World Applied Sciences Journal 32 (11): 2206-2212, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.11.91114

Effect of Chlorine on Noroviruses, Rotaviruses and Hepatitis E Virus in Drinking Water

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

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

Abstract: In the present study human norovirus genogroups I and II (GGI and GGII), Hepatitis E virus (HEV) and human and animal rotaviruses (Wa strain and simian rotavirus), were exposed to different chlorine concentrations in inoculated Nile water and drinking water samples. In raw water samples inoculated separately with viruses before autoclaving, the chlorine dose 5mg/l for 15 min was sufficient to remove 1 log_{10} from the initial doses of norovirus GGI and GGII and HEV, while sample inoculated after autoclaving, the chlorine dose 5mg/l for 15 min was sufficient to remove 2 log_{10} from the initial doses of norovirus GGI and GGII and HEV. While in drinking water samples, the chlorine dose 3 mg/l for 15 min was sufficient to remove 4 log_{10} from the initial dose of norovirus GGI and GGII and HEV. When we increased the chlorine dose to 4 mg/l, 6 log_{10} reduction of norovirus GGI while 7 log_{10} reduction from norovirus GGII and 6 log_{10} reduction of HEV was observed. Both human and animal Rotavirus are more resistant to chlorine in drinking water where only 3 log_{10} reduction under 3mg/l for 15 min was observed and 5 log_{10} reduction was observed under exposure to 4mg/l for 15 min.

Key words: *Noroviruses · Human rotaviruses · Animal rotaviruses · HEV · Chlorine Water*

INTRODUCTION

Disinfection of all surface water and most ground water is required to prevent the transmission of water-borne pathogenic microorganisms [1, 2]. Enteric viruses are among the agents that can be transmitted by fecally contaminated water. There are more than 140 different viruses excreted in the feces of humans [3]. Enteric viruses are highly infectious; ingestion of 1 to 10 viral particles is capable of having a significant probability of infection [4]. Current wastewater treatments do not ensure complete virus removal [5- 8], 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 [9]. An enteric virus concentration as low as 1 per 100 liters can pose a significant risk of infection to persons who consume the water [4].

Disinfection is a critical step in the drinking water treatment process to inactivate infectious viruses because primary treatment is less effective for the removal of viruses. Chlorine and monochloramine are the most widely used disinfectants in the United States [10]. Chlorine treatment can be used to inactivate bacteria, viruses and some protozoa. However, at the low levels of chlorine (5 mg/l) typically used for drinking water treatment, giardia spores will not be inactivated. Chlorine concentrations of 10 mg/l must be maintained for 30 minutes in order to inactivate giardia [11]. A number of variables influence the amount of chlorine necessary for disinfection. These include pH, water temperature and turbidity [11]. Longland [12] gave 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.

thorough explanation of tests that can be conducted in order to determine the chlorine demand in a given water supply.

The efficacy of chlorine disinfection for viruses has been evaluated in numerous studies over the years. Many early studies focused on the disinfection of polioviruses by chlorine [13-19]. Early investigators suggested a number of variables that must be controlled in the disinfection of viruses: contact time, temperature, ionic strength, pH, chlorine concentration and virus aggregation [16, 20].

The objective of this study was to estimate the effect of chlorine on genome copies and infectious units of noroviruses, rotaviruses and Hepatitis E virus in drinking water.

MATERIALS AND METHODS

Inoculation of Different Doses of Chlorine in Water Samples Spiked with Tested Viruses: Different doses to realize a final titers of ($1x10^5$, $1x10^6$, $1x10^7$, genome copies/liter), of positive norovirus GGI stool sample and ($1x10^4$, $1x10^5$, $1x10^6$ genome copies/liter) of positive norovirus GGII stool sample, human rotavirus Wa strain, simian rotavirus (kindly provided by Prof. Dr.Albert Bosch, University of Barcelona, Spain) and ($1x10^4$, $1x10^5$, $1x10^6$ genome copies/liter) of positive HEV sewage sample were inoculated separately in two types of water samples:

- 400 ml raw water (200 ml autoclaved sample to show the effect of organic matter and animal debris on viral protection against chlorine after dissolving by autoclaving and 200 ml non-autoclaved samples).
- 400 ml f drinking water (200 ml autoclaved sample to show the effect of organic matter and animal debris on viral protection against chlorine after dissolving by autoclaving and 200 ml non-autoclaved samples).
- Three doses from chlorine 5 mg/l [for raw water], 4 mg/l and 3 mg/l [for drinking water] and time of contact was 15 minutes.

