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International Journal of Current Research
Vol. 5, Issue, 10, pp.2724-2730, October, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

HUMAN ENTERIC VIRUS IN VARIOUS ENVIRONMENTAL MATRICES IN THE NORTHWEST OF MOROCCO

***[1,2]Laila Amar, [3]Ayalkibet Hundesa, [3]Sandra Fresno, [3]Jesús Rodriguez-Manzano, [1]Lahcen Wakrim, [3]Rosina Girones, and [2]Mustapha Lkhider**

[1]Virology Laboratory, Pasteur Institute of Morocco.1 Place Louis Pasteur, 20360, Casablanca, Morocco
[2]Department of Bioloby, Faculty of Science and Technology Mohammedia Hassan II, University Mohammedia, Mohammedia, Morocco
[3]Department of Microbiology, University of Barcelona, Avda Diagonal 645. 08028. Barcelona. Spain

ARTICLE INFO

Article History:
Received 14th July, 2013
Received in revised form
20th August, 2013
Accepted 19th September 2013
Published online 10th October, 2013

Key words:
Adenovirus, Enterovirus, Hepatitis virus A and E, Lagoon, Mussels, *Norovirus*, Seawater, Sediments.

ABSTRACT

A total of 72 samples were collected between 2006 and 2008 in the Mediterranean coast of Morocco (Smir lagoon water= 30, connected seawater =30, sediments=6, mussels = 6). The water samples were concentrated by glass wool method then analyzed for human enterovirus infectious by integrated cell culture-PCR (ICC/RT-PCR) and by qRT-PCR for other enteric viruses. Human adenovirus, polyomavirus JC, Norovirus GII and rotavirus A were detected in 63, 57, 43 and 36% of 30 lagoon samples respectively. For Norovirus GI, in 10 tested samples only 10 % revealed positive. Human adenovirus was detected in 60% while polyomavirus JC and Norovirus GII were detected in 10% of ten seawater samples. All were negative for hepatitis virus E and hepatitis virus A. The infectious enterovirus were detected in 40 and 36% of lagoon and seawater samples respectively and sequencing analyses showed that sequences belong to coxsackievirus B1. Mussels samples were positive for Rotavirus A (83%) and none of Norovirus were detected. Sediments were positive for Rotavirus A (33%) and Norovirus GI/GII (17%). This study revealed a human viral pollution of the environment and suggested Human adenovirus as a suitable viral indicator of human fecal pollution for recreational beaches quality.

INTRODUCTION

Human sewage is at least partly responsible for the degradation of water quality at costal beach (McQuaig *et al*., 2009) and several epidemiological studies showed that bathing in polluted recreational waters has been associated with an increased risk for transmission of infectious diseases including gastroenteritis and febrile, respiratory, skin, eye and ear illnesses (Halliday *et al*., 2011). Human enteric viruses are among other pathogens excreted in the feaces of infected individuals and may be dispersed into marine waters. The stability of these viruses in environmental water and their persistence for more extended periods than bacteria in the marine environment and in the digestive tracts of bivalves have been responsible for a great number of outbreaks (La Rosa *et al*., 2012; Vieira *et al*., 2012). The most commonly studied species of enteric viruses are *enterovirus* (EV), *adenovirus* (AdV), genogroup *A rotaviruses* (RoV A), *hepatitis A and E viruses* (HAV, HEV) and more recently *norovirus* (NoV) (Vecchia *et al*., 2012). They are associated primarily with diarrhea and self-limiting gastroenteritis in humans; and may also cause respiratory infections, conjunctivitis, hepatitis and diseases that have high mortality rates such as aseptic meningitis, encephalitis and paralysis notably in immunocompromised individuals (Fong *et al*., 2005). In the M'diaq city, part in the north region of Morocco, untreated sewage discharged into Smir lagoon which communicates with the mediterranean coast via a small bridge located near the marina Kabila. This situation has adversely affected the water quality of beaches (El fellah, 2005) and may affect the shellfish quality growing in this area.

