Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net

Comparison of Two Virus Concentration Methods for Enteric Viruses Detection in Moroccan Wastewater and Treated Effluent

Hasna A. Amdiouni[1,2] *PhD,* **Leena Maunula[3]** *PhD,* **Arwa M. Al-Shuwaikh[4]** *PhD,* **Jalal Nourlil[1]** *MD*

[1]Medical Virology and BSL3 Laboratory, Institut Pasteur du Maroc. Casablanca, Morocco, [2]Laboratory of Physiology and Molecular Genetics, Faculty of Sciences, Aîn chock, University Hassan II, Casablanca, Morocco, [3]Dept. of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland, [4]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background The effluents of wastewater treatment plants may contain infectious human viruses representing a major public health issue. Wastewater must be analyzed for viruses' detection using easy and rapid protocol.

Objective To compare between two viral concentration methods used for detection of enteric viruses in wastewater and treated effluent. Then assess the presence of viral genomes by cell culture and polymerase chain reaction (PCR).

Methods Fifty samples of wastewater were collected from two wastewater treatment plants during one year survey (January-December 2009) in order to compare two virus concentration methods; the polyethylene glycol (PEG) precipitation and the two-phase separation method, advised by the World Health Organization guidelines. Then assess the presence of viral genomes of human enteroviruses (EV), human adenoviruses (HAdV), hepatitis A and E viruses (HAV and HEV), human noroviruses (HuNoV), human rotaviruses (RV) and human astroviruses (HAstV) by cell culture and PCR technique.

Results This study, using three statistical tests, showed that there was no significant difference between the two concentration methods: the PEG precipitation and the two-phase separation ($P>0.05$).

Conclusion Considering this study with protocol time, cost and simplicity. The PEG precipitation seems to be an alternative method of the two-phase separation method.

Keywords Wastewater, enteric-virus, concentration methods, PCR, cell culture, Morocco

Citation Hasna A. Amdiouni, Leena Maunula, Arwa M. Al-Shuwaikh, Jalal Nourlil. Comparison of two virus concentration methods for enteric viruses detection in Moroccan wastewater and treated effluent. Iraqi JMS. 2017; Vol. 15(1): 27-38. doi: 10.22578/IJMS.15.1.5

List of abbreviation: PEG = polyethylene glycol, EV = Human enteroviruses, HAdV = Human adenoviruses, HAV = Hepatitis A, HEV = Hepatitis E viruses, HuNoV = Human noroviruses, RV = Human rotaviruses, HAstV = Human astroviruses

Introduction

A wide variety of pathogenic organisms pass through municipal wastewater treatment systems, including viruses [(1)]. The enteric viruses found in human feces belong to more than 140 types [(2)]. According to public health data, those types can colonize the gastrointestinal tracts and cause a wide variety of illnesses, inducing variable epidemiological features and some water-related diseases [(3)]. EV, HAV, HEV, HAdV, HuNoV, RV and HAstV have been detected in raw wastewater and treated effluent in different studies [(4-9)].

There are several studies, which have demonstrated the advantage of environmental surveillance as an additional tool to determine the epidemiology of different viruses circulating in a given community [10,11]. The availability of improved detection techniques, combined with an increased awareness of gastroenteritis-causing viral pathogens, has also led to the establishment of surveillance systems in various countries [12]. While a number of techniques have been developed and refined, it has been proven difficult to achieve the detection of all relevant virus types over the spectrum of water quality matrices that exist in nature and human-constructed facilities [13]. The detection of viruses in wastewater, especially at low concentrations, is a major challenge. Because relatively few viral particles are present in wastewater samples, it is necessary to concentrate the viruses in a large volume of water, commonly from 13 to 21 ml [14,15]. Hence, many virus concentration methods have been developed: the two-phase separation [16], methods using an electronegative filter [17], polyethylene glycol (PEG) precipitation [18,19], the ultracentrifugation [4] and methods using an electropositive filter [20]. Furthermore, two virus concentration methods from wastewater are advised by the WHO for environmental poliovirus surveillance [21]. The first one uses bags with sorbent macroporous glass to trap viruses and the second uses the two-phase separation, which is applied in this study. Regarding the comparison of virus concentration methods in water, several studies have been conducted using polymerase chain reaction (PCR) and cell culture [13,22].

In the present study, a one-year survey (January - December 2009) was conducted in order to compare two virus concentration methods, the PEG precipitation and the two-phase separation using three statistical tests, to analyze the correlation between the cell culture and PCR for both EV and HAdV and to assess the presence of viral genomes of EV, HAV, HEV, HAdV, HuNoV, RV and HAstV by each method. Furthermore, the presence of infectious EV and HAdV in raw wastewater and treated effluent was also evaluated.

Methods

Sample collection

From January to December 2009, fifty samples (25 raw wastewater and 25 treated effluents) were collected monthly from two wastewater treatment plants (WWTPs): Zone 1 (Z1) and Zone 2 (Z2). The plants are officially registered as secondary treatment plants by lagooning and located at 70 km from Casablanca. All 50 samples were collected in plastic bottles during sampling and delivered to the laboratory for immediate concentration.

Concentration methods

The wastewater samples were concentrated using two methods. The first one ‘‘PEG precipitation’’ was carried out as described elsewhere [18] with modifications. Five hundred milliliters of sample were clarified by centrifugation (30 min, 1000 g) and the pellet was resuspended in 10 ml of the supernatant. The remaining portion of supernatant was saved. Chloroform was added to the resuspended sample to a concentration of 10% (v/v, Sigma, USA) and mixed, and the mixture was centrifuged again (5 min, 1000 g). The first and second supernatants were combined. The volume was measured and the pH was adjusted to (7.5). The PEG–NaCl technique as described by [23] was used with modification. The combined supernatants were supplemented with NaCl and PEG until a final concentration of 2.2% (w) NaCl (Sigma, St Louis, USA) and 7% (w) PEG 6000 (Fluka, Steinheim, Germany) was obtained. The mixture was stirred 4 h at 4 °C and then centrifuged for 2 h at 2000 g at 4 °C. Supernatant was discarded and the pellet was suspended in 5 ml of phosphate buffer saline (PBS).

