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Multiplex Semi-Nested RT-PCR for Genotyping of Rotaviruses Group A in Giza Tap Water, Egypt

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Abstract: Rotavirus is one of the most common enteric viruses that can be transmitted via contaminated water globally. From the hygienic point of view, the occurrence of rotaviruses in drinking water is not acceptable. Investigations about the presence of rotaviruses in tap water are very limited in Egypt. Overall 72 tap water samples were collected and concentrated for the detection of rotavirus group A (RV-A) VP6 gene using RT-PCR. The positive samples for RV-A were further analyzed using multiplex semi-nested RT-PCR for identification P and G rotavirus genotypes. A total of 8.3% tap water samples were positive for rotavirus group A using nested RT-PCR. The temporal distribution showed that rotaviruses isolates were noticed in three seasons, 16.7% (3/18) in autumn, 11.1% (2/18) in winter and 5.6% (1/18) in spring, while in summer there were no positive samples. Statistically, seasonal variation had no considerable effect on the prevalence of RV-A in tap water samples. About 83.3% of G-type rotaviruses were belonging to G1-type, while 50% of the rotaviruses strains were P[8] and 16.7% were P[6]. In conclusion, the presence of RV-A into tap water represented a public health risk and our findings demonstrated the urgent need to add viral parameters to water quality surveillance.

Key words: Rotavirus, tap water, multiplex RT-PCR.

Introduction

The unique aspect of water as a vehicle for the transmission of disease is that a contaminated water supply can rapidly expose a large number of people to contaminants like bacteria, viruses and protozoa (WHO, 2011). The World Health Organization (WHO) classifies contaminated water by viral pathogens as high health significance (WHO, 2011). Rotaviruses as well as enteroviruses and noroviruses have been identified as potential reference pathogens (WHO, 2011). The presence of rotaviruses in drinking water has been reported in several studies (Craun et al., 2006; Matthijnssens et al., 2011; Yousuf et al., 2017). Rotaviruses can cause diarrhea as well as other symptoms including abdominal cramping, vomiting and fever (WHO, 2011). Globally, the leading cause of infant death is rotavirus which causes over half a million deaths annually (WHO, 2011; Matthijnssens et al., 2011).

Rotavirus is a non-enveloped RNA virus; the genome consists of 11 double-stranded segments, classified into P and G genotypes based on the two RNA segments no. 4 and 9, which encode for the two surface proteins VP4 (protease sensitive P) and VP7 (glycoprotein G), respectively. About 37 P-types and 27 G-types of RV-A have been identified. Globally, G1–G4, G9, G12, P[4] and P[8] are the most common serotype belonging to human rotavirus group A (Martella et al., 2010; Trojnar et al., 2013; Desselberger, 2014).

Rotavirus-contaminated water remains one of the most common sources of infection associated with

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gastroenteritis disease worldwide (Craun et al., 2006). According to WHO and other reports, these viruses have long persistence and more stable in waters which may facilitate their transmission into human by the fecal-oral route or indirectly by ingestion of contaminated water and food (WHO, 2011; Desselberger, 2014). In Egypt, several epidemiological studies have been reported on clinical and sewage samples and showed different genotypes of rotaviruses, but the available data concerning the prevalence of rotavirus genotypes in drinking water were much more limited (Villena et al., 2003; El-Senousy et al., 2015).

The present study aimed to investigate the incidence and genotyping of rotaviruses circulating in drinking water in Giza governorate, Egypt. Also, we analyzed prevalent G- and P-types of rotavirus to identify the predominant rotavirus genotype (which may differ between and inside the same geographic area from year to year), that is important for evaluating candidate rotavirus vaccine.