***Corresponding author: Laila Amar,** Virology Laboratory, Pasteur Institute of Morocco.1 Place Louis Pasteur, 20360, Casablanca, Morocco.*

The NHRI (National Fisheries Research Institute) was created in Morocco at 1996 to manage the public health and safety of recreational beaches and shellfish along the Atlantic and Mediterranean coasts. For this propose, bacterial indicator of fecal contamination were established as safety standard, according to Moroccan regulations (NM 03.7.200 and NM 08.0.147-2004). However, it has been clearly established that bacterial standards were not predictive of human virus to assess recreational water quality and the sanitary quality of shellfish (Wong *et al*., 2009; La rosa *et al*., 2012). Moreover, M'Diaq focuses on the development of molluscs because of excellent geographical conditions for marine organism cultures, especially mussels (Sentissi, 2010). On the other hand, this city is considered very important to the tourism industry because of the presence of marina kabila and beach (Dakki, 2004). The increasing pollution of the coastal waters of M'diaq requires monitoring of cultured bivalve molluscs to ensure health safety standards and recreational beaches to protect bathers' health. To the best of our knowledge, no investigation to date has studied the presence of viruses in seawater of Morocco. In this study, the distribution of human enteric viruses was evaluated in water samples, shellfish and sediments from M'diaq for more than year period, by enzymatic amplification (conventional PCR), quantification by real-time PCR and tested for viability by ICC-PCR. Recently, cleaning and clearing of the lagoon started at the Marina area and will continue progressively with a project by a private company.

MATERIALS AND METHODS

Study Area and sampling

Samples were collected from two sites (Figure 1) The first one (Z1) located at the Smir lagoon, small bridge closest to the mediterranean

which drains into Tamouda Bay through an opening of over 20 meters. The lagoon interacts with marine waters during all seasons with salinities ranging from 30 to 40 and temperature ranging from 12 °C in winter to 32 °C in summer (Benhissoune *et al*., 2005). The second one (Z2) located in Tamouda Bay between Sebta at the north and Cap Negron at the south in the Mediterranean coast of Morocco. A total of 72 samples, composed of lagoon water (n=30), seawater (n=30), mussels (n=6) and sediments (n=6) were collected from October 2006 to December 2008. Thirty liters volumes of samples water, 100 g of sediment and 10 to 15 mussels were collected and transported to the laboratory in sterilized plastic bottles and box at 4°C. Some physicochemical parameters were measured immediately after sample collection (pH and temperature) with pH meter (HANNA) while salinity and turbidity were determined at the laboratory with NM 03.07.019 and turbid meter (HACHA) respectively.

Virus concentration and RNA extraction from mussels and sediments samples

The outer surface of mussels was cleaned with water and ethanol (96%), dissected and digestive tissues (DT) were recovered. The viruses were concentrated and RNA was extracted from DT mussels and sediments by The Melt Total Nucleic Acid isolation system kit (Applied Biosystem, Lyon, France) according to the manufacturer's instructions. The RNA was eluted in 40 µl of tampon elution and conserved in -80°C until analyzed.

Virus Concentration in water

The samples were concentrated by a glass wool filter based on AFNOR norm XP T90-451. Briefly viruses in 30L samples were concentrated by adsorption to glass wool filters and eluted by saturating the filter with 3% beef extract (wt/vol) (Difco) containing 0.5mol l^{-1} glycine (pH 9.5) (Sigma). The eluted water sample was adjusted to pH 3.5 with 1 mol l^{-1} HCl and then flocculated with constant stirring for 1h at room temperature. The floc were pelleted by centrifugation at 4200×g for 48 min and the pellet was resuspended in sterile 0.15mol l^{-1} Na_2HPO_4 solution (pH 9). Fifty portion of this concentrate was used for cell culture analysis to detect *enterovirus* and nucleic acid extraction was performed directly on the other portion to analyze other enteric viruses.

