Asian Journal of Water, Environment and Pollution, Vol. 16, No. 4 (2019), pp. 17–22.
DOI 10.3233/AJW190044

Molecular Detection of Group C Rotavirus in Environmental Samples in Giza, Egypt

Mohamed N.F. Shaheen* and Elmahdy M. Elmahdy

Environmental Virology Laboratory, Water Pollution Research Department, Environmental Research Division, National Research Centre, Al-Buhouth Street, 12622 Dokki, Giza, Egypt
✉ m_nrc2007@yahoo.com

Received December 13, 2018; revised and accepted May 10, 2019

Abstract: Group C rotavirus (RVC) has been identified as important enteric pathogen associated with acute gastroenteritis in human, especially in outbreaks. To evaluate the presence of RVC in environmental samples, one-year study was performed in Giza, Egypt. A total of 64 sewage (raw and treated) with 32 sewage sludge samples from Zenin wastewater treatment plant (WWTP) and 72 drainage water with 24 sediment samples from El-Rahawy drain which receives the sewage effluent of Zenin WWTP were collected. After concentration of the collected samples, semi-nested RT-PCR using specific primers was used for amplification of the RVC VP7 gene. The overall detection rates were 21.9% (7 of 32) for the raw sewage, 6% (2 of 32) for the treated sewage, 12.5% (4 of 32) for the sewage sludge, 22% (16 of 72) for the drainage water, and 16.6% (4 of 24) for the drainage sediment. In addition, the virus was abundant in cold months (October-March) and less prevalent in hot months (April-September). The widespread occurrence of RVC in this study reflects that RVC circulates at a relatively high frequency in the Egyptian population.

Key words: Rotavirus, sewage, sludge, sediment, gastroenteritis.

Introduction

Acute gastroenteritis is the third common cause of childhood morbidity and mortality and rotavirus is the most enteric viral pathogen associated with acute gastroenteritis, being responsible for approximately 38.3% of children hospitalized with acute gastroenteritis, resulting in about 197,000 deaths of children under five years, annually (Lanata et al., 2013; Liu et al., 2015). Rotaviruses belong to the Reoviridae family and have a genome consisting of eleven double-stranded RNA (dsRNA) segments. They are classified into nine (sero) groups (RVA–RVI) (Matthijnssens et al., 2012; Mihalov-Kovacs et al., 2015). Group A rotavirus (RVA) is identified as the most important pathogen of acute infantile diarrhea, worldwide (Patton, 2012). However, group C rotavirus (RVC) is the second most frequent RV species detected in humans (Estes and Greenberg, 2013).

RVC has already been detected in several countries, either associated with sporadic cases of acute diarrhea in all age groups as reported in Japan (Kumazaki and Usuku, 2014), Slovenia (Steyer et al., 2006), and South Africa (Steele and James, 1999), or with outbreaks of acute diarrhea as in Sweden (Nilsson et al., 2000), Japan (Ishimaru et al., 1991), and Brazil (Gabbay et al., 1999). However, the detection rates of group C rotavirus infection is much lower than group A rotavirus, and prevalence of RVC antibodies usually peaks at an age of 40-50 years of age or later (James et al., 1997; Steele and James, 1999; Kuzuya et al., 2001).

*Corresponding Author

The difference in detection rates between the different groups of rotavirus may be due to differences in their stability and transmission mechanisms in the environment. Rotavirus infections are primarily transmitted via the fecal-oral contact, including person-to-person contact (Offit and Clark, 2000), swelling of contaminated food or water (Offit and Clark, 2000; Anderson and Weber, 2004), aerosol (Offit and Clark, 2000), and zoonotic transmission (Gentsch et al., 2005). Among a large number of environmental studies on the occurrence of RVA, few studies have assessed the presence of RVC in the environment. RVC has been detected in sewage and Nile water (Meleg et al., 2008; El-Senousy et al., 2015).