Concentration of Water Samples: After inoculation, the water samples either autoclaved or non-autoclaved were filtered through nitrocellulose membrane filter (Shleicher and Schuell, 0.45 µm pore size and 142 mm diameter filter series) [21, 22], followed by organic flocculation according to Katzenelson *et al.* [23].

Viral Nucleic Acid Extraction: It was done using BIOZOL Total RNA Extraction reagent (BIOFLUX, Japan) and according to the manufacturer's instructions.

Real Time Pcr for Quantification of Noroviruses, HEV and Rotaviruses in Water Samples: Two steps real time RT-PCR for quantification of noroviruses, HEV and rotaviruses were used where the first step RT occurred as following: NVs GGI RT at 55°C 1 hr using reverse primer (NV1LCR), NVs GGII RT at 55°C 1hr using reverse primer (COG2R), according to kegeyama *et al.* [24] and Kojima *et al.* [25], HEV RT at 42°C 1 hr using reverse primer (3157 N) according to Kasorndorkbua *et al.* [26], rotavirus RT at 50°C 1 hr using forward (VP6-3) and reverse (VP6-4) primers according to Gallimore *et al.* [27]. The second step of real time PCR was done using power SYBR green PCR master mix (Applied Biosystem UK) and forward and reverse primers(QNIF4, N1LCVR) for NVs GGI and (QNIF2, COG2R) for NVs GGII according to kegeyama *et al.* [24] and Kojima *et al.* [25], forward and reverse primers (3156N and 3157N) for HEV according to Kasorndorkbua *et al.* [26], and primers of VP6-3 and VP6-4 for rotavirus according to Gallimore *et al.* [27]. The condition for second step of real time RT-PCR was carried 45 cycles for following steps 95°C for 5 min, 95°C for 15 sec, 60 °C for 1min and finally 65°C for 1min.

CC-RT-PCR for Quantification of Infectious Rotavirus Particles: It was done according to Abad *et al.* [28], El-Senousy *et al.* [29] and Ghazy *et al.* [30] to estimate the initial doses of the infectious units of both rotavirus Wa strain and simian rotavirus and also the number of the infectious units after chlorine treatment. Rotavirus cell culture RT-PCR (CC-RT-PCR) assay was performed on suspensions of infected MA104 cells. Set of primers VP6-F and VP6-R were used. The RT-PCR method was the same as described previously. The detection limit in this tissue culture assay using 100 µl of inoculum was $1x10^1$ CC-RT-PCR units/ml (u/ml), where CC-RT-PCR u is the reciprocal end point dilution detectable by CC-RT-PCR.

RESULTS AND DISCUSSION

It was found that the chlorine dose 5mg/l for 15 min is sufficient to remove 1 log from the initial dose of norovirus GGI before autoclaving the raw water sample. While the same dose after autoclaving the raw water sample is sufficient to remove 2 $\log_{10}$ from the initial dose

Table 1: Effect of different doses of chlorine on different doses of Norovirus GGI genome copies/liter in raw Nile water and drinking water spiked samples.

	Raw Nile water		Drinking water	
	5 mg/l		3 mg/l	4 mg/l
Initial doses of the viral genome	Final viral genome titer in non-autoclaved samples	Final viral titer in autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples
1×10^5	2×10^4	1×10^3	0	0
1×10^6	3×10^5	1×10^4	2×10^2	0
1×10^7	3×10^6	3×10^5	1×10^3	1 x10

Slope:-3.315, Rsq :0.992, Ct ranged from 22.71 to 38.74

Table 2: Effect of different doses of chlorine on different doses of Norovirus GGII genome copies/liter in raw Nile water and drinking water spiked samples.

