not detected in this year, in the samples of the year 2002, 5/69 (7.25%) samples resulted positive in determining the presence of rotavirus in fresh water, but norovirus and astrovirus have been not detected in this year, in the samples of the year 2005, no rotavirus was found in any of the sampling places, while in the same year, 2/69 norovirus positive samples were obtained from fresh water taken from faucets at people homes and one astrovirus positive sample was obtained from the river water [41]. In the study of El-Senousy *et al.* [24], using TaqMan probe real time RT-PCR and using mengo virus as a control of extraction method efficiency and internal control as a control of real time RT-PCR efficiency to quantify norovirus GGI and GGII in spiked and naturally contaminated fresh produce and irrigation water samples, polyethylene glycol was 1 $\log_{10}$ more efficient than organic flocculation as secondary step concentration methods. In our study, using SYBR green real time RT-PCR to quantify norovirus GGI and GGII in spiked Nile water and negative samples in the RT-PCR survey, also, polyethylene glycol was 1 $\log_{10}$ more efficient than organic flocculation as secondary step concentration methods.

The incidence of noroviruses using RT-PCR in addition to using more efficient concentration method for the environmental samples, the noroviruses incidence was still low so that we used other more sensitive technique (real time RT-PCR) for noroviruses detection in the environmental samples concentrated using organic flocculation and polyethylene glycol. First we compared the efficiency of organic flocculation and polyethylene glycol as re concentration methods in the lab scale via inoculation (positive control) of human stool suspensions for norovirus GGI and norovirus GGII into two Nile water samples, one sample for norovirus GGI and another for GGII and each of sample was concentrated with two methods of organic flocculation and polyethylene glycol at the same time. The result after estimation of RNA copies/l of noroviruses GGI and GGII by real time RT-PCR, Polyethylene glycol method is one $\log_{10}$ more sensitive than organic flocculation method as reconcentration methods of noroviruses GGI and GGII from Nile water samples.

By real time RT-PCR, The negative environmental samples using RT-PCR concentrated by organic flocculation and polyethylene glycol were tested for noroviruses GGI and GGII, the results were six samples of Nile water concentrated by organic flocculation were positive for norovirus GGI, also the same six samples concentrated by polyethylene glycol were positive for norovirus GGI, also by real time RT-PCR there was one $\log_{10}$ more sensitivity in the positive samples concentrated by polyethylene glycol than concentrated by organic flocculation. On the other hand one Nile water sample was positive for norovirus GGII concentrated by organic flocculation and the same sample concentrated by polyethylene glycol was positive for norovirus GGII, also there was one $\log_{10}$ more sensitive in the positive sample concentrated by polyethylene glycol than concentrated by organic flocculation.

In the present study, Meet Khames WTP succeeded in the removal of the genome of rotavirus only in nine times (5 times rotavirus genome was detected in drinking water in RT-PCR screening/14 times rotavirus genome were detected in raw water samples). In Meet Khames WTP the incidence of infectious rotavirus was 2/14, in raw water September 2009 and November 2007 (2 times infectious rotavirus units were detected/14 times were positive for rotavirus genome in RT-PCR screening), also infectious rotavirus had been detected after sedimentation in one time of November 2007, also in one time after sand filtration in April 2008 while rotavirus infectious units had not been detected in drinking water samples. Shoha WTP succeeded in the removal of the genome of rotavirus only in two times (8 times rotavirus genome was detected in drinking water in RT-PCR screening/10 times rotavirus genome were detected in raw water samples). In Shoha WTP the incidence of infectious rotavirus was 2/10 in raw water in March 2008 and September 2009 (2 times infectious rotavirus units were detected/10 times were positive for rotavirus genome in RT-PCR screening), while rotavirus infectious units had not been detected in drinking water samples. Mahalet Damana WTP succeeded in the removal of the genome of rotavirus four times (3 times rotavirus genome were detected in drinking water in RT-PCR screening/7 times rotavirus genome were