The objective of this research was to investigate the occurrence of group C rotavirus in raw sewage, treated sewage, and sewage sludge of a large wastewater treatment plant as well as in drainage water and sediment samples of a large urban drain that receive the treated effluents of that WWTP.

Material and Methods

Sampling Area and Collection

Zenin WWTP, using the activated sludge process with a capacity of 330,000 m^3/day, is located in the west of Cairo. El-Rahawy drain receives daily about 1.90 million m^3 of drainage water from the Abu-Rawash and Zenin wastewater treatment plants, as well as from agricultural drainage. A one-year surveillance study was conducted to detect RVC in sewage samples (n = 32 for raw and n = 32 for treated samples) and sewage sludge samples (n = 32) collected from Zenin WWTP as well as in drainage water (n = 72) and drainage sediment (n = 24) samples collected from El-Rahawy drain.

Viral Concentration in the Collected Samples

Rotaviruses were concentrated from sewage, drainage, and sludge/sediment samples by methods described previously by USEPA, (2011), Katayama et al. (2002), and EPA (1992), respectively. The concentrated samples were stored at −20°C until the next step.

RNA Extraction and Complementary DNA (cDNA) Synthesis

Viral nucleic acids were extracted from 240 µl of the concentrate samples to obtain a final volume of 60 µl, using the QIAamp Viral RNA (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions. cDNA was synthesized from the extracted RNA by using random hexameric primers and Maxima Reverse Transcriptase Thermo scientific (200 U/µl). The cDNA synthesis was carried out by incubation for 60 min at 37 °C, followed by incubation for 5 min at 95°C. The cDNA was then utilized for group C VP7 gene amplification.

PCR Amplification of the RVC VP7 Gene

The full-length VP7 (1063 bp) gene was amplified using a semi-nested RT-PCR method. The first PCR mix was prepared in a final volume of 50 µl containing 15 µl of cDNA, 4 µl of 2.5 mM dNTPs, 4 µl of the 10x reaction buffer, 0.5 µl of VP7 FP-1 as forward primer (5′-GGC ATT TAA AAA AGA AGC TG-´3), 0.5 µl of BMJ-13 as reverse primer (5′-AGC CAC ATG ATC TTG TTT-′3), 0.5 µl of Taq DNA polymerase, and 26 µl of DDW. For nested-PCR, 2 µl of the first PCR product were mixed with 4 µl of 2.5mM dNTPs, 5 µl of 10x reaction buffer, 1 µl of BMJ-107 as forward primer (5′-TGT TTG GAG ATG TGA TGA-′3) and 1 µl of BMJ-13 as reverse primer (5′-AGC CAC ATG ATC TTG TTT-′3), 0.5 µl of the Taq DNA polymerase, and 36.5 µl of DDW. The first and semi-nested PCR was performed under the following conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 48°C for 2 min and 72°C for 3 min and a final extension at 72°C for 7 min. The PCR products were then analyzed by running on 2.5% agarose gel (Tiku et al., 2017).

Results and Discussion

Since the first group C rotavirus outbreak in the USA, in the year 1980, there have been several outbreaks documented from different regions of the world. The serosurveillance study performed in Sweden reported group C rotavirus prevalence ranging from 35%-45% depending on age (Nilsson and Svensson, 1993). A survey in the UK reported the group C rotavirus seroprevalence of 66% for 71-75 years age group and 43% in all age groups (James et al., 1997). Several studies for the detection and prevalence of group C rotavirus were performed, worldwide. However, the prevalence of RVC in Egypt remains largely unknown. As far as we know, there is only one study describing the detection of group C rotavirus in both environmental and clinical samples (El-Senousy et al., 2015). Therefore, there is insufficient data concerning the detection and prevalence of RVC in clinical samples and its spread in the water environment. Thus, the aim of this research is to study the occurrence of RVC in environmental samples.