	Raw Nile water		Drinking water	
	5 mg/l		3 mg/l	4 mg/l
Initial doses of the viral genome	Final viral genome titer in non-autoclaved samples	Final viral titer in autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples
1×10^4	2×10^4	1×10^2	0	0
1×10^5	3×10^4	1×10^3	2 x10	0
1×10^6	3×10^5	3×10^4	3×10^2	0

Slope:-3.315, Rsq :0.992, Ct ranged from 22.71 to 38.74

of virus. On contrary, in drinking water samples either before or after autoclaving the samples it was found that the chlorine dose 3mg/l for 15 min is sufficient to remove 5 logs from the initial dose of virus, but when we used higher the initial dose of the virus to 1×10^6 or 1×10^7 only 4 log reduction occurred. Also the chlorine dose when increased to 4 mg/l, completely viral removal occurred at initial dose 1×10^5 and 1×10^6 but only 6 $\log_{10}$ reduction occurred when we increased the initial dose of the virus to 1×10^7 (Table 1).

It was found that the chlorine dose 5mg/l for 15 min is sufficient to remove 1 log from the initial dose of norovirus GGII before autoclaving the raw water sample but no reduction occurred when the dose of the initial virus was 1×10^4 and this might be attributed to the low of the initial dose of the virus with high percentage from suspended solids. While the same dose after autoclaving the raw water sample is sufficient to remove 2 $\log_{10}$ from the initial dose of virus. On contrary, in drinking water samples either before or after autoclaving the samples it was found that the chlorine dose 3mg/l for 15 min is sufficient to make complete viral removal at low initial dose of the virus (1×10^4) but with higher concentration of the virus only 4 $\log_{10}$ reduction occurred. On the other hand complete removal for the virus occurred when increasing the chlorine dose to 4 mg/l in inoculated drinking water samples (Table 2). The inactivation of human adenoviruses 2, 40 and 41 (HAdV2, HAdV40 and HAdV41), coxsackieviruses B3 and B5 (CVB3 and CVB5), echoviruses 1 and 11 (E1 and E11) and murine norovirus (MNV) were compared using either 0.2 mg of free chlorine or 1 mg of monochloramine/liter at pH 7 and 8 in buffered reagent-grade water at 5 degrees C. CT values (disinfectant concentration x time) for 2- to 4-$\log_{10}$ (99 to 99.99%) reductions in virus titers were calculated by using the efficiency factor Hom model. The enteroviruses required the longest times for chlorine inactivation and MNV the least time. CVB5 required the longest exposure time, with CT values of 7.4 and 10 mg x min/liter (pH 7 and 8) for 4-$\log_{10}$ inactivation. Monochloramine disinfection was most effective for E1 (CT values ranged from 8 to 18 mg x min/liter for 2- and 3 $\log_{10}$ reductions, respectively). E11 and HAdV2 were the least susceptible to monochloramine disinfection (CT values of 1,300 and 1,600 mg-min/liter for 3 $\log_{10}$ reductions, respectively). Monochloramine inactivation was most successful for the adenoviruses, CVB5 and E1 at pH 7. A greater variation in inactivation rates between viruses was observed during monochloramine disinfection than during chlorine disinfection [31]. In the study of Kitajima *et al.* [32], the murine norovirus (MNV) was inactivated faster than Poliovirus 1 (PV1) and there was no significant difference in the viral RNA reduction rate between human norovirus (HuNoV) and MNV. The results suggested that

Table 3: Effect of different doses of chlorine on different doses of HEV genome copies/liter in raw Nile water and drinking water spiked samples.

	Raw Nile water		Drinking water	
	5 mg/l		3 mg/l	4 mg/l
Initial doses of the viral genome	Final viral genome titer in non-autoclaved samples	Final viral titer in autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples
1×10^4	1×10^3	2×10^2	0	0
1×10^5	3×10^4	2×10^3	3x10	0
1×10^6	1×10^5	3×10^4	3×10^2	0

Slope:-3.238, Rsq: 0.996, Ct ranged from 22.87 to 39.11

appropriate water treatment process with chlorination can manage the risk of HuNoV infection via drinking water supply systems.