Detection of Enteric Viruses in water

Enterovirus by ICC/RTPCR

Cell culture of concentrated water

Human epidermis carcinoma cells of the larynx (*HEp-2 cells*) and rabdomyosarcome (*RD*) were used to isolate the enterovirus in concentrated samples. Cells were grown in flasks containing MEM medium (PAN) supplemented with 10% (growth medium) or 2% (maintenance medium) heat inactivated fetal bovine serum (Eurobio.France), 1% of L glutamine 20 mg and 1% of penicillin-streptomycin (100 U/ml and 100 mg/ml). The eluates were immediately adjusted to neutral pH with 1 mol-1 HCl. The 300 ml volumes of eluate were incubated overnight with gentle stirring at 4°C after adding 13% (w/v) polyethylene glycol (PEG) 8000 (+0,2 mol -1 NaCl) at pH 7,2. After incubation, the eluates were centrifuged at 7000 x g for 30 min. The resulting pellets were resuspended in 6 ml of 10 mmol-l phosphate buffered saline (PBS). Before the inoculation, suspensions were treated with a penicillin-streptomycin-amphotericin B (Fungizone) mix (GIBCO) for 30 min at room temperature and then passed through a newborn calf serum pretreated 0,2µm filter (Sartorius) to remove any microbial contaminants. The fifty portion of the final concentrate was inoculated in plates 6 wells of Hep2c and RD cells with negative control cell and incubated for 45 min at 37°C in an atmosphere of 5% CO2. The plates were rocking every 15 min for efficient adsorption of viral particles then the sample inoculums was removed and the cells were washed twice with 3 ml PBS and re-fed with maintenance medium. The plates were incubated for 7 days at 37°C in a 5% CO2 and observed daily under microscopy inverted for a cytopathic effect (CPE). The cells plates were frozen and thawed twice and the previous supernatants with and without CPE were determined by using integrated cell culture RTPCR (ICC/RT-PCR) for *enterovirus human* (HEV).

Nucleic acids extraction

Viral nucleic acids were extracted from 140 µL of the concentrated water samples and cell culture lysates using a QIAamp Viral RNA mini kit (Qiagen) following the manufacturer's instructions to obtain a final volume of 80 µL. The extraction was performed with a QIACUBE (automated platform).

ICC/RT-PCR enterovirus

Two different regions in the enteroviral genome were amplified by a one-step RT-PCR (OneStep RT-PCR kit; Qiagen) with lysates from cell culture with and without CPE. Briefly, a 362-bp gene fragment of the 5'NCR was amplified with two 5'NCR-specific primers (P2/P3) as described previously by Leparc *et al.* (1993), to confirm that the virus specimens were enterovirus positive. For typing of the virus specimens, a VP1 RT-PCR was carried out with primers (222/292) as described previously by oberste *et al*. (2003). Amplification conditions were provided by 30 min of reverse transcription at 42°C, 5 min of denaturation at 94°C, 35 amplification cycles (94°C, 30 s; 45°C, 30s; 72°C, 30s)), and finally a 7-min extension cycle at 72°C. Each 25 µL PCR reaction mixture contained 12.5 µL of SuperMix, 400 nM of each primer, and 5 µL of template RNA. For each PCR run, corresponding positive (poliovirus 1 LSC-2ab) and negative (nuclease-free water) controls were included. Thermal cycling was carried out with a Gene Amp 9600 PCR system (Applied Biosystems). Amplified products were separated electrophoretically on a 2 % agarose gel and visualized by ethidium bromide staining.

Sequencing and phylogenetic analysis

The VP1 RT-PCR product was purified by exonuclease I and shrimp phosphatase alcaline enzymes (Promega). Sequencing was performed with BigDye Terminator Cycle Sequencing Kit using the ABI Prism 3130 automated sequencer (Applied Biosystems). The obtained VP1 consensus sequences were compared to all of the corresponding enterovirus sequences available in Gen-Bank by using FASTA analysis in order to identify the enterovirus type. Multiple sequence alignments were prepared by using Clustal W2 software ftp://ftp.ebi.ac.uk. Phylogenetic trees were constructed by the neighbor-joining method and numbers at nodes represent the percentage of 100 bootstrap replicates. Phylogenetic analyses were conducted by using MEGA version 4 (Tamura *et al*., 2007). The sequences obtained in this work have been deposited in the GenBank database under accession numbers: KF 199866, KF 199867, KF 199868, KF 199869 (data no still diffused).

Quantitative PCR and quantitative reverse transcription-PCR (qPCR and qRT-PCR)

