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Prevalence of Human and Animal Rotaviruses and HEV in Egyptian Nile Water Resources

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Abstract: The frequency of human and animal rotaviruses and Hepatitis E virus (HEV) in Nile water (inlets) and drinking water (outlets) of traditional water treatment plant (WTP, Meet Kames) and two small water treatment plants (compact units, Shoha and Mahalet Damana) in Nile Delta of Egypt (El-Dakahlia Governorate) was investigated. The efficiency of traditional water treatment plant and compact units in the removal of human and bovine rotaviruses and HEV was also compared. Using RT-PCR, the frequency of rotavirus in water samples collected from Meet Khames water treatment plant was 41.7% (10/24) and 29.2% (7/24) in raw Nile water and after final chlorine (drinking water), respectively. At the same time, the frequency of rotavirus was 20.8% (5/24), 16.7% (4/24) and 20.8% (5/24) and 4.2% (1/24) in raw Nile water and after final chlorine (drinking water), of the two compact units (Shoha and Mahalet Damana) respectively. The frequency of human and bovine rotavirus strains in the positive samples was compared. From the other hand, the capability of different studied WTPs to remove human and bovine rotavirus strains was also compared. Low prevalence of HEV and high prevalence of rotaviruses in water samples was observed. The most frequent rotavirus combination with human origin found was P[4]G1, followed by P[8]G1 and both P[4]G3, P[8]G3 and P[6]G1 in equal percentages. The only detected rotavirus combination with animal origin was P[11]G10.

Key words: *Human rotavirus · Animal rotavirus · HEV · Water resources · Domestic animals · Zoonotic viruses · Water treatment plants*

INTRODUCTION

Enteric viruses are excreted with human feces and have been found not only in wastewater but also in rivers, recreational waters and even treated drinking water. In the USA, 10% of waterborne outbreaks are reportedly associated with viral agents [1]. Over 100 virus species present in sewage and contaminated water and cause a wide variety of illnesses in man. These include hepatitis, gastroenteritis, meningitis, fever, conjunctivitis and also diabetes. It is estimated that 1 billion diarrheal illnesses and 3.3 million deaths from diarrhea occur annually in the under-5-year age group in developing countries of Africa, Latin America and Asia [2].

Hepatitis E virus (HEV) causes an acute form of hepatitis that is transmitted via the fecal–oral route. It has been responsible for sporadic waterborne epidemics of hepatitis, especially in developing countries and results in high mortality among pregnant women. There are accumulating lines of evidence that a number of animal species such as pigs, rats, mice, rabbits, dogs, cows, sheep and goats may act as natural reservoirs of HEV [3].

Rotaviruses are the most common causes of acute diarrhea in infants and young children, causing approximately 111 million episodes of gastroenteritis and 800.000 deaths in children (less than 5-years-old) worldwide each year [4]. Rotaviruses have been detected in wastewater [5- 8], river water and even in drinking water

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[6, 9, 10]. Current wastewater treatments do not ensure complete virus removal [11-14], hence viruses become environmental contaminants in numbers high enough to represent a public health threat although low enough to pose serious difficulties for their detection. Water-related diseases are associated with exposure to water environments especially, waters used for drinking [15].

Rotaviruses have a wide host range, infecting many animal species as well as humans. These unusual human rotavirus types may have arisen either as whole virions or as genetic reassortants between human and animal strains during coinfection of a single cell. Rotaviruses are able to form new strains by a mechanism of reassortment. Reassortment can occur when two rotaviruses of two different strains infect the same cell and during replication and packaging they exchange genome segments so zoonotic transmission may be occur [16]. Domestic animals produce large amounts of residues. These domestic animals are found in the majority of houses of Egyptian farmers in villages along the River Nile. Additionally, these enteric viruses which are excreted with human feces and also with animal residues constitute an additional viral load in the raw water which enters the water treatment plants.

So in the present study we made an investigation to show the incidence of human and animal rotavirus strains in addition to HEV in the water resources before and after treatment. HEV had been proved to be zoonotic virus. Also, there had been speculation on the role of animals as a source of rotavirus infection of human (different rotavirus strains may have a zoonotic nature and called human–animal reassortant strains) to show the role of animals in the contamination of water resources with zoonotic viruses [17-19].

MATERIALS AND METHODS

Water Samples: Water samples (20L each) were collected from Nile water (before treatment) and drinking water (after treatment) from Meet Khamees water treatment plant and Before treatment (Nile water) and after treatment (drinking water) from compact units water treatment plants (Shoha, Mahalet Demana in El-Daqahlia governorate villages in the Delta (north of Cairo) from October 2006 to September 2008.