In this study, we applied semi-nested RT-PCR for detection of RVC in the collected samples to increase the PCR sensitivity and avoid any false-positive results due to the interface of organic and inorganic inhibitors (e.g. heavy metals, polyphenols, and humic acids) present in sewage samples with the enzyme amplification. The semi-nested RT-PCR analysis identified RVC in 7 of 32 raw sewage (21.9%), 2 of 32 treated sewage (6%), 4 of 32 sewage sludge (12.5%), 16 of 72 drainage water (22%), and 4 of 24 drainage sediment (16.6%) (Table 1). The frequency of RVC detected in our current study is higher than those detected by El-Senousy et al. (2015) in raw and treated sewage samples from the same wastewater treatment plant in Egypt. However, it was lower than the frequency detected in a study performed in Hungary (Meleg et al., 2008).

Although this research was only one-year surveillance of group C rotavirus, we found that the prevalence of RVC was higher in raw sewage (5/7, 71.4%), treated sewage (2/2, 100%), sewage sludge (3/4, 75%), drainage water (11/16, 68.7%), and drainage sediment (3/4, 75%) samples collected during the cold seasons, October-March, than in raw sewage (2/7, 28.6%), treated sewage (0/2, 0%), sewage sludge (1/4, 25%), drainage water (5/16, 31.2%), and drainage sediment 1/4, 25%) samples collected during the hot months, April–September (Figure 1). This observation is similar to previous studies conducted on both environmental and clinical samples (Kuzuya et al., 1998; El-Senousy et al., 2015). Also, this study supports other epidemiological reports that group C rotavirus was detected more frequently in hospitalized patients' stools in the winter (Pereira et al., 1993; Kuzuya et al., 1998). Increased viral stability in the aquatic environment due to lowered temperature (Moresco et al., 2016), could increase water-borne viral gastroenteritis during winter, and thereby a higher viral concentration in sewage (Myrmel et al., 2006).

Increasing numbers of outbreak reports of viral gastroenteritis associated with swallowing of virus-contaminated water or eating of virus-contaminated food (Chitambar et al., 2012; Guo et al., 2014) suggest that it is significant to look for RVC in water, sewage, and food samples to investigate if this virus plays a role in water and food-borne causes of gastroenteritis. These studies can *contribute* to effective implementation strategies to prevent the spread of infection in the population. A previous study provided data on water-borne outbreak associated with a multiple of enteric viruses, based on clinical epidemiological investigation (Kukkula et al., 1997); RVC was detected in patients with acute diarrhea but it was not detected in the implicated water sample which may be due to the unavailability of sensitive

Table 1: Semi-nested RT-PCR results for the detection of group C rotavirus in raw sewage, treated sewage, sewage sludge, drainage water and drainage sediment samples

Sampling date (month/year)	Raw sewage	Treated sewage	Sewage sludge	Drainage water	Drainage sediment
01/2017	1/2	1/2	0/2	NC	NC
02/2017	1/3	0/3	1/3	NC	NC
03/2017	1/3	1/3	1/3	NC	NC
04/2017	0/2	0/2	0/2	0/6	0/2
05/2017	1/3	0/3	1/3	2/6	0/2
06/2017	0/3	0/3	0/3	1/6	0/2
07/2017	1/2	0/2	0/2	0/6	0/2
08/2017	0/3	0/3	0/3	2/6	1/2
09/2017	0/3	0/3	0/3	0/6	0/2
10/2017	1/2	0/2	0/2	1/6	0/2
11/2017	0/3	0/3	0/3	3/6	0/2
12/2017	1/3	0/3	1/3	1/6	1/2
01/2018	NC	NC	NC	2/6	0/2
02/2018	NC	NC	NC	2/6	1/2
03/2018	NC	NC	NC	2/6	1/2
Total	7/32	2/32	4/32	16/72	4/24