In the second method ‘‘two-phase separation’’ [16,21], 500 ml of sample was centrifuged for (10 min at 1000 g). The pellet was stored and the pH of the supernatant was adjusted to neutral

pH (7-7.5). The supernatant was mixed with 39.5 ml of 22 % dextran (Sigma Aldrich, St Louis, USA), 287 ml of 29 % PEG 6000 (Fluka, Steinheim, Germany), and 35 ml of (5 N) NaCl (Sigma, St Louis, USA) and kept in constant agitation for 1 h at 4 °C using a magnetic stirrer. After overnight incubation at 4 °C in a separation funnel, the entire lower layer and the interphase were collected. The pellet from the first centrifugation was resuspended in this concentrate and the suspension was extracted with 20% volume of chloroform by shaking vigorously, followed by centrifugation for (5 min at 1000 g). The supernatant (10 ml) was recovered and decontaminated by antibiotic (e.g., penicillin G and streptomycin to final concentrations of 100 IU/ml and 100 g/ml, respectively).

Detection of EV and HAdV by cell culture

Two cell lines, human rhabdomyosarcoma tumour tissue (RD) and laryngeal carcinoma cells (Hep2), were used for inoculation. Cells were cultivated in minimum essential medium (MEM; Gibco) supplemented with 2% fetal bovine serum (FBS), penicillin and streptomycin, then incubated at 37 °C with 5% CO_2 for 5 days. For cell culture infection, cells were grown in 24-well microplates. The water concentrates were filtered through a 0.22 µm-pore-size membrane filter, and 100 µl of filtered water samples was inoculated in duplicate wells. Virus replication was monitored daily under the microscope up to 7 days. All cultures with or without cytopathic effect (CPE) were frozen and thawed up to three times to release the virus, the debris was removed by centrifugation at 1500xg for 20 min and virus suspensions were stored at -80 °C until further processing. Echovirus 7 (EV-7) and adenovirus C5 (HAdV-C5) used as positive controls were obtained from patient fecal samples and maintained in Institut Pasteur du Maroc Medical Virology and BSL3 Laboratory Casablanca Morocco. Titers of EV-7 and HAdV-5 were 107 and 106 TCID50 per 100 µl, respectively, determined by 50% tissue culture infective dose (TCID50).

Detection of EV, HAV, HEV, RV, HuNoV GI, HuNoV GII and HAstV by PCR

All PCR done on cell culture supernatant even if the cells are not specific for viruses because The use of integrated cell culture/PCR (ICC-PCR) helps to dilute the PCR inhibitors present in environmental samples and allows the in vitro amplification of the virus on different cellular systems as reported by Reynolds, 2004 [24].

Nucleic acid extraction: The High Pure Viral Nucleic Acid Kit (Roche, California, USA) was used to extract the DNA and RNA from the samples according to manufacturer's instructions. DNA and RNA extracts obtained were performed on 200 µl of sample concentrate and eluted in 50 µl.

Positive controls: HAV type IB and RV type G9P [2] were obtained from patient fecal samples and maintained in Institut Pasteur du Maroc Medical Virology and BSL3 Laboratory. HEV type G3 was kindly provided by Dr. Leena Maunula from the Faculty of Veterinary Medicine, university of Helsinki, Finland. HAstV- 1 and NoV GII.4 were kindly provided by Dr. Nicole Gregoricus from the Calicivirus Laboratory, CDC, Atlanta, GA. These virus samples were used as positive controls during molecular detection. All virus stocks were stored at -80 °C until use.

PCR for adenovirus detection: Polymerase chain reaction for HAdV was performed following the protocol of Casas et al., 2005 [25]. The primers for the first round of PCR were ADHEX1F (5'-CAACACCTAYGASTACATGAA-3') and ADHEX1R (5'-KATGGGGTARAGCATGTT-3'), yielding an amplicon of 473 bp, and the primers for the second round of PCR were ADHEX2F (5'-CCCITTYAACCACCACCG-3') and ADHEX2R (5'-ACATCCTTBCKGAAGTTCCA-3') with a resulting amplicon of 168 bp. Seven microlitres of sample was used in 50 µl reaction mixture containing 1X PCR buffer, 2 mmol l-l 1 $MgCl_2$, 200 lmol l-l deoxynucleoside

triphosphate (dNTP), 0.5 µmol l-l concentration of each primer and 1.5 U of Taq DNA polymerase (Invitrogen, London, UK). The PCR cycles for the first round and nested PCR were 94°C for 5 min, followed by 35 cycles of 94 °C for 30s, 50 °C for 30s and 72 °C for 30s; and a final extension at 72 °C for 7 min. PCR products were resolved by electrophoresis on 2% agarose gel.

Reverse transcription PCR (RT-PCR) for EV, HAV, HEV, RV, HuNoV and HAstV detection: For RT-PCR reaction, cDNA synthesis was performed following the protocol of Amdiouni et al., 2012 [(26)]: a pd (N) 6 random primer and 5 µl of RNA were mixed, heated at 70°C for 5 min and quickly chilled on ice. 200 µmol l-1 of dNTP, 20 U of RNAsin, 20 U of reverse transcriptase (MMLV) and 5 µl of 5X buffer MMLV were added in the tubes and incubated at 37 °C for 60 min and 94 °C for 5 min.

For PCR reaction of all retrieved enteric viruses, 5 µl of cDNA was used as template for PCR amplification carried out in a final volume of 50 µl that included: 5 µl of 10X PCR buffer, 200 µmol l-1 of each dNTP, 2 mmol l-1 MgCl2, 1.5 U of Taq DNA polymerase, 0.3 lmol l-1 of each primers. The thermocycler profile was 5 min at 94 °C followed by 35 cycles of 20s at 94 °C, 30s at Tm and 30s at 72 °C; and a final extension of 10 min at 72 °C. The PCR products were analyzed on ethidium bromide (EtBr)-stained agarose gels. The Tm and primers used are listed in table 1.