Water Treatment Facilities: Two different types of drinking water treatment facilities (two compact units (CUs) and one conventional drinking water treatment plants (DWTPs) were compared on the bases of their production capacity. The two compact units (Shoha and Mahalet Damana) were supplied by a small freshwater canal (Tanah canal), serve two villages Shoha and Mahalet Damana in El-Dakahlia Governorate (10000-15000 persons for each) with flow rate 200 m3/day, 12 minutes sedimentation time and residual chlorine 2 ppm. The conventional DWTPs (Meet Khamees) is located in Mansoura city and supplied by the raw water from the Demiatta branch of the River Nile with flow rate 130000 m3/day, 2 hours sedimentation time and residual chlorine 2 ppm. It serves about 500000 persons in Mansoura city. The treatment processes in Meet Khamees water treatment plant include pre-chlorination, flocculation, sedimentation, sand filtration and finally post chlorination.

Sewage Samples: Wastewater samples (4L each) were collected from raw sewage (before treatment) and treated effluents (after treatment) from Meet Khamees wastewater treatment plant from Mansoura city, El-Daqahlia Governorate from October 2007 to September 2008.

Concentration of Rotavirus in Water and Sewage Samples: Water samples (20 liters) and sewage samples (4 liters) were concentrated by filtration through negatively charged nitrocellulose membrane filters (142 mm-diameter, 0.45 µm pore size, Alpet, Spain).and adsorbed viruses were eluted with 70 ml of 0.05 M glycine (Merck-Schuchardt) buffer, pH 9.5, containing 3% beef extract (Lab-Limco powder, Oxoid) [20, 21]. All samples were reconcentrated using an organic flocculation method [22]. Samples were neutralized and kept at -70 ºC until used.

Concentration of HEV in Sewage and Water Samples: According to Jothikumar *et al*. [23], briefly, 4 liters sewage samples and 20 liters water samples was blended for 5 min. Coarse material was removed through centrifugation in a 250-ml tube at 3,000 rpm for 20 min. The supernatant was supplemented with $MgCl_2.6H_20$ (Merck-Schuchardt) to a final concentration of 1,200 mg/liter. Samples were adjusted to pH 5.0 and then samples were filtered through negatively charged nitrocellulose membrane filters (142 mm-diameter, 0.45 µm pore size, Alpet, Spain). Eluates to microliter volumes Subsequent elution of viruses from the Millipore filters was performed with 100 ml of Urea (Panreac-spain) (1.5 M), Arginine (Merck-Schuchardt) (0.02 M) and Phosphate (Merck-Schuchardt)

(0.008 M) buffer (UAPB). Reconcentration of this primary eluate will be performed by addition of 1 ml of $MgCl_2.6H_20$ (Merck-Schuchardt) (1 M) to the eluate. A precipitate which subsequently formed after stirring of the eluate was recovered by centrifugation at 5,000 rpm for 30 min and was dissolved in 4 ml of Mcllvaines buffer (pH 5.0). Further concentration of viruses to microliter volumes from the Mcllvaines buffer (pH 5.0) was accomplished by passing the solution through smaller-diameter (47 mm) Millipore membrane filters (0.45 µm pore size) for adsorption of viruses, elution of that filter with 5 ml of UAPB and further reconcentration by adding 100 µl of 1M $MgCl_2.6H_20$ (Merck-Schuchardt) to the 5 ml of eluate. The resulting precipitate was collected though centrifugation at 7,000 rpm for 30 min and the precipitate was then dissolved in 400 µl of McIlvaines buffer (pH 5.0) and transferred to a 2-ml Eppendorf centrifuge tube.

Stool Samples: A total of 120 stool specimens were obtained from children of < 5 years of age who presented with acute diarrhea during March 2008 to March 2009 collected from Academic Mansoura hospital in Mansoura city, Dakahlia governrate. Approximately 0.1 g of stool samples was weighed; diluted 1:10 in nuclease-free H_2O and vortexed for 30s. 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**.**

Detection of Immunoglobulin G Antibodies to Hepatitis E Virus by Class Capture Enzyme Immunoassay: Blood samples were collected from 3 groups. First group included adults (35-40 years), second group included pregnant women (20-35 years) and third group included children (2-4 years) from two Egyptian communities. The communities were located in two geographically different regions of Egypt, one in Cairo Governorate (El Hussein hospital) and the other in the Nile delta (El-Dakahlia Governorate from El Dawly hospital in Mansoura city). Blood samples were collected in the year 2009. Blood was permitted to clot, centrifuged at 800 xg for 10 min. Sera from these samples were separated and aliquots were kept at -70°C. Anti-HEV IgG antibodies were determined using IgG ELISA kit (Adaltis-Italy, Gen Lab Co.) and according to manufacturer's instructions.