Materials and Methods

Samples Collection

Six drinking water samples (20 litre each) were collected monthly along one-year period starting in June 2016 until May 2017 from Giza governorate, Egypt. Samples were collected in clean sterile bottles and transported within three hours to Virology Laboratory, National Research Centre, Egypt

Samples Concentration

After adding $AlCl_3$ at final concentration of 0.5 mM to each water sample, the pH value of each sample was adjusted to acidified pH 3.5. Then, each sample was filtrated using negatively charged nitrocellulose filter membrane (0.45 µm pore size, and 142 mm diameter). 75 ml of 0.05 M glycine buffer, pH 9.5 containing 3% beef extract (Lab-Limco powder, Oxoid, UK) were added to elute the adsorbed viruses (Rose et al., 1984). According to Katzenelson et al. (1976), an organic flocculation method was used to re-concentrate the samples. Briefly, each concentrated sample was acidified to pH 3.5 and centrifuged at 3000 rpm for 15 min. The obtained pellet was dissolved in 1 ml of Na_2HPO_4 (0.14 N, pH 9) and kept at –70°C until use.

Viral Nucleic Acid Extraction

Viral nucleic acids were extracted from 200 µl of the supernatant using GeneJET Viral DNA and RNA Purification kit (Thermo Scientific-USA) according to the manufacturer's instructions. The obtained RNA was dissolved in 40 µl of Eluent and kept at –70°C until use.

Detection of Rotavirus Group A

The VP6 segment of rotavirus was detected by using the specific primers (Table 1) for nested RT-PCR according to Gray and Iturriza-Gomara (2011), to amplify 382bp and 147bp for the first and second rounds, respectively.

Rotavirus Genotyping

Multiplex semi-nested RT-PCR was performed for genotyping of RV-A in tap water samples based on the characterization of VP7 and VP4 genes into G-type and P-type, respectively. The cocktail of the primers were used to determine G1-G6, G8-G11, P[1], and P[4]-P[11] genotypes as shown in Table 1 (Gouvea et al., 1994a and b; Gray and Iturriza-Gomara, 2011). P and G genotypes were determined according to amplicon size. The amplicon sizes were estimated using a 100bp molecular weight ladder.

Statistical Analysis

The obtained data were analyzed using one-way analysis of variation (ANOVA) in Minitab statistical program (Minitab Inc., Pennsylvania, USA). A *P*-value less than 0.05 was considered significant (Wild, 2005).

Results

By using nested RT-PCR, rotavirus VP6 amplicon was detected in six (8.3%) out of 72 examined tap water samples. For rotavirus genotyping, rotavirus VP7 amplicon was detected in 83.3% (5/6) of positive rotavirus samples, while rotavirus VP4 amplicon has been detected in 66.6% (4/6) of positive rotaviruses as shown in Table 2. Multiplex semi-nested RT-PCR for VP7 amplicons showed that all positive rotaviruses belonged to G1-type, while VP4 amplicons were related to P[6] and P[8]-types. There was no mixed infection in all positive samples (Figure 2). The temporal distribution showed that rotaviruses isolates were found in three seasons, 16.7% (3/18) in autumn, 11.1% (2/18) in winter and 5.6% (1/18) in spring, but there was no rotavirus detected in summer (Figure 1). Seasons had no significant effect on the prevalence of RV-A using one-way ANOVA ($P = 0.330$).

Figure 2 showed that 83.3% (5/6) of rotavirus-positive samples were related to G1 using multiplex semi-nested RT-PCR, while 16.7% (1/6) of the same samples were non-typed G. Other rotavirus types as G2, G3, G4, G5, G6, G8, G9, G10 and G11 were not

Table 1: Primer sequences used for genotyping of VP7 and VP4 gene of rotavirus strains