It was found that the chlorine dose 5 mg/l for 15 min is sufficient to remove 1 $\log_{10}$ from the initial dose of HEV before autoclaving the raw water sample. While the same dose from chlorine after autoclaving the raw water sample iwas sufficient to remove 2 $\log_{10}$ from the initial dose of virus. On contrary, in drinking water sample either before or after autoclaving the samples it was found that the chlorine dose 3mg/l for 15 min is sufficient to remove 4 $\log_{10}$ from the initial dose of virus but when we increased the chlorine dose to 4mg/l, HEV genome was completely removed (6 $\log_{10}$ reduction) (Table 3). Girones *et al.* [33] reported that using immunofluorescence and quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays, HEV has been shown to be susceptible to chlorine disinfection and presented equivalent kinetics to human adenoviruses. The C(t) values observed for a 2-log reduction of HEV were 0.41 in buffered demand-free water and 11.21 mg/L × min in the presence of 1% sewage. The results indicated that the inactivation kinetics of HEV and HadV2 are equivalent and support the use of chlorine disinfection as an effective strategy to control HEV waterborne transmission.

It was found that the chlorine dose 5mg/l for 15 min is sufficient to remove 1 $\log_{10}$ either from the initial dose of the genome copies or the infectious units of both rotavirus Wa strain and simian rotavirus before autoclaving the raw water sample. While the same dose after autoclaving the raw water sample is sufficient to remove 2 $\log_{10}$ either from the genome copies of both rotaviruses and their infectious units. On contrary, in drinking water samples either before or after autoclaving the samples, it was found that the chlorine dose 3mg/l for 15 min is sufficient to remove 3 $\log_{10}$ either from the genome copies of the rotaviruses and their infectious units, but when increasing the chlorine dose to 4mg/l either rotaviruses genomes or infectious units were completely removed except for the higher initial dose of both genomes of rotavirus Wa strain and simian rotavirus (1×10^6 genome copies/liter) only 5 $\log_{10}$ from the initial doses of both rotaviruses were removed. The results showed complete similarity between human and animal rotavirus strains (Tables 4 and 5).

The efficacy of copper and silver ions, in combination with low levels of free chlorine (FC), was evaluated for the disinfection of hepatitis A virus (HAV), human rotavirus (HRV), human adenovirus and poliovirus (PV) in water. HAV and HRV showed little inactivation in all conditions. PV showed more than a 4 $\log_{10}$ titer reduction in the presence of copper and silver combined with 0.5 mg of FC per liter or in the presence of 1 mg of FC per liter alone. Human adenovirus persisted longer than PV with the same treatments, although it persisted significantly less than HRV or HAV. The addition of 700 µg of copper and 70 µg of silver per liter did not enhance the inactivation rates after the exposure to 0.5 or 0.2 mg of FC per liter, although on some occasions it produced a level of inactivation similar to that induced by a higher dose of FC alone. Virus aggregates were observed in the presence of copper and silver ions, although not in the presence of FC alone. This data indicated that the use of copper and silver ions in water systems may not provide a reliable alternative to high levels of FC for the disinfection of viral pathogens [34].

The reduction of rotaviruses, noroviruses and HEV in drinking water samples was higher than their reduction in raw Nile water samples and this might be due to the presence of high quantity of suspended solids in raw Nile water which play an important role in the protection of the viruses from exposure to the chlorine. From the same point of view, the reduction of rotaviruses, noroviruses and HEV was higher in the autoclaved raw Nile water samples than their reduction in the non-autoclaved samples as a result of the breakdown of the suspended

Table 4: Effect of different doses of chlorine on different doses of Human rotavirus Wa strain genome copies/liter and infectious units (CC-RT-PCR units/liter) in raw Nile water and drinking water spiked samples.