Extraction of Ribonucleic Acids: Extraction was made using TRIzol (BIOZOL Total RNA Extraction reagent) (Invitrogen Life Technologies, Paisley, Scotland,) according to the manufacturer's instructions.

RT-PCR for the Detection of HEV in Concentrated Water Samples: Two methods for detection of HEV were done. The first one was according to Jothikumar *et al*. [23] and the other one was according to Kasorndorkbua *et al*. [24].

RT-PCR of a Fragment of the VP6-Coding Gene of Rotaviruses: It was done according to Iturriza-Gomara *et al*. [25] and Gallimore *et al.* [26], using the first pair of primers VP6-F GACGGVGCRACTACATGGT and VP6-R GTCCAATTCATNCCTGGTGG to amplify a 379-bp region (nucleotides [nt] 747 to 1126, coding for amino acid 241 to 367) of the VP6 gene. The second-round primers were VP6-NF GCW AGA AATTTT GAT ACA and VP6-NR GATTCACAAACTGCAGA to amplify 155 bp fragment. PCR products (10 µl) were analyzed by electrophoresis on 3% agarose gels (Panreac-spain).

Human Rotavirus Typing: VP6-positive samples were analyzed further and the G type and P type were determined by using the methods described by Gouvea *et al*. [27] and Gentsch *et al*. [28], respectively. The cocktail of primers used in the multiplex reaction allowed determination of the G1, G2, G3, G4, G8 and G9 VP7 types [27, 29] and the P[4], P[6], P[8] and P[9] VP4 types [28].

Human Rotavirus G Typing: G typing was performed using a nested PCR [27]. The RT and the first round PCR amplified the whole length of the VP7 gene (1062 bp) using primers Beg9 and End9. The second-round typing PCR was a multiplex PCR and included the primer RVG9 and the G-type- specific primers aBT1 (G1 specific), with a predicted product size of 749 bp, aCT2 (G2 specific), with a predicted product size of 652 bp, aET3 (G3 specific), with a predicted product size of 374 bp, aDT4 (G4 specific), with a predicted product size of 583 bp, AT8 (G8 specific), with a predicted product size of 885 bp and AFT9 (G9 specific), with a predicted product size of 306 bp. The PCR mixture for the first round consisted of 5 µl of the PCR buffer (Fermentas-EU), 0.2 mM of each dNTPs, 1 U of Expand PCR enzyme (Fermentas-EU) and 100 µM concentrations of each primer. The first round PCR was performed by adding 5 µl of cDNA (primed with 1 µM of both Beg 9 and End 9, 0.2 mM of dNTPs and 3 U of RT enzyme (Fermentas-EU); RT was done at 50°C for 1h) to 45 µl of PCR mixture. After denaturation at 95°C for 9 min, 40 PCR cycles each consisting of 94°C for 1 min, 47°C for 2 min and 72°C for 5 min were performed, followed by an extension at 72°C for 10 min. The second-round PCR was performed using 2 µl of the first-round reaction product in

the same mixture described above but involving all the multiplex primers. The PCR protocol was as follows, denaturation at 95°C for 9 min, 35 PCR cycles each consisting of 94°C for 1 min, 45°C for 2 min and 72°C for 3 min followed by an extension at 72°C for 10 min. PCR products (10 µl) were loaded on 3% agarose (Panreac-spain)) containing 0.5 µg of ethidium bromide per ml. After electrophoresis at 120 V for 1 h, the gels were photographed under UV light.

Human Rotavirus P Typing: P typing was performed using a seminested PCR [28]. The RT combined with the first round PCR amplified an 876-bp fragment of the gene 4 of group A rotaviruses, by using the consensus primers Con2 and Con3. The second round PCR incorporated Con3 and the P type specific primers 1T-1 (P[8] specific), with a predicted product size of 346 bp, 2T-1 (P[4] specific), with a predicted product size of 483 bp, 3T-1 (P[6] specific), with a predicted product size of 267 bp, The RT reaction with primers Con2 and Con3 was performed in a similar way that in G typing. The PCR mix for the first and second rounds of amplification was the same as that for the G typing except for the annealing temperature of the second-round PCR that was 44°C. PCR products were examined as described above. PCR products (10 µl) were loaded on 3% agarose (Panreac-spain) containing 0.5µg of ethidium bromide per ml. After electrophoresis at 120 V for 1 h, the gels were photographed under UV light.