detected in raw water samples). In Mahalet damana WTP, the incidence of infectious rotavirus was 2/10 in raw water in November 2007 and March 2009 (2 times infectious rotavirus units were detected/7 times were positive for rotavirus genome in RT-PCR screening), while rotavirus infectious units had not been detected in drinking water samples. While in the Nile water of Salamon site one, the infectious rotavirus genome had been detected only in one month, January 2009 (1 time rotavirus genome was detected in RT-PCR screening/13 times rotavirus genome were detected in raw water samples), while the rotavirus infectious units had not been detected in Nile water of Salamon site two. On the other hand, Meet Khames wastewater treatment plant succeeded in the removal of the genome of rotavirus only in nine times (4 times rotavirus genome were detected in chlorinated effluent in RT-PCR screening/13 times rotavirus genome were detected in raw sewage samples). In Meet Khames WWTP the incidence of infectious rotavirus was 2/14 in raw sewage in September 2009 and March 2009 (2 times infectious rotavirus units were detected/13 times were positive for rotavirus genome in RT-PCR screening), while rotavirus infectious units had not been detected after primary sedimentation, after aeration and in chlorinated effluent samples. Also, Meet Khames WTP succeeded in the removal of the genome of astrovirus genogroup A only in 5 times (one time astrovirus genogroup A genome was detected in drinking water in RT-PCR screening/6 times astrovirus genome were detected in raw water samples). In Meet Khames WTP the incidence of infectious astrovirus genogroup A was 1/6 in raw water in January 2009 (1 time infectious astrovirus genogroup A units was detected/6 times were positive for astrovirus genogroup A genome in RT-PCR screening), while astrovirus genogroup A infectious units had not been detected after sedimentation, after sand filtration and in drinking water samples. Shoha WTP succeeded in the removal of the genome of astrovirus genogroup A only in two times (2 times astrovirus genogroup A genome were detected in drinking water in RT-PCR screening/4 times astrovirus genogroup A genome were detected in raw water samples). In Shoha, astrovirus genogroup A infectious units had not been detected neither in raw water nor in drinking water samples. Mahalet Damana WTP succeeded in the removal of the genome of astrovirus genogroup A at all times (astrovirus genogroup A genome had not been detected in drinking water in RT-PCR screening/2 times astrovirus genogroup A genome were detected in raw water samples). In Mahalet Damana WTP, astrovirus genogroup A infectious units had not been detected neither in raw water nor in drinking water samples. In the Nile water samples of Salamon, the infectious astrovirus genogroup A genome had not been detected neither in site one nor in site two. On the other hand, Meet Khames wastewater treatment plant succeeded in the removal of the genome of astrovirus genogroup A only in four times (2 times astrovirus genogroup A genome were detected in chlorinated effluents in RT-PCR screening/6 times astrovirus genogroup A genome were detected in raw sewage samples). In Meet Khames WWTP the infectious astrovirus genogroup A had not been detected in raw sewage, after primary sedimentation, after aeration and in chlorinated effluent samples. Also Meet Khames WTP succeeded in the removal of the genome of astrovirus genogroup B only in 6 times (3 times astrovirus genogroup B genome were detected in drinking water in RT-PCR screening/9 times astrovirus genome were detected in raw water samples). In Meet Khames WTP the incidence of infectious astrovirus B units was three times, one of them was in raw water sample in March 2009 and two times after sedimentation step, one time in March 2009 and one time after sedimentation in December 2007, while astrovirus genogroup B infectious units had not been detected neither after sand filtration nor in drinking water samples. Shoha WTP failed in the removal of the genome of astrovirus genogroup B at all times. In Shoha, astrovirus genogroup B infectious units were detected in one time in December 2008 (infectious astrovirus genogroup B units had been detected in one time/6 times were positive for astrovirus genogroup B genome in RT-PCR screening). Mahalet Damana WTP succeeded in the removal of the genome of astrovirus genogroup B in one time (2 times astrovirus genogroup B genome were detected in drinking water in RT-PCR screening/3 times astrovirus genogroup B genome were detected in raw water samples). In Mahalet Damana WTP, astrovirus genogroup B infectious units had not been detected neither in raw water nor in drinking water samples. In Nile water of Salamon site one the infectious astrovirus genogroup B units had been detected in one time in November 2007 (1 time infectious astrovirus genogroup B units detected/9 times were positive for astrovirus genogroup B genome in RT-PCR screening), also one infectious astrovirus genogroup B units was detected in Salamon site two of Nile water, in March 2009 (1 time infectious astrovirus genogroup B units was detected/8 times were positive for astrovirus genogroup B genome in RT-PCR screening), On the other hand, Meet Khames wastewater treatment plant succeeded in the removal of