Statistical analysis

All statistical analyses were carried out with GraphPad Prism software version 6.03 using binary variables (presence or absence) of independent groups, except the analysis results of the presence of infectious EV and HAdV in raw wastewater and treated effluent was calculated using the Excel program. The comparison of the ability of the two virus concentration methods to concentrate infectious EV and HAdV was carried out by Fischer exact test, the correlation between the cell culture and PCR was determined by chi-squared (X2) test and the assessment of the viral genome by method was performed by student test (t-test). Differences were considered significant if $P<0.05$.

Results

Presence of infectious EV and HAdV by cell culture

To detect both infectious and non-infectious HAdV and EV on the Hep2 and RD monolayer, all cultures with or without CPE were analysed by PCR (Fig. 1). The results obtained by cell culture infection showed that 12% (6/50) of samples induced positive CPE for culturable EV on the RD cell line and 10% (5/50) of samples on the Hep2 cell line using the PEG precipitation method and 8% (4/50) and 2% (1/50) of samples were positive on the RD and Hep2 cell lines respectively using the two phase separation method. HAdV was detected on the RD and Hep2 cell lines, in 8% (4/50) and 12% (6/50) of samples, using the PEG precipitation method, and in 6% (3/50) and 12% (6/50) of samples using the two phase separation, respectively (Fig. 2). However, the difference between the two methods was statistically not significant for the EV and HAdV concentration ($n=50$, $P = 0.24$ and $P = 0.74$, respectively, Fisher exact test).

Correlation between the cell culture and PCR

Among the fifty wastewater samples analyzed for EV and HAdV, 26% (13/50) and 30% (15/50) samples were positive by PCR, whereas 22% (11/50) and 20% (10/50) were positive for the cell culture, respectively using the PEG precipitation method. However, the results of the two-phase separation method showed that 22% (11/50) and 26% (13/50) of samples were positive by PCR for EV and HAdV, while 10% (5/50) and 18% (9/50) were positive by cell culture, respectively (Fig. 3 and 4). This study found that there is no correlation between the detection of infectious EV and HAdV and viral genomes, for both methods: the PEG precipitation and the two phase separation ($n = 50$, $P = 0.34$ & $P = 0.74$, respectively, X2 test).

Table 1. Primers used for the various RT-PCR

Virus	Sequence	Region	Tm (°C)	Amplicon (pb)	References
EV	**292** (MIGCIGYIGARACNGG)	VP1	45	340 appr	Obertse *et al.* 2003 [27]
	222 (CICCIGGIGGIAYRWACAT)				
HAV	**HAVU2167** (GTTTTGCTCCTCTTTACCATGCTATG)	VP3-VP1	54	247	Hot *et al.* 2003 [28]
	HAVL2413 (GGAAATGTCTCAGGTACTTTCTTTG)				
HEV	**ConsORF2-s1** (GACAGAATTRATTTCGTCGGCTGG)	ORF2	54	197	Wang *et al.* 1999 [29]
	ConsORF2-a1 (CTTGTTCRTGYTGGTTRTCATAATC)				
RV	**ROT2U23** (GCTTTAAAARMGAGAATTTCCGT)	VP7	58	376 appr	Hot *et al.* 2003 [28]
	ROT376L23 (TAAACWGAWCCWGTYGGCCAWCC)				
	VP4-F (TATGCTCCAGTNAATTGG)	VP4	50	663 appr	Simmonds e*t al.* 2008 [30]
	VP4-R (ATTGCATTTCTTTCCATAATG)				
HuNoV GI	**G1SKF** (CTGCCCGAATTYGTAAATGA)	VP1	50	330	Kojima *et al.* 2002 [31]
	G1SKR (CCAACCCARCCATTRTACA)				
HuNoV GII	**G2SKF** (CNTGGGAGGGCGATCGCAA)	VP1	50	344	
	G2SKR (CCRCCNGCATRHCCRTTRTACAT)				
HAstV	**Mon269** (CAACTCAGGAAACAGGGTGT)	ORF2	50	449	Matias *et al.* 2007 [32]
	Mon270 (TCAGATGCATTGTCATTGGT)				

Assessment of the viral genome by PCR

The viral genome was detected by PCR from cell supernatant in 28%, 30%, 4%, 10%, 2%, 6% and 2% of samples for EV, HAdV, HAV, HEV, RV, HuNoV GII and HAstV respectively, for PEG precipitation method, while 20%, 26%, 8%, 0%, 10%, 4% and 2% were positive by PCR for EV, HAdV, HAV, HEV, RV, HuNoV GII and HAstV, respectively using the two phase-separation. No sample was found positive for HuNoV GI for both methods. The difference between the two methods was not statistically significant to detect viral genome (n = 50, P= 0.5, t-test) (Fig. 5).

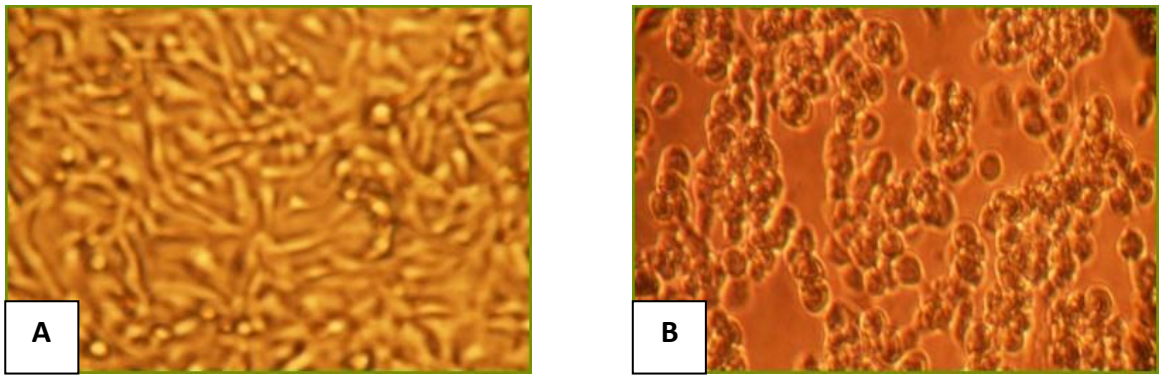

Figure 1. Typical cytopathic effect on the Hep-2 cell line infected by the propagation of the Enterovirus. (A) Normal Hep-2 cell line, (B) Enteriviruses infected Hep-2 cell line showed rounding of the cells, shrinkage and detachment of surface