HAdV, JCPyV, NoV GII and HAV quantitation were based on the assays described previously by Hernroth *et al.* (2002), Pal *et al*. (2006). Da Silva *et al*. (2007) and Jothikumar *et al.* (2005) respectively. Standards for the real-time PCR assays were created using transformated *E. coli* JM109 cells (Promega. Madison. WI. USA) with pBR322 plasmid (Promega. Madison. WI. USA) containing a sequence of the adenovirus type 41 or JCPyV Mad1 genome, with pTrueBlue-Pvu II plasmid (Promega. Madison. WI. USA) containing a sequence of norovirus capsid protein region or with pGEM-T Easy plasmid (Promega. Madison. WI. USA) containing a sequence of the HAV 5'-untranslated region (5'UTR) following manufacturer's instructions. Amplification was performed in a 25 µl reaction mixture with TaqMan PCR master mix system (Applied Biosystems) for HAdV and JCPyV or with RNA Ultrasens quantitative RT-PCR One-step system (Invitrogen) for NoV. HAV were carried out with a QIAGEN OneStep RT-PCR kit (QIAGEN). The standard

dilutions (5 µL) from 10^3 to 10^6 copies μL^{-1} were used in triplicate for the standard RNA or DNA. A negative amplification control (water) and positive amplification controls (HAdV 2, JCPyV, NoV GII.13 and HAV) were included in each amplification series at 10^2-10^4 GC/reaction. For the specific detection and quantitation of genomes, 10 µl of undiluted samples, 1:10 dilutions of every DNA or RNA extraction and 10 µl of a quantified DNA plasmid–1X TaqMan master mix were tested. These dilutions were made to detect and reduce amplification inhibition due to the potential presence of inhibitory substances that may interfere with the quantitative PCR (qPCR). Known quantities of target DNA were added to a parallel amplification reaction containing the qPCR mix and the sample. In every assay, the amplification plots of samples and standard dilutions were compared. A sample was considered positive if it produced correct amplification curves and the quantitation data was within the detection limit. All reactions were performed with an MX3000P sequence detector system (Stratagene, La Jolla, CA).

Qualitative reverse transcription-PCR (qRT-PCR)

The qRT-PCR was performed with primers and probes for HEV, RoV A and NoV GI respectively as previously described (Jothikumar *et al*., 2006; Jothikumar *et al*., 2009; da Silva *et al*., 2007). The amplification was performed in a 25 µL volume containing 10µL of RNA with TaqMan One step Qualitative RT-PCR kit with Rox (Invitrogen) for RoV, quantitec probe RT-PCR kit (Qiagen) for HEV and RNA Ultrasens quantitative RT-PCR One-step system (Invitrogen) for NoV
All reactions were performed with an MX3000P sequence detector system (Stratagene, La Jolla, CA) except for rotavirus which carried out with a Gene Amp 9600 PCR system (Applied Biosystems).

Detection of Enteric virus in sediments and mussels samples Rotavirus et norovirus GI/GII

The RT-PCR was performed with primers for RoV A and NoV GI/GII respectively as previously described (Jothikumar *et al*., 2009; da Silva *et al*., 2007). The amplification was carried out in a 25 µL volume containing 5µL of RNA using access quick one step RT-PCR kit (Promega) following manufacturer's instructions. The cycling conditions were as follows: reverse transcription at 45°C for 40 min and for PCR, initial denaturation for 2 min at 94°C, 45 amplification cycles with denaturation for 30 s at 94°C, annealing for 30 s at 55°C for RoV , 59°C for NoV GI and 61°C for NoVGII, extension for 1 min at 72°C, and a final cycle of incubation at 72°C for 10 min.
Thermal cycling was carried out with a Gene Amp 9600 PCR system (Applied Biosystems) and amplified products were separated electrophoretically on a 2 % agarose gel and visualized by ethidium bromide staining.

RESULTATS

Enteric virus in water

Enterovirus in seawater and lagoon

ICC/RT-PCR was performed to detect EV in samples from the lagoon Smir and Kabila Beach (Fig. 2). EV infectious was detected by ICC/RT-PCR in twelve of thirty (40%) lagoon samples and in eleven of thirty (36%) seawater samples (Tab.2). Four samples (13%) exhibited CPE in two cell lines was confirmed coxsackivirus B1 by sequencing.

Other Enteric virus in seawater

Six of ten seawater samples collected between November 2006 and April 2008 were tested positive for HAdV with genome copies ranged from 26 to 750 (GC l^{-1}). The mean value was 68 genome copies and the highest concentration of HAdV was observed in spring. For JCPyV only one sample of ten tested revealed positive in spring with 37 genome copies. Norovirus GI (NoV GI) was also detected in one of ten samples analyzed in winter but neither HAV nor HEV were detected.