VP4 Typing of Bovine Group A Rotaviruses: P typing was performed using a seminested PCR [28]. The PCR mixture for the first round consisted of 5 µl of the PCR buffer (Fermentas-EU), 0.2 mM of each dNTPs, 1 U of Taq DNA polymerase (Fermentas-EU) and 100 µM concentrations of each primer. The first round PCR was performed by adding 5 µl of cDNA (primed with 1 µM of both Con3/Con2, 0.2 mM of dNTPs and 3 U of RT enzyme (Fermentas-EU); RT was done at 50°C for 1h) to 45 µl of PCR mixture. After denaturation 9 min. incubation at 95°C, followed by 40 cycles of PCR (94°C for 1 min, 47°C for 2 min and 72°C for 5min) and a final 10-min incubation at 72°C.. The seminested PCR amplification performed from dsDNA that was obtained from the first amplification of the entire gene 4. In this case, 5 µl of the dsDNA product served as the template for this second typing amplification. The same reaction buffer was used, but with the addition of the primers mix containing all serotype-specific primers pNCDV, pUK, pB223 and the common primer Con2 [24]. The PCR program was 9-min incubation at 95°C, followed by 35 cycles of PCR (94°C for 1 min, 45°C for 2 min and 72°C for 3min) and a final 10-min incubation at 72°C. From each PCR product, 10µ1 were analyzed on 3% agarose gels (Panreac-spain) stained with ethidium bromide.

VP7 Typing of Bovine Group A Rotaviruses: For the detection of group A rotaviruses, the VP7 gene was amplified using the primer pair Beg9/End9 [27]. The PCR mixture for the first round consisted of 5 µl of the PCR buffer (Fermentas-EU), 0.2 mM of each dNTPs, 1 U of Expand PCR enzyme (Fermentas-EU) and 100 µM concentrations of each primer. The first round PCR was performed by adding 5 µl of cDNA (primed with 1 µM of both Beg 9 and End 9, 0.2 mM of dNTPs and 3 U of RT enzyme (Fermentas-EU); RT was done at 50°C for 1h) to 45 µl of PCR mixture. After denaturation at 95°C for 9 min, 40 PCR cycles each consisting of 94°C for 1 min, 47°C for 2 min and 72°C for 5 min were performed, followed by an extension at 72°C for 10 min.

The seminested PCR amplification performed from dsDNA that was obtained from the first amplification of the entire gene 9. In this case, 5µl of the dsDNA product served as the template for this second typing amplification. The same reaction buffer was used, but add the primers mix containing all serotype-specific primers DT6 and ET10 and the common primer Beg9 [29, 30]. The PCR program was 9-min incubation at 95°C, followed by 35 cycles of PCR (94°C for 1 min, 45°C for 2 min and 72°C for 3min) and a final 10-min incubation at 72°C. From each PCR product, 10µ1 were analyzed on 3% agarose gels (Panreac-spain) stained with ethidium bromide.

Quantitation of HEV using SYBR Green Method in Water Samples: Real time RT-PCR was done for two positive samples and some selected negative samples for HEV RNA in a previous RT-PCR screening. The RT mixture contained 10 pmol of 1x reverse transcriptase buffer (Fermentas-EU) containing a 10 p.M concentration of each deoxynucleoside triphosphate (dNTP) (Fermentas-EU), 0.4 µl of the antisense primer (3157N), 20 pmol of a heat-denatured and quick-chilled test sample containing RNA and 20 U of Moloney Murine Leukemia Virus reverse transcriptase at 42°C for 1hr. Real time PCR was done using power SYBR green PCR master mix (Applied Biosystem, UK) with 0.5µl of forward primer 3156N and reverse primer 3157N according to Kasorndorkbua *et al*. [24] in a total volum of 25µl. Amplification was performed with 45 cycles in a Real time PCR machine (Step one, Applied Biosystem). The cycle

involved denaturation at 95°C for 5 min, primer annealing at 95°C for 15 sec. and primer extension at 56°C for 1 min. The final extension step was at 65°C for 1 min. Positive control used was a plasmid (pCR-XL-TOPO, Invetrogen, USA) containing cDNA from a genotype 1 HEV isolate (dilution from 10^{-1} to 10^{-6}).

Quantitation of Rotavirus using SYBR Green Method in Water Samples: Real time RT-PCR was done for positive samples in previous RT-PCR screening. The RT mixture contained10 pmol of 1x reverse transcriptase buffer (Fermentas-EU) containing a 10 p.M concentration of each deoxynucleoside triphosphate (dNTP) (Fermentas-EU), 0.4 µl of the sense and antisense primer VP6-3 and VP6-4 according to Villena *et al*. [7], 20 pmol of a heat-denatured and quick-chilled test sample contained RNA and 20 U of Moloney Murine Leukemia Virus reverse transcriptase**.** Real time PCR was done using power SYBR green PCR master mix (Applied Biosystem, UK) with 0.5 µl of forward primer VP6-3 and reverse primer VP6-4 in a total volume of 25µl. Amplification was performed with 45 cycles in a Real time PCR machine (Step one, Applied Biosystem). The cycle involved denaturation at 95°C for 5 min, primer annealing at 95°C for 15 sec. and primer extension at 60°C for 1 min. The final extension step was done at 65°C for 1 min. Positive control used was a plasmid (pCR2.1-TOPO, Invetrogen, USA) containing full length of cDNA of rotavirus VP-6 genome (dilution from 10^{-1} to 10^{-6}).