NC: Not collected; -: Not detected; +: Detected

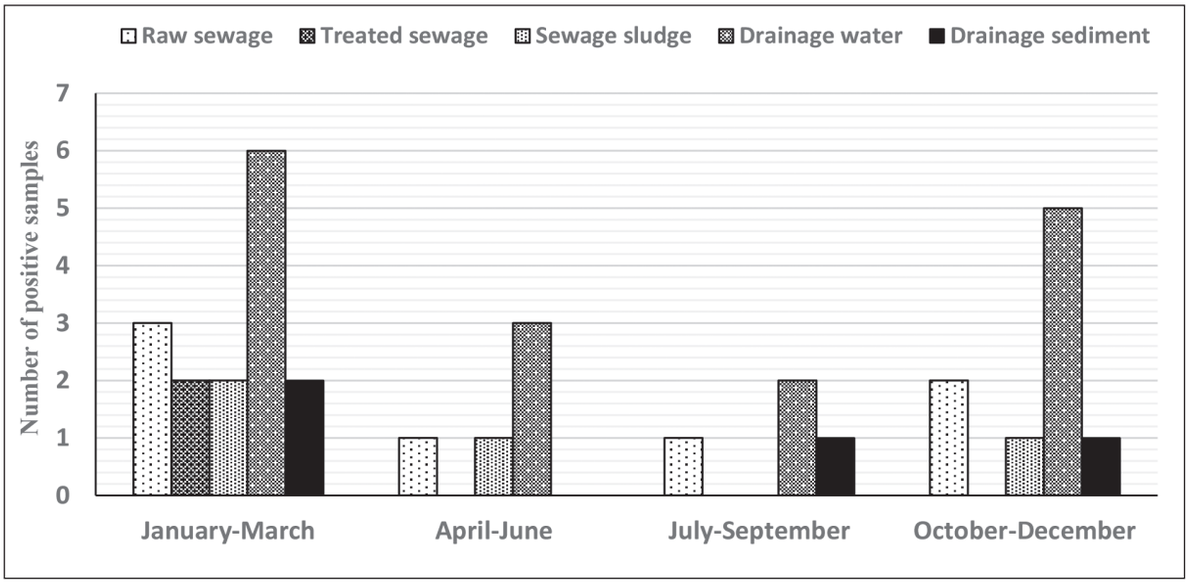

Figure 1: Distribution of group C rotaviruses in the samples according to the month of collection.

and adequate detection method. With the advent of molecular techniques for virus diagnosis, it is now possible to perform fast detection and characterization tests of RVC in samples suspected to be contaminated. However, the possibility of detecting RVC in sewage depends on several factors such as the number of shedders, the concentration of virus shed, and the sewage volume.

It was very difficult to eliminate RVC of the raw sewage by the sewerage system and the virus also occurred in the treated sewage of the sewerage system, which exhibited the permanence property of this virus and the dissemination possibility by the reclaimed treated sewage of sewerage system. Molecular methods used for RVC detection cannot differentiate between infectious and non-infectious particles, although they provide a fast and sensitive method to detect enteric viruses as an alternative tool to overcome the limitations of traditional techniques (e.g. cell cultures) since rotaviruses are considered fastidious viruses. However, the detection of a single-strand RNA (ssRNA) genome in the aquatic environment has indicated the occurrence of infective viruses, since ssRNA molecule is sensitive to environmental conditions (Rutjes et al., 2009).

This study shows a wide circulation of RVC in wastewater and, therefore, in the Egyptian population. This could mean that RVC infection circulating silently does not necessarily associate with illness status, or it causes potentially undiagnosed infections. Further studies are required to investigate the exact epidemiological and clinical roles of RVC. Moreover, the potential role of the water environment in the transmission of RVC should be investigated.

Conclusion

We have studied the presence and seasonal frequency of group C rotavirus in the Egyptian environment by semi-nested RT-PCR. Although the absence of clinical data of RVC, we believe that the frequency of RVC detection in environmental samples reflects the frequency of RVC detection in population. Furthermore, frequent detection of RVC RNA in the treated effluent of sewage treatment implies widespread dispersion of human enteric viruses in the environment.

References

Anderson, E.J. and S.G. Weber (2004). Rotavirus infections in adults. *Lancet Infect. Dis.*, **4:** 91-99.