Table 1. Quantification of HAdV and JCPyV by qPCR and NoV GG-II by qRT-PCR of the samples collected from lgoaon water

Dates of collection (day/mo/yr)	Genome copies L^{-1} of		
	HAdV	JCPyV	NV- GG II
10/11/2006	7.00E+03	1.06E+05	3.37E+03
22/11/2006	2.64E+01	9.12E+00	9.40E+02
08/12/2006	-	-	-
21/12/2006	-	-	-
11/01/2007	9.62E+01	2.37E+01	-
29/01/2007	2.27E+01	3.03E+01	-
08/02/2007	-	-	-
02/03/2007	1.80E+01	-	-
06/04/2007	-	-	-
27/04/2007	-	6.55E+02	-
10/05/2007	3.18E+01	-	3.14E+01
24/05/2007	8.18E+01	8.78E+01	1.54E+03
07/06/2007	-	2.15E+01	4.13E+03
21/06/2007	2.93E+01	-	-
20/07/2007	-	1.58E+01	-
15/08/2007	-	-	-
03/09/2007	3.88E+01	2.90E+01	2.70E+01
03/10/2007	1.23E+02	-	-
29/10/2007	-	-	2.90E+01
13/11/2007	4.18E+01	3.51E+01	-
06/12/2007	1.25E+02	8.67E+01	6.77E+02
26/12/2007	-	2.88E+01	-
09/01/2008	6.02E+01	3.12E+01	2.11E+02
24/01/2008	1.70E+01	-	8.38E+02
06/02/2008	1.72E+01	1.17E+02	2.10E+02
27/02/2008	2.97E+02	5.24EE+02	8.38E+01
13/03/2008	7.07E+01	1.03E+02	-
27/03/2008	8.57E+02	2.03E+01	2.34E+02
09/04/2008	0.78E+01	-	-
08/05/2008	-	-	-
Mean	6.42E+02	1.07E+04	1.22E+03
SD	1.65E+03	3.35E+04	1.43E+03

Table 2. Results of rotavirus and enterovirus analyzes in lagoon and seawater samples

Nature Samples	Hep2 cell	RD cell	ICC/RTPCR EV	qRTPCR RoV
Lagoon water	4/30 (13%)	4/30 (13%)	12/30 (40%)	10/30 (33%)
Seawater	4/30 (13%)	4/30 (13%)	11/30 (36%)	nt

nt : no treated

Table 3. Results of rotavirus and norovirus analyzes in sediments and mussels samples

Samples	NoV		RVA
	GI	GII	
Sediments	1/6 (17%)	1/6(17 %)	2/6 (33%)
Mussels	0/6	0/6	5/6 (83%)

Other Enteric virus in lagoon

Thirty lagoon water samples were analyzed by quantitative qPCR assay during this study for HAdV, JCPyV and Norovirus GII and at least one virus was detected in 24 (80%) of out 30 samples. All four groups of viruses investigated were detected with HAdV (63%) as the most prevalent, followed by JCPyV (56%) and NoV GII (43%) and ranged from 0.78 to 7 $x10^3$, 9.1 to $1x10^5$ and 2 $x10^1$ to 4x 10^3 GC L^{-1} respectively (Tab. 1). 10 samples of 30 were analyzed for NoV GI, and only one sample collected in November was positive. In the present study, NV GII was more detected than NV GI in lagoon samples. RoV A was present in 36% of lagoon samples but neither HAV nor hepatitis virus E (HEV) were detected by the real-time PCR method. According to the collection period, qPCR showed that a high prevalence of viruses correlated with rainfall.