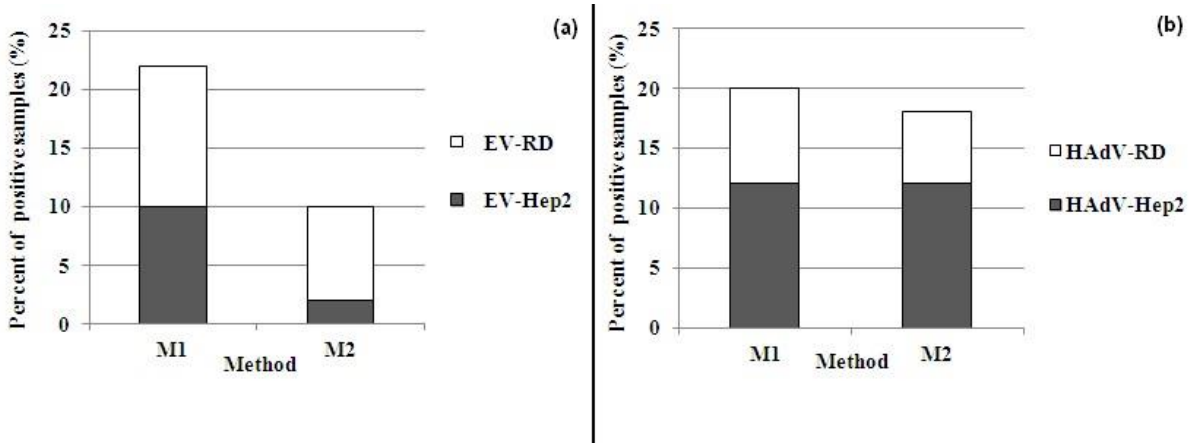

Figure 2. Comparison of infectious EV (a) and HAdV (b) using the PEG precipitation method and the two phase separation method with cell culture CPE. M1. PEG precipitation. M2. Two phase-separation

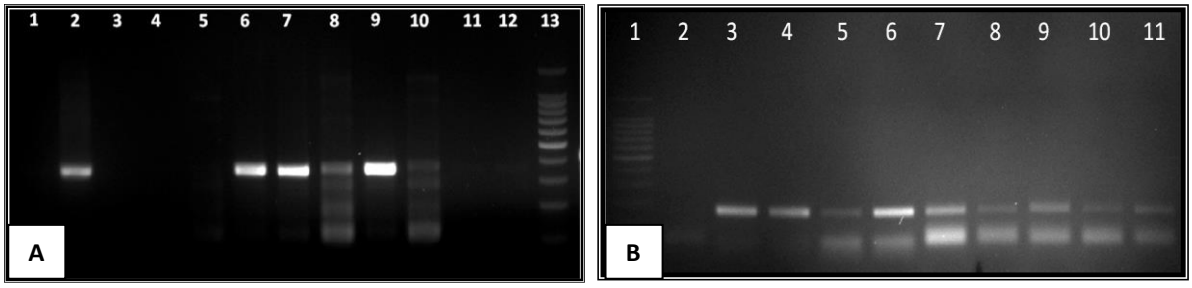

Figure 3. A: PCR of EV detection. Line 1: Negative control. Line 2: Positive control. Lines 3, 4, 5, 8, 10, 11, 12: Negative samples. Lines 6, 7, 9: Positive samples. Line 13: 100 pb ladder. B: PCR of HAdV detection. Line 1: 100 pb ladder. Line 2: Negative control. Line 3: Positive control. Lines 3 to 11: Positive samples