Primer	*Sequence 5′–3′*	*Target gene*	*Ref.*	*Primer set*	*Amplicon Length*
VP6-F	GACGGVGCRACTACATGGT	VP6	Gray and Iturriza-Gomara, 2011	VP6-F/ VP6-R	382
VP6-R	GTCCAATTCATNCCTGGTG				
VP6-NF	GCWAGAAATTTTGATACA	VP6		VP6-NF/ VP6-NR	147
VP6-NR	GAT TCA CAA ACT GCA GA				
VP7-F	ATGTATGGTATTGAATATACCAC	VP7	Gray and Iturriza-Gomara, 2011	VP7-F/ VP7-R	881
VP7-R	AACTTGCCACCATTTTTTCC	VP7			
aBT1	CAAGTACTCAAATCAATGATGG	VP7		aBT1/VP7-R	618
aCT2	CAATGATAT TAACACATTTTCTGTG	VP7		aCT2/VP7-R	521
G3	ACGAACTCAACACGAGAGG	VP7		G3/VP7-R	682
aDT4	CGTTTCTGGTGAGGAGTTG	VP7		aDT4/VP7-R	452
G8	TTRTCGCACCATTTGTGAAAT	VP7		G8/ VP7-R	756
G9	CTTGATGTGACTAYAAATAC	VP7		G9/ VP7-R	179
G10	ATGTCAGACTACARATACTGG	VP7		G10/VP7-R	266
FT5	CATGTACTCGTTGTTACGTC	VP7	Gouvea et al., 1994a	VP7-F/ FT5	729
DT6	CTAGTTCCTGTGTAGAATC	VP7		VP7-F/ DT6	449
BT11	GTCATCAGCAATCTGAGTTGC	VP7		VP7-F/ BT11	286
VP4-F	TATGCTCCAGTNAATTGG	VP4			
VP4-R	ATTGCATTTCTTTCCATAATG	VP4	Gray and Iturriza-Gomara, 2011	VP4-F/ VP4-R	663
2T-1	CTATTGTTAGAGGTTAGAGTC	VP4	Gray and Iturriza-Gomara, 2011	VP4-F/2T-1	483
3T-1	TGTTGATTAGTTGGATTCAA	VP4		VP4-F/3T-1	267
1T-1D	TCTACTGGRTTRACNTGC	VP4		VP4-F/1T-1D	345
4T-1	TGAGACATG CAATTGGAC	VP4		VP4-F/4T-1	391
5T-1	ATCATAGTTAGTAGTCGG	VP4		VP4-F/ 5T-1	583
P(11)	GTAAACATCCAGAATGTG	VP4		VP4-F/ P(11)	312
pNCDV	CGAACGCGGGGGTGGTAGTTG	VP4	Gouvea et al., 1994b	pNCDV / VP4-R	526
pUK	GCCAGGTGTCGCATCAGAG	VP4		pUK /VP4-R	459
pOSU	CTTTATCGGTGGAGAATACGTCAC	VP4		pOSU /VP4-R	406

Table 2: Detection of rotavirus in tap water in Giza, Egypt

Seasons	*Samples*	*Positive of VP6 amplicons rotaviruses*	*Positive of VP7 amplicons (G-type)*		*Positive of VP4 amplicons (P-type)*	
			Typed	*Non-typed*	*Typed*	*Non-typed*
Winter	18	2	2	0	0	2
Spring	18	1	1	0	1	0
Summer	18	0	0	0	0	0
Autumn	18	3	2	1	3	0
Total (n) %	72	(6/72) 8.3%	(5/6) 83.3%	(1/6) 16.7%	(4/6) 66.7%	(2/6) 33.3%

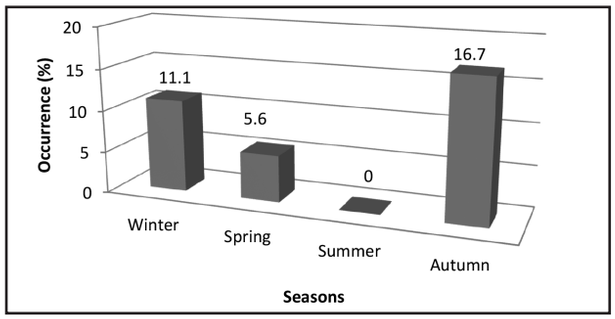

Figure 1: Seasonal variation of rotavirus in tap water.