the genome of astrovirus genogroup B only in five times (3 times astrovirus genogroup B genome were detected in chlorinated effluents in RT-PCR screening/8 times astrovirus genogroup B genome were detected in raw sewage samples). In Meet Khames WWTP the infectious rotavirus was not detected in raw sewage, after primary treatment, after aeration and in chlorinated effluent samples. Generally the infectious units for rotavirus and astrovirus genogroups A and B were 1 $\log_{10}$ more in the samples concentrated by polyethylene glycol than the same samples concentrated by organic flocculation.

In the present investigation, the peak incidence of rotavirus genome in the Nile water of the three WTPs and Salamon Nile water was more frequent in the cooler months, where it had been detected 37 times in the cooler months (autumn and winter) while detected only 18 times in spring and summer. Also, the peak of incidence of astrovirus genogroup A in the Nile water of the three WTPs and Salamon Nile water was more frequent in the cooler months, where it had been detected 17 times in cooler months (autumn and winter) while detected only 6 times in spring and summer. Also, the peak of incidence of astrovirus genogroup B genome in the raw water of the three WTPs was more frequent in the cooler months, where it had been detected 23 times in the cooler months (autumn and winter) while had been detected only 8 times in spring. The two years survey gave us an opportunity to confirm the incidence of rotavirus in the same months in the two years.

In our study, the rotavirus was the most frequent than astroviruses then noroviruses and this agrees with Villena *et al.* [42], which in raw sewage samples collected from Egypt (1998-1999) rotavirus was the most frequent (85.71%) followed by HAV, enterovirus, astrovirus and caliciviruses with frequent percentage 71.43, 57.14, 45.71 and 28.57% respectively. Also by El-Senousy *et al.* [43], in the Nile water samples collected from Cairo, rotavirus was the most frequent (66.7%) followed by HAV 50%, enterovirus 41.7%, astrovirus 8.3% and caliciviruses 0%. Also our results agree with Marize *et al*. [44], in Brazil, during one year, a total of 52 river water samples was obtained from collections performed in August (beginning of the dry season) and November (dry season), 2004 and in February (beginning of the wet season) and June (wet season), 2005. Using RT-PCR, rotavirus was the most frequent (44.2%), followed by human adenovirus (30.8%), human astrovirus (15.4%) and norovirus (5.8%).

Our result agrees with La Rosa *et al.* [45], in Italy, none of the 15 river, estuarine and seawater samples collected during June 2004 were negative for GI or GII NoV. In addition to study done in Egypt by El-Senousy *et al.* [43], noroviruses neither GGI nor GGII were detected in Nile water or drinking water. In contrast, our results were greatly lower than the study of Cheonghoon Lee *et al*. [46], in south Korea, 58 surface water samples were collected 15 times monthly or semimonthly between May 2002 and March 2003 at each of four river tributaries (the Sanbon, Hwajeong, Ansan and Siheung rivers), a total of 58 water samples were analyzed for norovirus GI and GII by RT-nested PCR. Among them, 32 (55.2%) and 26 (44.7%) showed positive results for GI and GII, respectively. Also, our results are greatly lower than that of Aw *et al.* [47], in Singapore, among the 60 water samples collected, noroviruses were detected in 43 (71.7%) of these samples. The samples were collected from tropical countries, such as Singapore and from the site of samples, there are many old buildings within the catchment and the sewers were laid more than 40 years ago.

In our results, astrovirus genogroup B was more frequent than astrovirus genogroup A in sewage samples and this agreed with the study of El-Senousy *et al*. [29], in Cairo, Egypt.