Chitambar, S., Gopalkrishna, V., Chhabra, P., Patil, P., Verma, H., Lahon, A., Arora, R., Tatte, V., Ranshing, S., Dhale, G. and R. Kolhapure (2012). Diversity in the enteric viruses detected in outbreaks of gastroenteritis from Mumbai, Western India. *International Journal of Environmental Research and Public Health*, **9(3):** 895-915.

El-Senousy, W.M., Ragab, A.M.E.S. and E.M.A.E.H. Handak (2015). Prevalence of rotaviruses groups A and C in Egyptian children and aquatic environment. *Food and Environmental Virology*, **7(2):** 132-141.

EPA – Environmental Protection Agency (1992). Standards for the disposal of sewage sludge, federal register. Washington, **503:** 9387-9404.

Estes, M.K. and H.B. Greenberg (2013). Rotavirus. *In:* Knipe, D.M. and Howley, P.M. (eds). Fields Virology. Philadelphia: Lippincott Williams & Wilkins, Inc.

Gabbay, Y.B., Jiang, B., Oliveira, C.S., Mascarenhas, J.D., Leite, J.P.G., Glass, R.I. and A.C. Linhares (1999). An outbreak of group C rotavirus gastroenteritis among children attending a day-care center in Belem, Brazil. *J. Diarrhoeal Dis. Res.*, **2:** 69-74.

Gentsch, J.R., Laird, A.R., Bielfelt, B., Griffin, D.D., Bányai, K., Ramachandran, M., Jain, V., Cunliffe, N.A., Nakagomi, O., Kirkwood, C.D. and T.K. Fischer (2005). Serotype diversity and reassortment between human and animal rotavirus strains: Implications for rotavirus vaccine programs. *Journal of Infectious Diseases*, **192(1):** S146-S159.

Guo, Z., Huang, J., Shi, G., Su, C.H. and J.J. Niu (2014). A food-borne outbreak of gastroenteritis caused by norovirus GII in a university located in Xiamen City, China. *International Journal of Infectious Diseases*, **28:** 101-106.

Ishimaru, Y., Nakano, S., Nakano, H., Oseta, M. and Y. Yamashita (1991). Epidemiology of group C rotavirus in Matsuyama, Japan. *Acta Paediatr. Jpn*., **33:** 50-56.

James, V.L.A., Lambden, P.R., Caul, E.O., Cooke, S.J. and I.N. Clarke (1997). Seroepidemiology of human group C rotavirus in the UK. *J. Med. Virol*., **52:** 86-91.

Katayama, H., Shimasaki, A. and S. Ohgaki (2002). Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. *Applied and Environmental Microbiology*, **68:** 1033-1039.

Kukkula, M., Arstila, P., Klossner, M.L., Maunula, L., Bonsdorff, C.H.V. and P. Jaatinen (1997). Waterborne outbreak of viral gastroenteritis. *Scand. J. Infect. Dis.*, **29:** 415-418.

Kumazaki, M. and S. Usuku (2014). Epidemiological and genetic analysis of human group C rotaviruses isolated from outbreaks of acute gastroenteritis in Yokohama, Japan, between 2006 and 2012. *Archives of Virology*, **159(4):** 761-771.

Kuzuya, M., Fujii, R., Hamano, M., Ohata, R., Ogura, H. and M. Yamada (2001). Seroepidemiology of human group C rotavirus in Japan based on blocking enzyme-linked immunosorbent assay. *Clin. Diagn. Lab. Immunol.*, **8:** 161-165.

Kuzuya, M., Fujii, R., Hamano, M., Yamada, M., Shinozaki, K., Sasagawa, A., Hasegawa, S., Kawamoto, H., Matsumoto, K., Kawamoto, A. and A. Itagaki (1998). Survey of human group C rotaviruses in Japan during the winter of 1992 to 1993. *J. Clin. Microbiol.*, **36:** 6-10.

Lanata, C.F., Fischer-Walker, C.L., Olascoaga, A.C., Torres, C.X., Aryee, M.J. and R.E. Black Child Health Epidemiology Reference Group of the World Health Organization and UNICEF (2013). Global causes of diarrheal disease mortality in children <5 years of age: A systematic review. *PloS ONE*, **8:** e72788.

Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J.E., Cousens, S., Mathers, C. and R.E. Black (2015). Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: An updated systematic analysis. *Lancet*, **385:** 430-440.

Matthijnssens, J., Otto, P.H., Ciarlet, M., Desselberger, U., Van Ranst, M. and R. Johne (2012). VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Arch Virol*., **157:** 1177-1182.

Meleg, E., Bányai, K., Martella, V., Jiang, B., Kocsis, B., Kisfali, P., Melegh, B. and G. Szűcs (2008). Detection and quantification of group C rotaviruses in communal sewage. *Applied and Environmental Microbiology*, **74(11):** 3394-3399.

Mihalov-Kovács, E., Gellért, Á., Marton, S., Farkas, S.L., Fehér, E., Oldal, M., Jakab, F., Martella, V. and K. Bányai (2015). Candidate new rotavirus species in sheltered dogs, Hungary. *Emerg. Infect. Dis*., **21:** 660-663.

Moresco, V., Damazo, N.A. and C.R.M. Barardi (2016). Thermal and temporal stability on the enteric viruses infectivity in surface freshwater. *Water Science and Technology: Water Supply*, **16(3):** 620-627.

Myrmel, M., Berg, E.M., Grinde, B. and E. Rimstad (2006). Enteric viruses in inlet and outlet samples from sewage treatment plants. *J. Water Health*, **4:** 197-209.

Nilsson, M. and L. Svensson (1993). Antibody prevalence and specificity against group C rotavirus. *In:* Glasgow: Program and Abstracts of the IXth International Congress of Virology.

Nilsson, M., Svenungsson, B., Hedlund, K.O., Uhnoo, I., Lagergren, A., Akre, T. and L. Svensson (2000). Incidence and genetic diversity of group C rotavirus among adults. *The Journal of Infectious Diseases*, **182(3):** 678-684.

Offit, P.A. and H.F. Clark (2000). Rotavirus. *In:* G.L. Mandel, J.E. Bennet and R. Dolin (eds), Principles and practice of infectious diseases, 5th ed. Churchill Livingstone, New York, NY.

Patton, J.T. (2012). Rotavirus diversity and evolution in the postvaccine world. *Discov. Med.*, **13:** 85-97.

Pereira, H.G., Linhares, A.C., Candeias. J.A.N. and R.I. Glass (1993). National laboratory surveillance of viral agents of gastroenteritis in Brazil. *Bull. Pan. Am. Health Organ*, **27:** 224-233.

Rutjes, S.A., Lodder, W.J., Van Leeuwen, A.D. and A.M. de Roda Husman (2009). Detection of infectious rotavirus in naturally contaminated source waters for drinking water production. *Journal of Applied Microbiology*, **107(1):** 97-105.

Schlindwein, A.D., Rigotto, C., Simões, C.M.O. and C.R.M. Barardi (2010). Detection of enteric viruses in sewage

sludge and treated wastewater effluent. *Water Science and Technology*, **61(2):** 537-544.

Steele, A.D. and V.L.A. James (1999). Seroepidemiology of human group C rotavirus in South Africa. *J. Clin. Microbiol.*, **37:** 4142-4144.

Steyer, A., Poljšak-Prijatelj, M., Bufon, T., Sedmak, M., Vidmar, L., Mijovski, J.Z. and J. Marin (2006). First detection of group C rotavirus in patients with gastroenteritis in Slovenia. *Journal of Medical Virology*, **78(9):** 1250-1255.

Tiku, V.R., Jiang, B., Kumar, P., Aneja, S., Bagga, A., Bhan, M.K. and P. Ray (2017). First study conducted in Northern India that identified group C rotavirus as the etiological agent of severe diarrhea in children in Delhi. *Virology Journal*, **14(1):** 100.

USEPA (2011). Manual of methods for virology. EPA/600/4-84/013. USEPA, Cincinnati, USA.