CC-RT-PCR for Quantification of Infectious Rotavirus Particles: It was done according to El-Senousy *et al*. [31] and Ghazy *et al*. [32].

RESULTS

Molecular Detection for Hepatitis E virus in Water Samples: Using RT-PCR, HEV RNA was detected only in one (Raw of WTP of Mahalet Damana in October, 2008) out of 72 of total inlet water samples (1.4%) which were collected from these water treatment plants during October 2006 until September 2008, HEV was not detected in all drinking water samples (after final chlorine).

Quantitation of HEV in Some Water Samples Using Real Time RT-PCRL: The positive sample of HEV RNA in RT-PCR screening contained $1x10^2$ RNA copies/l. From the eight negative samples chosen and quantified using sensitive real time RT-PCR, only one sample had HEV genome ($3x10^1$ RNA copies /l) (raw water of Meet Khamees WTP, February, 2007).

Detection of Immunoglobulin G Antibodies to Hepatitis E Virus by Class Capture Enzyme Immunoassay: In Dakahlia governorate 18 adult serum samples out of 90 (20%) were positive for Anti-HEV IgG, 22 pregnant serum samples out of 73 (30.14%) were positive for Anti-HEV IgG and 15 child serum samples out of 86 (17.44%) were positive for Anti-HEV IgG. While in Greater Cairo 11 adult serum samples out of 60 (18.3%) were positive for Anti-HEV IgG, 10 pregnant serum samples out of 36 (27.78) were positive for Anti-HEV IgG and 7 child serum samples out of 50 (14%) were positive for Anti-HEV IgG (Table 1). Overall the frequency of anti-HEV IgG in pregnant was more than adult and children.

Molecular Detection of HEV in Sewage Samples: HEV was detected in only one (Inlet of wastewater treatment plant of Meet Khamees in Oct., 2008) (8.3%) out of 12 of total sewage samples collected from this wastewater treatment plant during October 2007 until September 2008 and complete absence of HEV in treated effluents was observed.

Molecular Detection for Human Rotavirus in Water Samples: The prevalence of human rotavirus in water samples collected from Meet Khames water treatment plant from October 2006 until September 2008 was 41.7% (10/24) and 29.2% (7/24) in raw Nile water and in drinking water (after final chlorine) respectively. At the same time, the prevalence of human rotavirus in Shoha water treatment plant was 20.8% (5/24) and 16.7% (4/24) in raw Nile water and in drinking water (after final chlorine) respectively. The prevalence of rotavirus in Mahalet Damana water treatment plant was 20.8% (5/24) and 4.2% (1/24) in raw Nile water and in drinking water (after final chlorine) respectively (Table 2).

Table 1: Anti-HEV IgG positive samples in Egyptian human groups.

Adults Number (%)		Pregnant women Number (%)		Children Number (%)	
Dakahlia	Cairo	Dakahlia	Cairo	Dakahlia	Cairo
18/90 (20%)	11/60 (18.3%)	22/73 (30.14%)	10/36 (27.78)	15/86 (17.44%)	7/50 (14%)

Table 2: Frequency of human rotavirus in Meet Khames water treatment plant (Oct.2006-Sep.2008)

Sampling date	Raw Nile water	Drinking water (after final chlorine)
Oct. 2006	-	-
Nov. 2006	-	-
Dec. 2006	+G1P4, G10P11	-
Jan. 2007	+G1P8, G10P11	+G1P8
Feb. 2007	-	-
Mar. 2007	+G3P4	+ G3P4
Apr. 2007	+G1P4	+P4
May.2007	-	-
Jun. 2007	-	+G1P4
Jul.2007	-	-
Aug. 2007	-	-
Sep. 2007	+G3P8	+P8
Oct. 2007	-	+G1P6
Nov. 2007	+G1P4	+G1P4
Dec. 2007	+G1P4, G10P11	-
Jan. 2008	+G1P4	-
Feb. 2008	-	-
Mar. 2008	+G1P8	-
Apr. 2008	+G1P8	-
May 2008	-	-
Jun. 2008	-	-
Jul. 2008	-	-
Aug. 2008	-	-
Sep.2008	-	-
No .of samples	10/24	7/24
% +ve	41.7%	29.2%

At the same time, the frequency of human rotavirus in Shoha water treatment plant (First compact unit) from October 2006 until September 2008 were 20.8% (5/24) and 16.7% (4/24) in raw water and drinking water (after final chlorine), respectively and the frequency of rotavirus in Mahalet Damana water treatment plant (second compact unit) from October 2006 until September 2008 was 20.8% (5/24) and 4.2% (1/24) in raw Nile water and drinking water (after final chlorine), respectively (Table 3).