CONCLUSIONS

We could conclude that norovirus genogroup I was less frequent than rotaviruses and astroviruses in both Egyptian clinical and raw sewage samples while, both norovirus genogroups I and II were completely absent in Nile water, drinking water and treated sewage effluents. From other hand, polyethylene glycol method was 1 $\log_{10}$ more efficient than organic flocculation method as a secondary concentration step for viral concentration from sewage samples before and after treatment, Nile water samples and water samples after treatment steps.

REFERENCES

1. Pallansch, M.A. and R.P. Roos, 2001. Enteroviruses: polioviruses, coxsackieviruses, echoviruses and newer enteroviruses, In: Virology. Ed., Fields, B.N.Lippincott Williams & Wilkins, Philadelphia, Pa. pp: 723-775.
2. Moore, B.E., 1993. Survival of human immunodeficiency virus (HIV), HIV-infected lymphocytes and poliovirus in water. Appl. Environ. Microbiol., 59: 1437-1443.
3. Gilgen, M., B. Wegmüller, P. Burkhalter, H.P. Bühler, U. J. Müller Lüthy and U. Candrian, 1995. Reverse transcription PCR to detect enteroviruses in surface water. Appl. Environ. Microbiol., 61: 1226-1231.

4. Chapron, C.D., N.A. Ballester, J.H. Fontaine, C.N. Frades and A.B. Margolin, 2000. Detection of astroviruses, enteroviruses and adenovirus types 40 and 41 in surface water collected and evaluated by the information collection rule and an integrated cell culture-nested PCR procedure. Appl. Environ. Microbiol., 66: 2520-2525.
5. Greening, G.E., J. Hewitt and G.D. Lewis, 2002. Evaluation of integrated cell culture-PCR (C-PCR) for virological analysis of environmental samples. Appl. Microbiol., 93: 745-750.
6. Lee, S.H. and S.J. Kim, 2002. Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. Water Res., 36: 248-256.
7. El-Senousy, W.M., A.B. Barakat, H.E. Ghanem and M.A. Kamel, 2013. Molecular Epidemiology of Human Adenoviruses and Rotaviruses as Candidate Viral Indicators in the Egyptian Sewage and Water Samples. World Applied Sciences Journal, 27: 1235-1247.
8. Schwab, K.J., R. Leon and M.D. Sobsey, 1995. Concentration and purification of beef extract mock eluates from water samples for the detection of enteroviruses, hepatitis A virus and Norwalk virus by reverse transcription-PCR. Appl. Environ. Microbiol., 61: 531-537.
9. Kukkula, M., L. Maunula, E. Silvennoinen and C.H. Von Bonsdorff, 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. J. Infect. Dis., 180: 1771-1776.
10. Anderson, E.J. and S.G. Weber, 2004. Rotavirus infection in adults. Lancet Infect Dis., 4: 91-99.
11. Muscillo, M., A. Carducci, G. La Rosa, L. Cantiani and C. Marianelli, 1997. Enteric virus detection in Adriatic seawater by cell culture, polymerase chain reaction and polyacrylamide gel electrophoresis. Water Res., 31: 1980-1984.
12. Spinner, M.L. and G.D. Di Giovanni, 2001. Detection and identification of mammalian reoviruses in surface water by combined cell culture and reverse transcription-PCR. Appl. Environ. Microbiol., 67: 3016-3020.
13. Muscillo, M., G. La Rosa, C. Marianelli, S. Zaniratti, M. R. Capobianchi, L. Cantiani and A. Carducci, 2001. A new RT-PCR method for the identification of reoviruses in seawater samples. Water Res., 35: 548-556.
14. La Rosa, G., M. Pourshaban, M. Iaconelli and M. Muslillo, 2008. Recreational and drinking waters as a source of norovirus gastroenteritis outbreaks. Appl. Environ. Microbiol., 4: 15-24.
15. Patel, M.M., A.J. Hall, J. Vinje and U.D. Parashar, 2009. Noroviruses: a comprehensive review. J. Clin. Virol., 44: 1-8.
16. Hansen, S., S. Stamm-Balderjahn, I. Zuschneid, H. Behnke Ruden, R.P. Vonberg and P. Gastmeier, 2007. Closure of medical departments during nosocomial outbreaks: data from a systematic analysis of the literature. J. Hosp. Infect., 65: 348-353.
17. Patel, M.M., M.A. Widdowson, R.I. Glass, K. Akazawa, J. Vinje and U.D. Parashar, 2008. Systematicliterature review of role of noroviruses in sporadic gastroenteritis. J. Emerg Infect Dis., 14: 1224-31.
18. Koopmans, M., 2008. Progress in understanding norovirus epidemiology. Curr. Opin Infect Dis., 21: 544-52.
19. Donaldson, E.F., L.C. Lindesmith, A.D. Lobue and R.S. Baric, 2008. Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. Immunol Re., 225: 190-211.
20. Smith, E.M. and C.P. Gerba, 1982. Development of a method for detection of human rotavirus in water and sewage. Appl. Environ. Microbiol., 43: 1440-1450.
21. Rose, J.B., S.N. Singh, C.P. Gerba and L.M. Kelley, 1984. Comparison of microporous filters for concentration of viruses from wastewater. Appl. Environ. Microbiol., 47: 989-992.
22. Katzenelson, E., B. Fattal and T. Hostovesky, 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol., 32: 838-839.
23. Lewis, G.D. and T.G. Metcalf, 1988. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water and sediment samples. Appl. Environ. Microbiol., 54: 1983-1988.
24. El-Senousy, W.M., M.I. Costafreda, R.M. Pinto and A. Bosch, 2013. Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. International Journal of Food Microbiology, 167: 74-79.
25. Kageyama, T., S. Kojima, M. Shinohara, K. Uchida, S. Fukushi, F.B. Hoshino, N. Takeda and K. Katayama, 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J. Clin. Microbiol., 41: 1548-1557.
26. Kojima, S., T. Kageyama, S. Fukuda, F. Hoshino, M. Shinohara, K. Uchida, K Natori, N. Takeda and T. Kageyama, 2002. Genogroup-specific PCR primer for detection of Norwalk-like viruses. J. Virol. Methods., 100: 107-114.