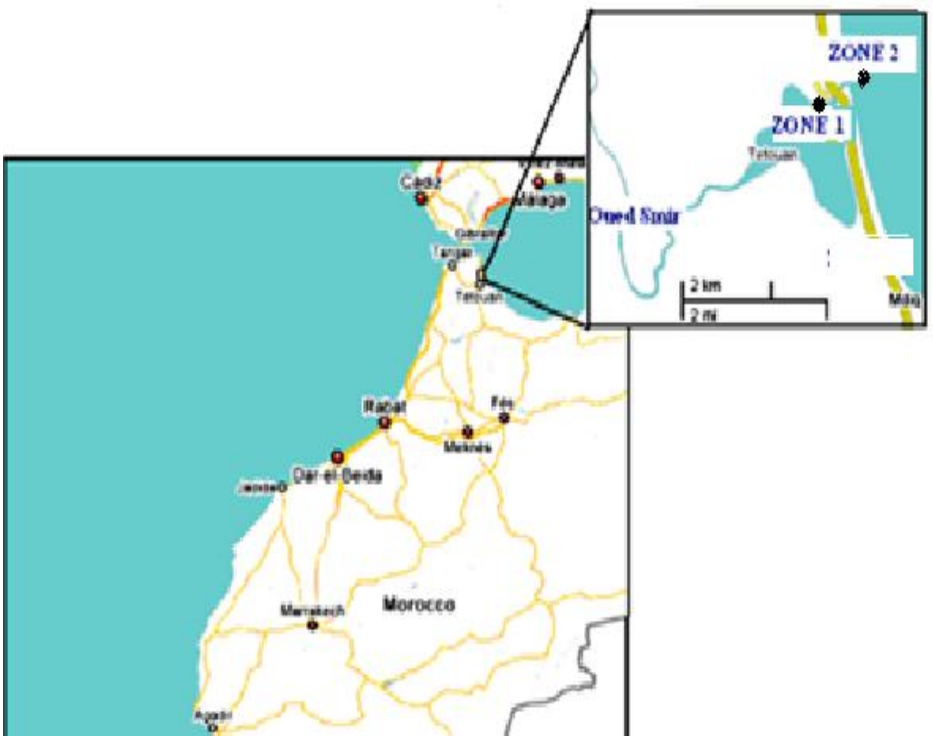

Figure 1. Sampling sites Zone 1 (25°43' N -5°20'W), Zone 2 (N: 35° 41' 402'' W: 005° 18' 226'')

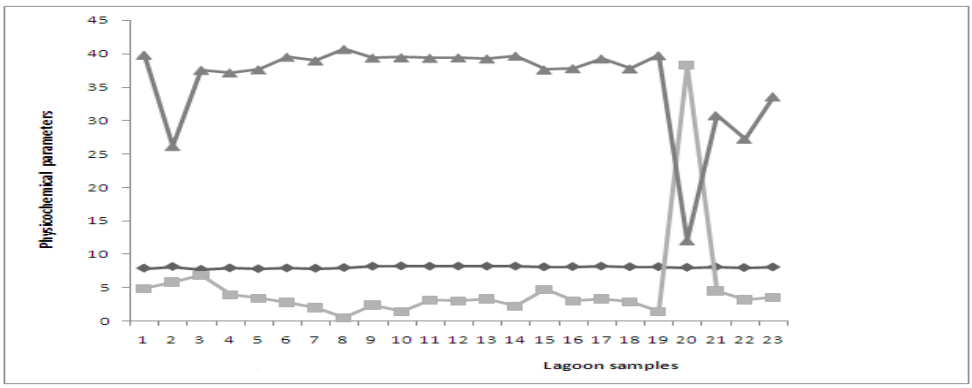

Figure. 2 Measures of pH ( ), Turbidity ( ), Salinity ( ) in lagoon water samples

Enteric virus in sediments and mussels samples

RVA was most frequent in mussels (83%) but no NoV were detected (Tab.3). The dilution of mussels samples showed presence of qPCR inhibitors but not in sediments samples. Marine sediments show a high contamination for both RVA (33%) following by low incidence for NoVGI/II (17%) (Tab.3). Both presence of RVA and NoVGI were detected in one sediments sample and all samples were collected in winter. Three of six sediments samples (50%) were negative for both virus while three of six (50%) were positive at least for one virus.

qPCR sensitivity

Standard curves constructed from analysis of HAdV or JPCyV or NoV GII viral genomes displayed a linear relationship for the qPCR assay with an average Rseq value 0.99, 0.96 and 1 respectively. For all standard curves generated during the present study the average values for the variables in the linear regression were as follows: the y intercept was 40. 38 and 40 and the slope were 3.41, 3.07 and 3.41 respectively for HAdV, JCPyV and Norovirus GII. The calculated efficiency values of the assay were 0.96, 1.11 and 0.96 based on the slopes of the standard curves of the above strains. Serial dilutions of prequantified plasmids (10^9 to 10 copies per reaction) revealed that the real-time PCR assay reliably detects less than 10 copies per reaction of HAdV, JCPyV and NoV GII DNA.

PCR inhibitions

For each sample the value from the dilution showed the absence of inhibition and no contamination was found neither in negative control and nor matrix sample.