Quantification of Some Positive Samples for Rotavirus Genome by Real Time RT-PCR and Rotavirus Infectious Units Using CC-RT-PCR in Three WTPs: The results of quantification of rotavirus genome using real time RT-PCR in some samples positive for rotavirus (By RT-PCR screening) and the results of the number of infectious units in the same samples are shown in Tables (4 and 5). The number of RNA copies/l ranged from 3x10 to $7x10^5$, 2x10 to $8x10^3$ and $8x10^2$ to $9x10^3$ in Meet Khamees, Shoha and Mahalet Damana WTPs respectively. The results of CC-RT-PCR u/l ranged from 0.5x10 to $0.5x10^3$ in Meet Khamees WTP. In Shoha and Mahalet Damana, the number of CC-RT-PCR u/l was 0.5x10 in all samples tested.

Human Rotavirus G Types in Water Samples: A total of 29 out of 32 rotavirus positive samples could be G typed, so 9.44% of the samples were untypeable. Overall, the most frequent type was G1 (21/29) (72.4%), followed by G3 (8/29) (27.6%). There were not any samples containing more than one type. Complete absence of types G2, G4, G8 and G9 was observed.

Human Rotavirus P Types in Water Samples: A total of 30 out of 32 rotavirus positive samples could be P typed, so 6.3% of the samples were untypeable. Overall, the most frequent type was P[4], 56.7% followed by (17/30); P[8], 33.3%; (10/30) and P[6], 10%. (3/30). Mixed types were not detected in the samples.

Combinations of P and G Types: The human genotypes (G and P) of rotaviruses were found in 19 samples from 72 raw Nile water samples (26.4%) and only G type in one more sample. Human genotypes of rotaviruses (G and P) were found in 8 samples from 72 drinking water samples (11.1%) and only P types in three more samples and only G type in one more sample. The most frequent rotavirus combination with human origin found was P[4]G1 (47.4%),

Table 3: Frequency of human rotavirus in two compact units Shoha and Mahalet Damana water treatment plants (2006-2008).

	Shoha WTP		Mahalet Damana WTP	
Sampling date	Raw Nile water	Drinking water (after final chlorine)	Raw Nile water	Drinking water (after final chlorine)
Oct. 2006	-	-	-	-
Nov. 2006	-	-	-	-
Dec. 2006	-	-	-	-
Jan. 2007	G1P4+ G10P11	G1P4+	G1P4+	-
Feb. 2007	-	-	-	-
Mar. 2007	G1P4+ G10P11	G1P4+	-	-
Apr. 2007	G1P8+	-	G3P4+	G3+
May.2007	-	-	-	-
Jun. 2007	-	-	-	-
Jul.2007	-	-	-	-
Aug. 2007	-	-	-	-
Sep. 2007	-	-	-	-
Oct. 2007	-	-	-	-
Nov. 2007	-	-	G1P4+	-
Dec. 2007	-	-	-	-
Jan. 2008	G3+	P8+	G1P6+G10P11	-
Feb. 2008	-	-	-	-
Mar. 2008	G3P8+	+G3P8	G1P6+	-
Apr. 2008	-	-	-	-
May 2008	-	-	-	-
Jun. 2008	-	-	-	-
Jul. 2008	-	-	-	-
Aug. 2008	-	-	-	-
Sep.2008	-	-	-	-
No .of samples	5/24	4/24	5/24	1/24
% +ve	20.8%	16.7%	20.8%	4.2%

Table 4: Number of RNA copies and infectious units in samples positive for rotavirus genome In Meet Khamees WTP.