27. Iturriza Gomara, M., C. Wong, S. Blome, U. Desselberger and J. Gray, 2002. Molecular characterization of *VP6* genes of human rotavirus isolates: Correlation of genogroups with subgroups and evidence of independent segregation. J. Virol., 76: 6596-6601.
28. Gallimore, C.I., C. Taylor, A.R. Genney, A.J. Cant, A. Galloway, M. Iturriza-Gomara and J.J. Gray, 2006. J. Clin. Microbiol., 44: 395-399.
29. El-Senousy, W.M., S. Guix, I. Abid, R.M. Pintó and A. Bosch, 2007. Removal of Astrovirus from Water and Sewage Treatment Plants, Evaluated by a Competitive Reverse Transcription-PCR. Appl. Environ. Microbiol., 73: 164-167.
30. Ghazy, M.M.E., W.M. El-Senousy, A.M. Abdel-Aatty, B. Hegazy and M. Kamel, 2008. Performance evaluation of a waste stabilization pond in a rural area in Egypt. American Journal of Environmental Sciences, 4: 316-326.
31. Dominguez, A., N. Torner, L. Ruiz, A. Martinez, I. Barrabeig, N. Camps, P. Godoy, S. Minguell, I. Parron, A. Pumares, M.R. Sala, R. Bartolome, U. Perez, M. de Simon, R. Montava and J. Buesa, 2008. Aetiology and epidemiology of viral gastroenteritis outbreaks in Catalonia (Spain) in 2004–2005. J. Clin. Microbiol., 43: 126-131.
32. Roger, D., B. Kate, F. Rachel, H. Kylie, L. Victoria, E. John and F. Morey, 2002. Incidence of Human Astrovirus in Central Australia (1995 to 1998) and Comparison of Deduced Serotypes Detected from1981 to 1998. J. Clin. Microbiol., 40: 4114-4120.
33. Coluchi, N., V. Munford, J. C. Vazquez, M. Escobar, E. Weber, P. Mármol and M.L. Rácz, 2002. Detection, subgroup specificity and genotype diversity of rotavirus strains in children with acute diarrhea in Paraguay. J. Clin. Microbiol., 40: 1709-1714.
34. Rosa e Silva, M.L., I.P. De Carvalho and V. Gouvea, 2002. 1998-1999 Rotavirus Seasons in Juiz de Fora, Minas Gerais, Brazil: Detection of an Unusual G3P [4] Epidemic Strain. J. Clin. Microbiol., 40: 2837-2842.
35. Antunes, H., A. Afonso, M. Iturriza, I. Martinho, C. Ribeiro, S. Rocha, C. Magalhães, L. Carvalho, F. Branca and J. Gray, 2009. G2P[4] the most prevalent rotavirus genotype in 2007 winter season in an European non-vaccinated population. J. Clin. Virol., 45: 76-78.
36. Kamel, A.H., M.A. Ali, H.G. El-Nady, A. de Rougemont, P. Pothier and G. Belliot, 2009. Predominance and circulation of enteric viruses in the region of Greater Cairo, Egypt. J. Clin. Microbiol., 47: 1037-1045.
37. Nicholas, M., M. Jason, N. Atunga, K. Julia, D. Andrew and B. Maureen, 2007. Astrovirus Infection in Young Kenyan Children with Diarrhoea. Journal of Tropical Pediatrics, 53: 206-209.