Physicochemical parameters analysis

During summer, the water temperature averaged 22°C approximately 5 to 7°C higher than in winter. Salinity values ranged from 12 to 40 ppt while turbidity values ranged from 0.3 to 38 NTC. Seasonal salinity differences reflect seasonal rainfall with salinity averaging 23 ppt during January through march (autumn) while significant dips in salinity were found in December and March which corresponded with rainstorms (Figure 2). Figure 2 shows the seasonal distribution of average pH, salinity and turbidity values during the study in the lagoon. There were no significant differences in water pH among sampling sites located in the Beach.

DISCUSSION

This study is among the first to detect and quantify viral pathogen in recreational water of Morocco. Among the most finding, high

frequency of adenovirus detected in lagoon and seawater samples, should be considered as a potential indicator of fecal environmental contamination in recreational water as reported by several studies (Wyer *et al*., 2012; Wyn-Jones *et al.*, 2011). JC Polyomavirus most strongly correlated with adenovirus except in a six sites suggest that JCPyV should therefore be interpreted carefully as a molecular index of fecal/urine contamination of human origin in conjunction with HAdV (Hundesa *et al*., 2006). In addition, the frequency of human markets detection at this site strongly suggests contamination from human source. In the present study, NV GII was more detected than NV GI in lagoon samples and this data seem to be in line with other studies (Steyera *et al*., 2011; Tian *et al*., 2012). In recreational water-borne diseases, noroviruses are believed to be the single largest cause of documented outbreaks, followed by adenovirus (Sinclair *et al*., 2009). Circulation of rotavirus (RoV A) in environmental suggest the presence of high concentration shedding (Ruggeri and Fiore, 2012) or low rotavirus vaccine coverage and support the use of enteric viral detection in water as a useful tool to measure the impact of specific interventions. RoV has been described as the most important virus in cases of acute gastroenteritis, since it is responsible for a third of these cases and caused waterborne infections in recreational waters (Greenberg and Estes, 2009; Hamza *et al*., 2009; Patel *et al*., 2009). 2916 cases of gastroenteritis reported in 2012 in Morocco with 38% of cases confirmed for rotavirus in children aged < 5 years and death rate 5 to 1000 (Bulletin épidemiologique juin., 2012).Vieira *et al*. (2012) were detected 24,3% of samples positive for RoV A in lagoon less than detected in our study. This difference may be depends on factors like population density, infection prevalence within a given community and on the protocol that is used for virus detection (Ruggeri and Fiore, 2012; Girones *et al*, 2010). Our study found that frequency and concentration of virus were higher in lagoon than in its inter-connected seawater (only 2/30 samples were positive for Norovirus GII and negatives for HAdV, being 11/30 positive for both). This finding implicated the lagoon as an important, but not only, source of viral at beach. The presence of virus was indicated along the year may indicate human fecal pollution from non communal source like boat discharge from marina kabila and human bather shedding (Jiang *et al*., 2007; Abdelzaher *et al*., 2011). However, our study revealed the dissemination of virus in seawater that conformed to quality guidelines for thermotolerant coliforms used for recreational water (Rapport national, 2011-2012).These data corroborate with previous studies that have shown no association between bacterial indicators and viral contamination (Carducci *et al*., 2008; Espinosa *et al*., 2009).

The high concentration of virus detected in our study, correlated with rainfall period as reported by Haramoto *et al*. (2006). Moreover, the majority of documented outbreaks of waterborne disease increase in this period (Curriero *et al*., 2001). Hepatitis virus A is endemic in Morocco; however, we noted absence of HAV and HEV, which is not surprising, due to low potential to be excreted in urban sewage (Rodriguez-Manzano *et al*., 2010). Inhibitory problems were noticed during the real-time PCR runs in this study. It has been reported that complex environmental samples like seawater may contain diverse PCR inhibitors including humic and fulvic acids (Xagoraraki *et al*., 2007). Diluting the lake extracts were carried out in this study and could overcome the inhibitory problems for sediment and shellfish PCR. However, no inhibition of PCR was observed with water samples, showed that glass wool appeared to diminish PCR inhibition, an observation also noted by van Heerden *et al*. (2005a) and Lambertini *et al*. (2008). Interestingly, ICC-PCR results showed that both lagoon and interconnected seawater were polluted with infectious EV (Table 3). The presence of infectious EV in both of sites indicated a recent viral contamination (Gantzer *et al*., 1999) and may include in some cases, lead to outbreaks associated with swimming (Begier *et al*., 2008; Leveque *et al*., 2008). Abbaszadegan *et al*.(1999) detected 8.7% of CPE on BGM cells against 30.1% by ICC / RT-PCR, which is close to our results in the seawater The presence of same serotype of EV (Coxsackievirus B1) in both lagoon and seawater indicated that are likely able to survive in the lagoon, survive sunlight inactivation, and be transported to the beach area. CB1 detected in our study showed 95% similarity to CB1 that was detected in outbreak in Spain during the same period of collection (Costán Longares A *et al*., 2008). This groups as the dominant types in environmental samples (Aw and Gin, 2010) can cause a risk by swimming (Mocé-Llivina *et al*., 2005), could be associated with neonatal infections, which in some cases can be fatal (Wikswo *et al*., 2009) and sometimes caused outbreaks (Baek K *et al*., 2011).