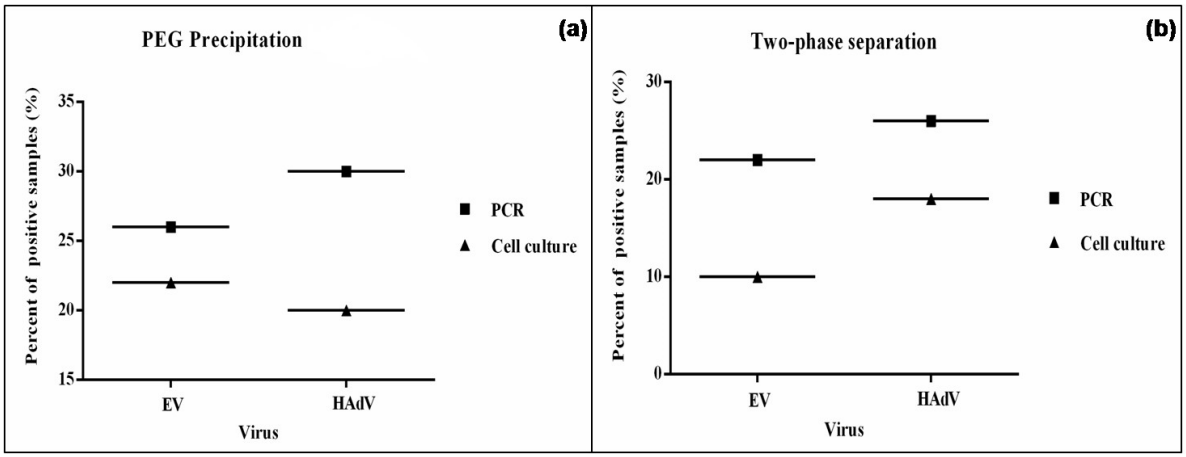

Figure 4. Comparison of the correlation between cell culture and PCR

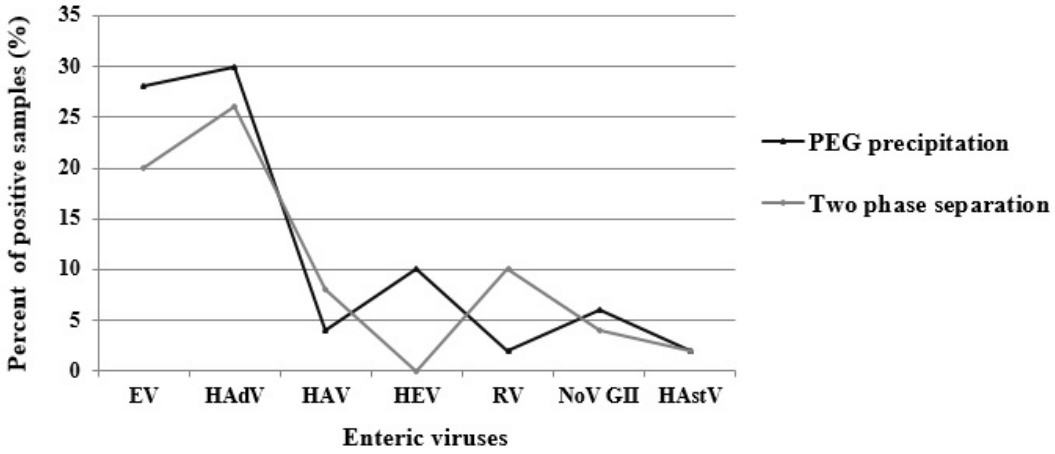

Figure 5. Comparison of PCR positive samples for the PEG precipitation and the two phase separation methods

Presence of infectious EV and HAdV in raw wastewater and treated effluent

By combining the cell culture results of EV and HAdV for both methods. In the Z1, HAdV were found in 31% (4/13) and 38.5% (5/13) of positive raw wastewater and treated effluent samples, respectively. While, the EV were detected in 46.5% (6/13) and 8% (1/13) of raw wastewater and treated effluent samples, respectively. HAdV were the most infectious viruses detected in treated effluents of this zone. The reverse results were found in the Z2. Indeed, infectious EV were more common in treated effluent than wastewater with 42% (5/12) and 25% (3/12) positive samples respectively, while HAdV were found in 33% (4/12) and 8.5% (1/12) of raw wastewater and treated effluent (Fig. 6).

Discussion

Presence of infectious EV and HAdV by cell culture

The obtained results showed that Hep2 cells were more suitable for adenovirus detection as reported in previous study [22]. The use of both cell lines was useful for us to isolate the most of HAdV and EV in samples. Current virus concentration methods are founded on cell culture to detect infectious enteric viruses that can grow on lines selected from host cells.

There are a number of cellular systems in which different viruses found in aquatic environments are likely to multiply, causing a specific cytopathic effect.

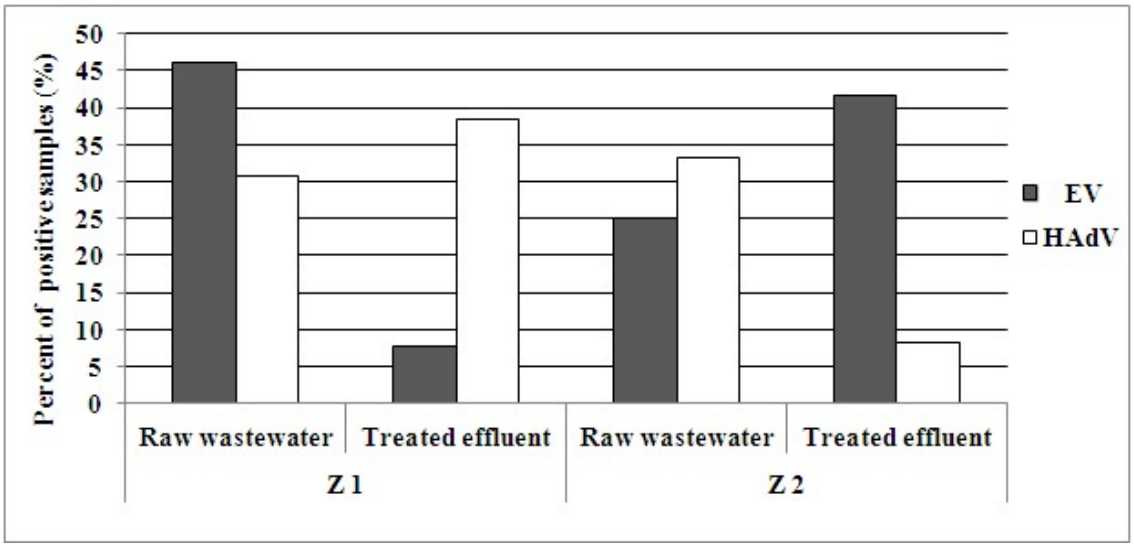

Figure 6. Comparison of the presence of infectious EV and HAdV in the two studied zones

The cell culture sensitivity can be increased by a combination of different cell lines for virus surveillance in water [33]. However, to detect a wide range of infectious HAdV and EV. Several cell lines were used: Hep 2 and BGM [34], RD and L20B [35] and Vero [36].