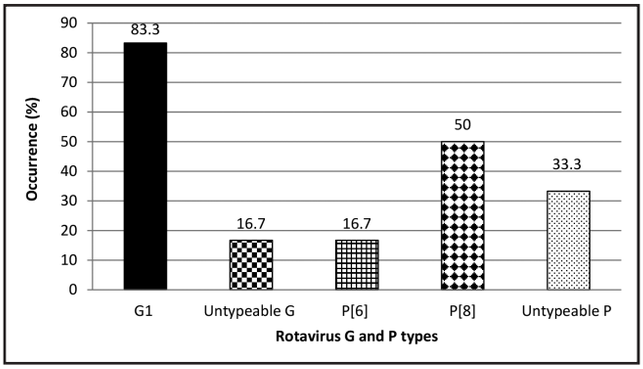

Figure 2: Occurrence of rotavirus genotypes in tap water.

detected in the collected water samples. The results showed that 66.7% of samples containing RV-A could be *P* typed, and thus the percentage of non-typed samples reached 33.3%. The *P*-typed samples were belonging to 50% (3/6) P[8] and 16.7% (1/6) P[6] genotypes, respectively. Other rotaviruses types P[1], P[4], P[5], P[7], P[9], P[10] and P[11] were not detected throughout the current study.

Discussion

The present study provided data concerning the incidence of RV-A genotypes in tap water samples. Our results showed that the incidence of RV-A reached 8.3% (6/72) in examined tap water samples. These results were relatively similar with the little incidence of rotaviruses in drinking water of the reports conducted in Egypt, France and Brazil (Gratacap-Cavallierg et al., 2000; Kluge et al., 2014; El-Senousy et al., 2015). However, our findings disagreed with the high incidence of rotaviruses in water collected from taps of schools and distribution network in southern Brazil and Ghana, respectively (Dongdem et al., 2010; Spilki et al., 2013). In another study in Pakistan, rotavirus was detected in a lower percentage (5%) in the tested drinking water samples (n = 20) (Yousuf et al., 2017). The differences in results of the present study and the study in Pakistan might be due to the sample volume (one litre), which was lower than that used in the present study.

Also lower percentages of rotavirus (1.4% and 2%) were detected in water samples analyzed in South Africa and Slovenia, respectively. While a higher occurrence percentage (11.8%) of rotavirus was detected in water samples collected from water purification plants (Gutiérrez et al., 2007; Dongdem et al., 2010). In different areas of China (Beijing), rotavirus was observed in 11.7% and 22.4% of treated drinking water and tap water samples, respectively (He et al., 2009). In Ghana, 35% of water samples from drinking water treatment plants were contaminated by rotavirus (Yousuf et al., 2017). This might be due to different methodologies that were used to detect rotavirus.

To the best of our knowledge, the published data concerning the genotypes of rotaviruses in drinking water are much more limited worldwide and in Egypt are nearly absent. Our outcomes showed that the positive samples of rotaviruses belonged to single genotype (G1) for G types, and for P types were P[6] and P[8] which were common predominant genotypes for rotaviruses in Egypt according to several reports conducted on different types of samples (Villena et al., 2003; El-Senousy et al., 2015; El-Senousy et al., 2017). In Ghana, the most predominant genotypes were G2 (8/21) and P[8] (6/21), while in the same study P[6] (3/21), G1 (1/21), G3 (1/21) and G9 (2/21) genotypes were also detected (Dongdem et al., 2010). Finally, the temporal distribution of rotaviruses in this study showed that the positive samples have been identified in autumn, winter and spring, but not in summer season. Moreover, this observation was nearly comparable to what identified earlier in several reports conducted in Egypt (El-Senousy et al., 2013; El-Senousy et al., 2015).

Recommendations

Our outcomes administrated the presence of rotaviruses in tap water samples. These results, with other published reports in Egypt, should raise awareness and highlight the necessity for implementing routine national vaccination against rotaviruses.

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