It is not surprising that the virus concentrations at the studied recreational beaches were lower than those in sewage and river water duo to the dilution that is apparent at ocean added to the mixture between lagoon and ocan (McQuaig *et al*., 2009). Although the ocean offers relatively rapid dilution of sewage, it appears that dilution phenomena and the inactivation of viruses is not enough to minimize the impact of virus pollution when contaminant effluents discharge at or within a short distance of the fecal contamination (Bofill-Mas *et al*., 2010; Wyn-Jones *et al*., 2011). Different viral concentration methods in seawater associated with conventional viral isolation in cell cultures and/or molecular methods for virus detection have been developed and showed variable levels of recovery efficiency (Victoria *et al*., 2009; Sassoubre *et al*., 2012). The glass wool method was optimized in seawater by Vilaginès *et al*. (1997) and some studies showed that was not as efficient as for freshwater (Griffin *et al*., 2003; Calgua *et al*., 2008; Girones *et al*., 2010). However, a few studies compared glass wool with other methods in seawater and more studies are needed to further clarify this observation. Our results provided a evidence presence of viral contamination in a maximum of water samples analyzed and reported similar observations with other previous studies (Wyer *et al*., 2012; Wyn-Jones *et al*., 2011; Hundesa *et al*., 2006). There were no significant differences in water pH among sampling sites located in the Bay. In contrast there were clear seasonal differences in water turbidity and salinity with a possible correlation between virus presences as demonstrated in beach by previous studies (Wong *et al*., 2009; Victoria *et al*., 2010). These parameters, in addition to temperature and other environmental components, such as pH, proteases and RNAses, have been described as factors that can influence the resistance and inactivation of viruses in aquatic environments (Carter, 2005; Fong and Lipp, 2005 a). Sediments represented as reservoir of virus than the overlying seawater and resuspension of contaminated sediments in recreational may release significant numbers of enteric viruses to the water body (Bosch *et al*., 2005). Our results showed a higher incidence of rotavirus and norovirus in sediments than in shellfish. The site of collected sediments was different than the one of shellfish thus explain the discordance of results between them. The contamination with rotavirus and norovirus were observed in both sediments and seawater collected in same sites. The ability of mussels in accumulating and retaining different species of enteric microorganisms confirmed their utility as bio-monitor organisms enables testing for viral marine pollution (De Donno *et al*., 2012). In home or restaurant cooked mussels continue to feature occasionally in the disease statistics as a cause of outbreaks of gastroenteritis (lees, 2000) and rotavirus was detected with few outbreak (Le guyader *et al*., 2008).

Conclusion

The spread of viral diseases in recreational water and shellfish revealed the importance of assessing the sources of environmental contamination. Because recreational waters are not subjected to any treatment and are considered suitable for swimming at certain bacterial levels, the presence of viruses presents a potential burden to public health that cannot be disregarded. Exposure to contaminated bathing water and ingestion of contaminated shellfish is a primary public health debate and need to include virological parameters for water and shellfish quality control to reduce the potential exposure of users.

Conflict of interest disclosure

All authors declare that there is any financial or otherwise conflict of interest.

Acknowledgements

The authors thank Dr. Cohen Nozha and Dr. Nader Hassan (Division of Microbiology and Product Safety, Food and Environment) and Dr Nourlil (Laboratory of medical virology) for supplying us with samples.

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