Site of sample	Time of sample taken	RNA copies/l	CC-RT-PCR unit/l
Raw water Meet Khamees	Dec. 2006	$7x10^5$	$0.5x10^3$
Outlet water Meet Khamees	Dec.2006	0	0
Raw water Meet Khamees	March 2007	$7x10^4$	$0.5x10^2$
Outlet water Meet Khamees	March 2007	3x10	0.5x10
Raw water Meet Khamees	April 2007	$6x10^4$	0.5x10
Outlet water Meet Khamees	April 2007	9x10	0
Raw water Meet Khamees	Sep 2007	$8x10^4$	$0.5x10^2$
Outlet water Meet Khamees	Sep 2007	5X10	0
Raw water Meet Khamees	Nov 2007	$6x10^5$	$0.5x10^3$
Outlet water Meet Khamees	Nov 2007	9X10	0

Slope:-3.383, Rsq :0.989, Ct ranged from 26.62 to 38.94

Table 5: Number of RNA copies and infectious units in samples positive for rotavirus genome In Shoha and Mahalet Damana WTPs

Site of sample	Time of sample taken	RNA copies/l	CC-RT-PCR unit/l
Raw water Shoha	March 2007	$6x10^3$	0.5x10
Outlet water Shoha	March 2007	2x10	0.5x10
Raw water Shoha	April 2007	$5x10^3$	0.5x10
Outlet water Shoha	April 2007	0	0
Raw water Shoha	March 2008	$8x10^3$	0.5x10
Outlet water Shoha	March 2008	6X10	0
Raw water Mahalet Damana	April 2007	$8x10^2$	0.5x10
Outlet water Mahalet Damana	April 2007	4X10	0
Raw water Mahalet Damana	Nov. 2007	$9x10^3$	0.5x10
Outlet water Mahalet Damana	Nov.2007	0	0
Raw water Mahalet Damana	March 2008	$1x10^3$	0.5x10
Outlet water Mahalet Damana	March 2008	0	0

Slope:-3.383, Rsq :0.989, Ct ranged from 33.35 to 38.94

followed by P[8]G1 (21.1%), P[4]G3, P[8]G3 and P[6]G1 (10.5%) in the raw Nile water samples. In the drinking water samples, it was found that P[4]G1 (50%) was the most frequent followed by P[8]G1, P[4]G3, P[8]G3 and P[6]G1 (12.5%).

Molecular Detection for Animal Rotavirus in Water Samples: Seventy two Nile water samples from El-Dakahlia Governorate were investigated to G type and P type with animal origin of rotavirus, 6 out of 72 (8.3%) could be positive for G type with animal origin (these six samples were positive also for human G rotavirus) and complete absence of G6 was observed. All the six samples contained G10. They could be positive for P type with animal origin (these six samples were positive also for human P rotavirus). All the six samples contained P[11] and complete absence of P[5] and p[1] was observed. Complete absence of animal rotaviruses was observed in the 72 drinking water samples.

Molecular Detection for Human and Animal Rotavirus in Clinical Samples: Using RT-PCR, rotavirus was detected in 29.2% (35/120) of total stool samples with human origin collected from Academic Mansoura hospital in El-Mansoura city. Total G-types of rotavirus detected in stool samples were 29.2% (35/120) with human origin and the most frequent G type was G1 (25.7%) (9/35) and G3 (17.1%) (6/35), while G2, G4, G8, G9 were completely absent. While, total P-types of Rotavirus in stool were 29.2% (35/120) with human origin and the most frequent P type was P[4] (25.7%) (9/35) and P[8] (17.1%) (6/35). Overall, the most frequent rotavirus combination with human origin found was G1P[4] and G3P[8] were 25.7 and 17.1%, respectively.

Rotavirus was detected in 1.7% (2/120) of total stool samples with animal origin and these two stool samples were negative to Human P/G rotavirus typing (non-bacterial/non-parasite) collected from Academic Mansoura hospital in Mansoura city. Total G-types of Rotavirus was detected in stool samples were 1.7% (2/120) with animal origin and G10 type was the only G type was detected. While, total P-types of Rotavirus in stool were 1.7% (2/120) with animal origin and P[11] type was the only P type detected. Overall, the rotavirus combination with animal origin was found P[11]G10 (2/120)(1.7%), respectively.

DISCUSSION

In the present investigation, low incidence of HEV was observed in raw Nile water samples (1.4%) and complete absence of the virus was observed in chlorinated drinking water. This low incidence was confirmed using real time RT-PCR for negative samples where low number of genome copies/l was quantified ($1X10^2$ and 3X10). This may indicate the low prevalence of HEV in Delta region of Egypt. In the same time IgG results for adults, pregnant women and children indicated slightly higher percentage of HEV IgG in Dakahlia in relation to Cairo. Pregnant women had the highest percentage of HEV IgG followed by adults and finally children. Among blood donors attending blood transfusion Center of Suez Canal University Hospital from March to September, 2010, Hepatitis E virus antibodies (IgG) prevalence was 20.9% ([33] which is very similar to our results. Rotavirus genome was detected in the raw water of the three studied WTPs 15 times in cooler months (autumn and winter) while it was detected 5 times in spring and summer. The two years survey gave us an opportunity to confirm the incidence of rotavirus in the same months in the two years. Our results agree with the study results of El-Senousy *et al*. [6], which the peak of rotavirus incidence in Cairo River Nile water samples was in autumn and winter months. Also they agree with the study results of Villena *et al.* [7], they found that the peak of rotavirus incidence in Cairo wastewater was in autumn and winter months. Mehnert and Stewien [34] recorded a higher rate of positivity for rotavirus in wastewater samples collected during autumn and winter months than those collected in spring and summer.