		Raw water				Drinking water			
		5 mg/l				3 mg/l		4 mg/l	
Initial doses of the viral genome	Initial doses of rotavirus infectious units	Final viral genome titer in non-autoclaved samples	Final viral infectious units in non-autoclaved samples	Final viral genome titer in autoclaved samples	Final viral infectious units in non-autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples	Final viral infectious units in autoclaved and non-autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples	Final viral infectious units in autoclaved and non-autoclaved samples
$1 x10^4$	$1 x10^2$	$2 x10^3$	1x10	$3 x10^2$	0	$5 x10^1$	0	0	0
$1 x10^5$	$1 x10^3$	$2 x10^4$	$1x10^2$	$2 x10^3$	1x10	$3 x10^2$	0	0	0
$1 x10^6$	$1 x10^4$	$4 x10^5$	$1x10^3$	$1 x10^4$	$1x10^2$	$3 x10^3$	1x10	1 x10	0

Slope:-3.315, Rsq :0.992, Ct ranged from 22.71 to 38.7

Table 5: Effect of different doses of chlorine on different doses of animal (simian rotavirus SA11 strain) genome copies/liter and infectious units (CC-RT-PCR units/liter) in raw Nile water and drinking water spiked samples.

		Raw water				Drinking water			
		5 mg/l				3 mg/l		4 mg/l	
Initial doses of the viral genome	Initial doses of rotavirus infectious units	Final viral genome titer in non-autoclaved samples	Final viral infectious units in non-autoclaved samples	Final viral genome titer in autoclaved samples	Final viral infectious units in non-autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples	Final viral infectious units in autoclaved and non-autoclaved samples	Final viral genome titer in autoclaved and non-autoclaved samples	Final viral infectious units in autoclaved and non-autoclaved samples
$1 x10^4$	$1 x10^2$	$2 x10^3$	1x10	$3 x10^2$	0	$5 x10^1$	0	0	0
$1 x10^5$	$1 x10^3$	$2 x10^4$	$1x10^2$	$2 x10^3$	1x10	$3 x10^2$	0	0	0
$1 x10^6$	$1 x10^4$	$4 x10^5$	$1x10^3$	$1 x10^4$	$1x10^2$	$3 x10^3$	1x10	1 x10	0

Slope:-3.315, Rsq :0.992, Ct ranged from 22.71 to 38.74

solids during the autoclaving process and this lead to exposure of the virus to high quantity of the chlorine in the autoclaving raw water samples than non-autoclaving.

CONCLUSIONS

Rotaviruses were more resistant to chlorine than noroviruses and HEV. On the other hand the genomes of rotaviruses were more resistant than the infectious unites of rotaviruses.

ACKNOWLEDGMENT

This work was supported by International Foundation for Science (IFS), Sweden, Grant agreement number: W/4327-1, Principal Investigator (PI), Prof.Dr.Waled Morsy El-Senousy. Authors thank Prof.Dr.Gamila Hussein Ali, Professor of Hydrobiology, Water Pollution Research Department, National Research Center, for her great assistance in this waork.

REFERENCES

1. United States Environmental Protection Agency (USEPA), 2002. National primary drinking water regulations: long term enhanced surface water treatment rule. Fed. Regist., 67: 1811-1844.
2. United States Environmental Protection Agency (USEPA), 2006. National primary drinking water regulations: ground water rule. Fed Regist., 71: 65574-65660.
3. Maier, R.M., I.I. Pepper and C.P. Gerba, 2000. Environmental Microbiology, San Diego, CA: Academic Press.
4. Gerba, C.P., J.B. Rose, C.N. Haas and K.D. Crabtree, 1996. Waterborne rotavirus: a risk assessment. Water Res., 30: 2929-2940.
5. Blatchley, E.R., W.L. Gong, J.E. Alleman, J.B. Rose, D.E. Huffman, M. Otaki and J.T. Lisle, 2007. Effects of wastewater disinfection on waterborne bacteria and viruses. Water Environment Research, 79: 81-92.
6. Bosch, A., 2007. Human viruses in water, 1st ed. Elsevier, Amsterdam; Boston.
7. El-Senousy, W.M., M. 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.
8. Tawfik, A., N. Bader, E. Abou Taleb and W. El-Senousy, 2012. Sewage treatment in an up-flow anaerobic sponge reactor (UASR) followed by moving bed biofilm (MBBR) reactor. Desalination and Water Treatment, 37: 350-358.