38. Mustafa, H., E.A. Palombo and R.F. Bishop, 2000. Epidemiology of astrovirus infection in young children hospitalized with acute gastroenteritis in Melbourne, Australia, over a period of four consecutive years, 1995 to 1998. J. Clin. Microbiol., 38: 1058-1062.
39. Douglas, K., M. Stephan, S. Monroe, X. Jiang, O. David, M. Roger, I. Glass and K. Larry, 1995. Virologic Features of an Astrovirus Diarrhea Outbreak in a Day Care Center Revealed by Reverse Transcriptase-Polymerase Chain Reaction. The Journal of Infectious Diseases, 172: 1437-1444.
40. Kittigul, L., K. Porntip, S. Dusit, U. Fuangfa, C. Kitja, C. Nattasai and V. Kanda, 2001. An Improved Method for Concentrating Rotavirus from Water Samples. Mem Inst Oswaldo Cruz, Rio de Janeiro, 96: 815-821.
41. Gutierrez, M.F., M. Alvarado, E. Martinez and N.J. Ajami, 2007. Presence of viral proteins in drinkable water-Sufficient condition to consider water a vector of viral transmission. Water Res., 41: 373-378.
42. Villena, C., W.M. El-Senousy, F.X. Abad, R.M. Pintó and A. Bosch, 2003. Group A rotavirus in sewage samples from Barcelona and Cairo: Emergence of unusual genotypes. J. Appl. Environ. Microbiol., 69: 3883-389.
43. El-Senousy, W.M., R.M. Pintó and A. Bosch, 2004. Epidemiology of human enteric viruses in the Cairo water environment. The 1st International Conference of Environmental Research Division on Sustainable Development Environmental Challenges Facing Egypt. National Research Centre, Cairo, Egypt.
44. Marize, P., F.M. Fabiana, R. Flavia, M. Tu´lio, D. Leonardo, B. Sergio Luiz, A. Luciete and G. Jose Paulo, 2008. Molecular Detection and Characterization of Gastroenteritis Viruses Occurring Naturally in the Stream Waters of Manaus, Central Amazonia, Brazil. J. Appl. Environ. Microbiol., 74: 375-382.

45. La Rosa, G., S. Fontana, A. Di Grazia, M. Iaconelli, M. Pourshaban and M. Muslillo, 2007. Molecular identification and genetic analysis of norovirus genogroups I and II in water environments: comparative analysis of different reverse transcription-PCR assays. J. Appl. Environ. Microbiol., 73: 4152-4161.
46. Cheonghoon, L. and K. Sang-Jong, 2008. The genetic diversity of human noroviruses detected in river water in Korea. Water Res., 42: 4477-4484.
47. Aw, T.G., K.Y. Gin, L.L.E. O on, E.X. Chen and C.H. Woo, 2009. Prevalence and Genotypes of Human Noroviruses in Tropical Urban Surface Waters and Clinical Samples in Singapore. Appl. Environ. Microbiol., 75: 4984-4992.