The rotavirus incidence in raw water of Meet Khamees WTP was more than other two compact units. There were high pollution factors with high viral load entered Meet Khamees WTP than other two compact units (Shoha and Mahal Damana). Although, some WTPs could have higher viral removal efficiency, viruses could be appeared in their outlets with higher chances because of the higher viral loads in their inlets. Meet Khames could remove 3 to 5 $\log_{10}$ of rotavirus genome while Shoha could remove 2 to 3 $\log_{10}$ and Mahalet Damana could remove 1 to 3 $\log_{10}$ of rotavirus genome. On the other hand, Meet Khames could remove 1 to 3 $\log_{10}$ of rotavirus infectious units while Shoha could remove 0 to 1 $\log_{10}$ and Mahalet Damana could remove 1 $\log_{10}$ of rotavirus infectious units. Meet Khames serves about 500000

persons in El-Mansoura city while other 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 could not be operated correctly because it needs to supply drinking water to large population). So, the viral load in the inlet of WTPs plays an important role in the chance of viral appearance in the outlets. The presence of the viruses in the drinking water (after final chlorine) may also depend on the resistance of the virus to the treatment processes of the treatment plants [35-37]. We can also easily notice that there is a direct proportional relationship between the number of RNA copies/l and the number of infectious units/l. RT-PCR results (qualitative) and real time PCR results (quantitative) do not imply the presence of infectious viruses which the detection or quantification of genome usually occurs. So, the quantification of infectious units is very important to express the capability of WTPs to remove infectious viruses and not only the viral genome. Detection of rotavirus genome or infectious units in drinking water samples indicates the resistance of rotavirus to treatment processes (especially chlorine as a disinfectant) in the water treatment plants. The high stability of rotavirus in front of disinfectants (free chlorine, copper, silver) was previously studied at laboratory scale [35-37]. This may explain the high positivity of rotavirus in drinking water samples. El Senousy *et al*. [6] reported that rotavirus is the most resistant enteric virus to treatment processes in WTPs among all RNA enteric viruses studied.

In the present study we noticed that the most frequent human rotavirus strain was P[4]G1 (47.4%), followed by P[8]G1 (21.1%), P[4]G3, P[8]G3 and P[6]G1 (10.5%) in the raw Nile water samples. In the drinking water samples, it was found that P[4]G1 (50%) was the most frequent followed by P[8]G1, P[4]G3, P[8]G3 and P[6]G1 (12.5%, each). In the study done by El Senousy *et al*. [6], the most frequent human rotavirus strain found in Cairo wastewater was P[8]G1 (38.5%), followed by P[6]G1 (27%), P[4]G1 and P[8]G3 (11.5% each), P[6]G4 (7.7%) and P[8]G9 (3.8%), Human rotavirus strain P[4]G1 was more frequent in our study than P[8]G1 which was detected by El-Senousy *et al*. [6] and that may be due to the reassortment which usually happens to rotavirus genome, also different samples sites (Dakahlia and Greater Cairo), year of samples collection may be other reasons. P[4]G1 was also the most frequent strain in the stool samples collected from Academic Mansoura hospital in El-Mansoura city, Dakahlia Governorate.

In water samples the rotavirus genotype with animal origin found was P[11]G10 (bovine strain). The incidence of animal rotavirus P[11]G10 strain in raw Nile water samples was 8.3% (6/72). Complete absence of animal rotaviruses was observed in the 72 drinking water samples which is less frequent than the human genotypes (G and P) of rotaviruses which were found in 19 samples from 72 raw Nile water samples (26.4%). Also, human genotypes of rotaviruses (G and P) were found in 8 samples from 72 drinking water samples (11.1%). Bovines are frequent animals in the Egyptian rural areas and this may indicate a role of bovines and may be other rural animals in the contamination of water resources with some zoonotic viruses however, it is less than the contamination usually occurred by the human rotaviruses. Other evidence for the possible zoonotic transmission may be the presence of animal rotavirus in 2 human stool samples from 120 samples collected (1.7%).

CONCLUSIONS

Low prevalence of HEV and high prevalence of rotaviruses in the Nile water of Egyptian rural areas were concluded. Also, some evidences of the role of domestic animals in the contamination of Egyptian water resources with zoonotic viruses were recorded.

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