Taking into account the difficulty to detect all infectious enteric viruses, virological surveillance of water could be based on the detection of virological indicator for the presence of human enteric viruses. Indeed, EV and HAdV have been proposed and used as indicators of viral contamination or treatment efficiency, because they are relatively easy to grow in cell cultures because of their presence at higher rates than other enteric viruses [21,37,38,39]. The difference between the two methods to concentrate infectious EV and HAdV was not significant, which explains that the two methods were able to concentrate EV and HAdV similarly. Rodriguez et al., 2008 [40] showed that the organic flocculation method, could also concentrate infectious EV and HAdV similarly using the PLC/PRF/5 and BGM cells, while Schlindwein et al., 2010 [41] were able to detect a higher rate of HAdV grown on three cell lines: FRhK-4 cells (derived from monkey kidney epithelial cells), A549 (basal epithelial cells of the human alveolar carcinoma) and Hep-2 than PV on Vero cells (derived fibroblast African green monkey kidney cells), using the electronegative membrane-elution. The use of cell culture followed by PCR helps to dilute the PCR inhibitors present in environmental samples and allows the in vitro amplification of viruses in different cell systems [24].

Correlation between the cell culture (CC) and PCR

The results of cell culture and PCR are different and depend on the state of the viral particle. To explain the simultaneously negative and positive results of the CC and PCR, This could be due to that during the transport and treatment of wastewater, some viral particles undergo alterations that reduce their infectivity in vitro which decrease the positive samples by CC [22]. The inactivation of viruses could be caused by virus contact with wastewater treatment lagoons sides or natural inactivation by sunshine [26]. To increase the sensitivity of the cell culture, the ICC-PCR has been widely

used for the detection of EV, HAdV, HAstV and HAV from environmental samples [36,42].

The correlation between cell culture and PCR has been reported previously in different studies while a Tunisian study showed that there is no correlation between the cell culture and PCR of HEV in raw sewage using the adsorption-elution (n = 26 technical, P = 0.077, χ2 test) [36]. Hewitt et al., 2011 [43] detected HAdV in 90% (27/30) samples of raw sewage by PCR while 80% (24/30) were positive by cell culture, and EV in 100% (30/30) and 60% (18/30) by PCR and cell culture, respectively, using the beef extract extraction - precipitation with PEG. All the results highlight a significant increase in sensitivity of detection of pathogenic viruses in relation to the only technique CC. In fact, the ICC-PCR is a perfectly interesting technique for enteric viruses that do not cause CPE in cell lines [44]. PCR can recover defective or non-culturable strains of virus particles but it is sensitive for the inhibitors which are present in the environmental samples.

Assessment of the viral genome by PCR

The results correlate with those obtained in our previous study, in which we have detected more HAdV than HEV using the PEG precipitation method [26]. The same results were obtained by Kokkinos et al., 2010 [4] when the ultracentrifugation method was used, with 40% (10/25) rate of EV and HAdV, and 4% (1/25) of HAV. Using the same method, Kokkinos et al., 2011 [2] detected HAdV in 45.8% (22/48) of samples, followed by 8.3% (4/48) for HAV, 6.3% (3/48) for HuNoV, with none of HEV. A Tunisian study detected a prevalence of RV with 32% (80/250) of wastewater samples, followed by HuNoV in 4.4% (11/250) and one HAdV [45].

Hugues et al., 1993 [46] showed that according to the technique used to concentrate the EV in treated wastewater (adsorption-elution on glass wool or glass powder), 94% or 46% of samples were found positive. Until now, there are no technical consensus protocols for the extraction and amplification of viral genomes. However, the PCR is the only technique used to isolate all enteric viruses in all types of environmental samples.

Presence of infectious EV and HAdV in raw wastewater and treated effluent

Many studies of HAdV and EV in wastewater were conducted previously. In previous study [26], we have seen a decrease in infectious viruses in treated effluent for EV and HAdV in 11.5% (1/9) and 22.5% (2/9), respectively. Otherwise, Hewitt et al., 2011 [43] reported that the rate of positive treated effluents samples from activated sludge WWTP has decreased to half for HAdV and quarterback for EV. The efficiency of wastewater treatment by lagooning fluctuates enormously (from 0 to 99.9%) and depends particularly on the duration of the treatment [47].

The impact of temperature on the inactivation of enteric viruses has been widely reported with a different survival. In this study, the temperature for both studied WWTPs ranged from 24.5 to 25°C for waste water and from 23 to 24.9 °C for treated effluent. In the same temperature, the differences in virus survival were observed between the PV and HAdV where PV was the most sensitive and HAdV the most resistant Enriquez et al., 1995 [48]. Actually, the temperature parameter is difficult to setting because it may have a role in some chemical reactions [49].

The comparison between the presence of infectious EV and HAdV in sewage and treated effluent provides us with information about their presence in both sewage and treated effluent. It can be suggested that EV and HAdV could be very interesting indicators of viral contamination as reported previously by several studies in different countries. Some of them advised EV [37,50] and others advised HAdV [38,51].

Current study results are in agreement with those obtained in previous study when the same methods were compared using Echovirus 7 as a model of enteroviruses [22].

This follow-up study confirms that, considering the protocol time, the cost and the no significant difference between the two methods, the PEG precipitation seems to be an alternative method of the two-phase separation method.

Acknowledgments

Thanks for Dr. Nozha Cohen and Dr. Kaoutar Hajjami for their assistance in wastewater samples collection.

Author contributions:

All authors contributed to this manuscript. Prof. Amdiouni performed the laboratory work, implementation and progress of this study, Dr. Al-Shuwaikh interpreted and arranged drafting of this paper, Dr. Maunula and Dr. Nouril supervise, conception and design of study.

Conflict of interest

There is no conflict of interest.

Funding

This study was funded by the Medical Virology & BSL3 Laboratory, Institut Pasteur du Maroc, Casablanca, Morocco.