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. American Water Works Association. 2000. Committee report: disinfection at large and medium-size systems. J. Am Water Works Assoc., 92: 32-43.
11. Extension Bulletin 795, 2003. "Ultraviolet." The Ohio State University. Site Visited March 26, 2003. Available: http://ohioline.osu.edu/b795/b795_9.html.
12. Longland, F., 1983. Field Engineering: An introduction to development work and construction in rural areas. Intermediate Technology Publications, London.
13. Hejkal, T.W., F.M. Wellings, P.A. LaRock and A.L. Lewis, 1979. Survival of poliovirus within organic solids during chlorination. Appl. Environ. Microbiol., 38: 114-118.
14. O'Brien, R.T. and J. Newman, 1979. Structural and compositional changes associated with chlorine inactivation of polioviruses. Appl. Environ. Microbiol., 38: 1034-1039.
15. Scarpino, P.V., G. Berg, S.L. Chang, D. Dahling and M. Lucas, 1972. A comparative study of the inactivation of viruses in water by chlorine. Water Res., 6: 959-965.
16. Sharp, D.G., D.C. Young, R. Floyd and J.D. Johnson, 1980. Effect of ionic environment on the inactivation of poliovirus in water by chlorine. Appl. Environ. Microbiol., 39: 530-534.
17. Varma, M.M., B.A. Christian and D.W. McKinstry, 1974. Inactivation of Sabin oral poliomyelitis type I virus. J. Water Pollut. Cont. Fed., 46: 987-992.
18. Weidenkopf, S.J., 1958. Inactivation of type 1, poliomyelitis virus with chlorine. Virology, 5: 56-67.
19. Young, D.C. and D.G. Sharp, 1977. Poliovirus aggregates and their survival in water. Appl. Environ. Microbiol., 33: 168-177.
20. Sharp, D.G. and J. Leong, 1980. Inactivation of poliovirus I (Brunhilde) single particles by chlorine in water. Appl. Environ. Microbiol., 40: 381-385.
21. 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.
22. 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.
23. 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.
24. 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.
25. 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.
26. Kasorndorkbua, C., T. Opriessnig, F.F. Huang, D.K. Guenette, P.J. Thomas, X.J. Meng and P.G. Halbur, 2005. Infectious swine Hepatitis E virus Present in pig manure storage facilities on United States Farms, but evidence of water contamination is lacking. Appl. Environ. Microbiol., 71: 7831-7837.
27. 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.
28. Abad, F.X., R.M. Pintó, C. Villena, R. Gajardo and A. Bosch, 1997. Astrovirus survival in drinking water. Appl. Environ. Microbiol., 63: 3119-3122.
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. Cromeans, T.L., A.M. Kahler and V.R. Hill, 2010. Inactivation of adenoviruses, enteroviruses and murine norovirus in water by free chlorine and monochloramine. Appl. Environ. Microbiol., 76: 1028-1033.
32. Kitajima, M., Y. Tohya, K. Matsubara, E. Haramoto, E. Utagawa and H. Katayama, 2010. Chlorine inactivation of human norovirus, murine norovirus and poliovirus in drinking water. Lett Appl Microbiol., 51: 119-121.

33. Girones, R., A. Carratalà, B. Calgua, M. Calvo, J. Rodriguez-Manzano and S. Emerson, 2014. Chlorine inactivation of hepatitis E virus and human adenovirus 2 in water. J Water Health. 12: 436-424.

34. Abad, F.X., R.M. Pinto, J.M. Diez and A. Bosch, 1994. Disinfection of Human Enteric Viruses in Water by Copper and Silver in Combination with Low Levels of Chlorine. CAppl. Environ. Microbiol., 60: 2377-2383.