References

1. Sinclair RG, Choi CY, Riley MR, et al. Pathogen surveillance through monitoring of sewer systems. Adv Appl Microbiol. 2008; 65: 249-69. doi: 10.1016/S0065-2164(08)00609-6.
2. Kokkinos PA, Ziros PG, Mpalasopoulou A, et. al. Molecular detection of multiple viral targets in untreated urban sewage from Greece. Virol J. 2011; 8: 195. doi: 10.1186/1743-422X-8-195.
3. World Health Organization (WHO). Waterborne zoonoses: identification, causes and control. London: World Health Organization; 2001 p. 1-528. Available from: http://www.who.int/water_sanitation_health/diseases/zoonoses.pdf
4. Kokkinos K, Filippidou S, Karlou K, et al. Molecular typing of enteroviruses, adenoviruses, and hepatitis A viruses in untreated and treated sewage of a biological treatment plant in Greece. Food Environ Virol. 2010; 2(2): 89-96. doi: 10.1007/s12560-010-9036-3.
5. Fumian TM, Vieira CB, Leite JP, et al. Assessment of burden of virus agents in an urban sewage treatment plant in Rio de Janeiro, Brazil. J Water Health. 2013; 11(1): 110-9. doi: 10.2166/wh.2012.123.
6. Masclaux FG, Hotz P, Friedli D, et al. High occurrence of hepatitis E virus in samples from wastewater treatment plants in Switzerland and comparison with other enteric viruses. Water Res. 2013; 47(14): 5101-9. doi: 10.1016/j.watres.2013.05.050.
7. Momou KJ, Akoua-Koffi C, Dosso M. Detection of enteroviruses in water samples from Yopougon, Côte d'Ivoire by cell culture and polymerase chain reaction. Food Environ Virol. 2014; 6 (1): 23-30. doi: 10.1007/s12560-013-9130-4.
8. Tonani KA, Padula J, Juliao FC, et al. Persistence of giardia, cryptosporidium, rotavirus and adenovirus in treated sewage in São Paulo State, Brazil. J Parasitol. 2013; 99(6): 1144-7. doi: 10.1645/12-121.1.
9. Han TH, Kim SC, Kim ST, et al. Detection of norovirus genogroup IV, klassevirus, and pepper mild mottle virus in sewage samples in South Korea. Archives of Virology. 2014; 159 (3): 457-63. doi: 10.1007/s00705-013-1848-7.
10. Pinto RM, Alegre D, Dominguez A, et al. Hepatitis A virus in urban sewage from two Mediterranean countries. Epidemiol Infect. 2007; 135: 270-3. doi: 10.1017/S0950268806006753.
11. La Rosa G, Pourshaban M, Iaconelli M, et. al. Detection of genogroup IV noroviruses in environmental and clinical samples and partial sequencing through rapid amplification of cDNA ends. Arch Virol. 2008; 53(11): 2077-83. doi: 10.1007/s00705-008-0241-4.
12. Bosch A, Guix S, Sano D, et. al. New tools for the study and direct surveillance of viral pathogens in water. Curr Opin Biotechnol. 2008. 19 (3): 295-301. doi: 10.1016/j.copbio.2008.04.006.
13. Ikner LA, Gerba CP, Bright KR. Concentration and recovery of viruses from water: a comprehensive review. Food Environ Virol. 2012. 4 (2): 41-67. doi: 10.1007/s12560-012-9080-2.
14. Myrmel M, Berg EM, Grinde B, et al. Enteric viruses in inlet and outlet samples from sewage treatment plants. J Water Health. 2006; 4(2): 197-209.
15. Kamel AH, Ali MA, El-Nady HG, et al. Evidence of the co-circulation of enteric viruses in sewage and in the population of greater Cairo. J Appl Microbiol. 2010; 108: 1620-9. doi: 10.1111/j.1365-2672.2009.04562.x.
16. Hovi T, Stenvik M, Partanen H, et al. Poliovirus surveillance by examining sewage specimens: quantitative recovery of virus after introduction into sewerage at remote upstream location. Epidemiol Infect. 2001; 127: 101-6. doi: 10.1017/S0950268801005787.
17. Haramoto E, Katayama H, Oguma K, et. al. Recovery of naked viral genomes in water by virus concentration methods. J Virol Methods. 2007; 142: 169-73. doi: 10.1016/j.jviromet.2007.01.024.
18. Meleg E, Jakab F, Kocsis B, et al. Human astroviruses in raw sewage samples in Hungary. J Appl Microbiol. 2006; 101: 1123-9. doi: 10.1111/j.1365-2672.2006.02997.x.
19. Melegh B, Szucs G. Detection and quantification of group C rotaviruses in communal sewage. Appl

Environ Microbiol. 2008; 74: 3394-9. doi: 10.1128/AEM.02895-07.
20. Lee H, Kim M, Paik SY, et al. Evaluation of electropositive filtration for recovering norovirus in water. J Water Health. 2011; 9: 27-36. doi: 10.2166/wh.2010.190.
21. World Health Organization (WHO). Guidelines for environmental surveillance of poliovirus circulation. Geneva: World Health Organization; 2003 p. 1-28. Available from: http://apps.who.int/iris/bitstream/10665/67854/1/WHO_V-B_03.03_eng.pdf.
22. Amdiouni H, Maunula L, Hajjami K, et al. Recovery comparison of two virus concentration methods from wastewater using cell culture and real-time PCR. Curr Microbiol. 2012; 65(4): 432-7. doi: 10.1007/s00284-012-0174-8.
23. Minor PD. Growth, assay and purification of picornaviruses. In: Mahy BWJ. (ed.) Virology: A practical approach. Oxford, Washington: IRL Press; 1985. p. 25–41.
24. Reynolds KA. Integrated cell culture ⁄ PCR for detection of enteric viruses in environmental samples. Methods Mol Biol. 2004; 268: 69-78. doi: 10.1385/1-59259-766-1:069.
25. Casas I, Avellon A, Mosquera M, et al. Molecular identification of adenoviruses in clinical samples by analyzing a partial hexon genomic region. J Clin Microbiol. 2005. 43: 6176-82. doi: 10.1128/JCM.43.12.6176-6182.2005.
26. Amdiouni H, Faouzi A, Fariat N. Detection and molecular identification of human adenoviruses and enteroviruses in wastewater from Morocco. Lett Appl Microbiol. 2012. 54(4): 359-66. doi: 10.1111/j.1472-765X.2012.03220.x.
27. Oberste MS, Nix WA, Maher K. Improved molecular identification of enteroviruses by RTPCR and amplicon sequencing. J Clin Virol. 2003. 26: 375-7.
28. Hot D, Legeay O, Jacques J, et al. Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. Water Research. 2003. 37: 4703-10. doi: 10.1016/S0043-1354(03)00439-1.
29. Wang Y, Ling R, Erker JC, et al. A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. J. Gen Virol. 1999; 80 (1): 169-77. doi: 10.1099/0022-1317-80-1-169.
30. Simmonds MK, Armah G, Asmah R, et al. New oligonucleotide primers for P-typing of rotavirus strains: Strategies for typing previously untypeable strains. Clin Virol. 2008. 42 (4): 368-73. doi: 10.1016/j.jcv.2008.02.011.
31. Kojima S, Kageyama T, Fukushi S, et al. Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods. 2002; 100(1-2): 107-14. doi: 10.1016/S0166-0934(01)00404-9.
32. Matias V, Carvalho-Costa FA, Heinemann MB, et al. Genotypes and molecular epidemiology of human astroviruses in hospitalized children with acute gastroenteritis in Rio de Janeiro. Brazil J Med Virol. 2007; 79(7): 939-44. doi: 10.1002/jmv.20814.
33. Sedmak G, Bina D, Macdonald J, et al. Nine-year study of the occurrence of culturable viruses in source water for two drinking water treatment plants and the influent and effluent of a waste water treatment plant in Milwaukee, Wisconsin (August 1994 through July 2003). Appl Environ Microbiol. 2005; 71: 1042-50. doi: 10.1128/AEM.71.2.1042-1050.2005.
34. Puig M, Jofre J, Lucena F, et al. Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. Appl Environ Microbiol. 1994; 60: 2963-70.
35. World Health Organization (WHO). Polio laboratory manual. Geneva: World Health Organization; 2004 p. 1-166. Available from: http://apps.who.int/iris/bitstream/10665/68762/1/WHO_IVB_04.10.pdf.
36. Sdiri K, Khelifi H, Belghith K, et al. Comparison of cell culture and RT–PCR for the detection of enterovirus in sewage and shellfish. Pathologie Biologie. 2006. 54: 280-4. doi: 10.1016/j.patbio.2005.12.001.
37. Hovi T. Surveillance of poliovirus. Biologicals. 2006; 34: 123-6. doi: 10.1016/j.biologicals.2006.02.009.
38. Silva DH, García-Zapata MTA, Anunciação CE. Why the use of adenoviruses as water quality virologic marker? Food Environ Virol. 2011; 3(3-4): 138-40. doi: 10.1007/s12560-011-9069-2.
39. United States Environmental Protection Agency (USEPA). Guidelines for water reuse. Washington: US Environmental Protection Agency; 2004 p. 1-28. Available from: http://www.waterboards.ca.gov/water_issues/programs/grants_loans/water_recycling/docs/econ_tskfrce/14.pdf.
40. Rodriguez RA, Gundy PM, Gerba CP. Comparison of BGM and PLC/PRC/5 cell lines for total culturable viral assay of treated sewage. Appl Environ Microbiol. 2008; 74: 2583-7. doi: 10.1128/AEM.00626-07.
41. Schlindwein AD, Rigotto C, Simões CM, et al. Detection of enteric viruses in sewage sludge and treated wastewater effluent. Water Sci Technol. 2010; 61 (2): 537-44. doi: 10.2166/wst.2010.845.
42. Chapron CD, Ballester NA, Fontaine JH, et al. Detection of astroviruses, enteroviruses, and adenovirus type 40 and 41 in surface waters collected and evaluated by the information Collection rule and an integrated cell culture-nested PCR procedure. Appl Environ Microbiol. 2000; 66: 2520-5. doi: 10.1128/AEM.66.6.2520-2525.2000.
43. Hewitt J, Leonard M, Greening GE. Influence of wastewater treatment process and the population size on human virus profiles in wastewater. Water Res. 2011; 45(18): 6267-76. doi: 10.1016/j.watres.2011.09.029.
44. Vivier JC, Ehlers MM, Grabow WO. Detection of enteroviruses in treated drinking water. Water Res. 2004; 38 (11): 2699-705. doi: 10.1016/S0043-1354(01)00433-x.

45. Sdiri-Loulizi K, Hassine M, Aouni Z. Detection and molecular characterization of enteric viruses in environmental samples in Monastir, Tunisia between January 2003 and April.2007 J Appl Microbiol. 2010; 109(3): 1093-104. doi: 10.1111/j.1365-2672.2010.04772.x.

46. Hugues B, Andre M, Plantat JL. Comparison of glass wool and glass powder methods for concentration of viruses from treated waters. Zentralbl Hyg Umweltmed. 1993; 193: 440-9.

47. Hurst CJ. Fate of viruses during wastewater sludge treatment processes. Crit. Rev Env Control. 1989; 18: 317-43. doi: 10.1080/10643388909388352.

48. Enriquez C, Hurst CJ, Gerba CP. Survival of the enteric Adenovirus 40 and 41 in Tap Sea and wastewater. Water Res. 1995; 29: 2548-53.

49. Beaudeau P, Tousset N, Lefèvre A, et al. Disparition des Escherichia coli dans les rivières normandes, rapprot de l'agence de l'eau Seine-Normandie. 1998; 92.

50. Hovi T, Shulman LM, van der Avoort H, et al. Role of environmental poliovirus surveillance in global polio eradication and beyond. Epidemiol Infect. 2012; 140(1): 1-13. doi: 10.1017/S095026881000316X.

51. Tong HI, Lu Y. Effective detection of human adenovirus in Hawaiian waters using enhanced PCR methods. Virol J. 2011; 8: 57. doi: 10.1186/1743-422X-8-57.

Correspondence to Prof. Hasna A. Amdiouni
E-mail: hasna.amdiouni@gmail.com,
hasna.amdiouni@yahoo.fr
Received 6th Oct. 2016
Accepted 15th Jan. 2017