Detection and phylogenetic analysis of hepatitis A virus and norovirus in marine recreational waters of Mexico

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ABSTRACT

An investigation was conducted to determine hepatitis A virus (HAV) and norovirus (NV) presence in marine recreational waters (MRWs) from two Mexican tourists beaches (Altata and Mazatlan), located at the northwestern state of Sinaloa, Mexico. Also, Binary Logistic Regression (BLR) analyses were conducted between physicochemical parameters (temperature, turbidity and salinity) and viral organisms (HAV and NV). A total of 32 MRWs samples were collected from April to July of 2006. Samples were processed according to the Environmental Protection Agency (EPA) adsorption-elution method. Overall, 18 MRWs samples (56.3%) were positive for HAV and NV; 4 (22.2%) were obtained from Altata and 14 (77.8%) from Mazatlan. HAV was detected in 3 MRWs samples (9.4%) and NV in 15 samples (46.8%). Phylogenetic analysis showed the presence of genotype I sub genotype B for HAV and NV genogroup II. BLR analysis showed significant correlations between NV and physicochemical parameters (temperature, turbidity and salinity) (p=0.017, $p = 0.08$, $p = 0.048$, respectively). No significant correlation between physicochemical parameters and HAV was observed. The results indicated that MRW quality of Sinaloa beaches is affected by human faecal pollution. Viral surveillance programs should be implemented to minimize health risks to bathers.

Key words | adsorption-elution method, hepatitis A virus, marine recreational waters, norovirus, public health risk

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INTRODUCTION

Fast-growing population, agriculture expansion and coastal development have increased wastewater discharge to the ocean, impacting marine recreational waters (MRWs) quality (Griffin *et al.* 1999). MRWs harbouring enteric viruses represent a public health risk (WHO 2003; SEMARNAT 2005), due to accidental water ingestion during recreational activities (Griffin *et al.* 2003; Bofill *et al.* 2005). Epidemiological studies have shown positive correlation between contaminated marine water with Enteroviruses (Adenoviruses, Enteroviruses, reoviruses, coxsackieviruses, Norwalk viruses, Rotavirus and hepatitis A virus) and health risks to bathers (Griffin *et al.* 2003).

NV and HAV are commonly transmitted by faecally contaminated water (Parshionikar *et al.* 2003; Kittigul *et al.* 2006). NV causes the most frequent viral waterborne gastroenteritis worldwide (Vinjé *et al.* 2004). NV is divided into five genogroups (GGI, GGII, GGIII, GGIV and GGV), which consist of great genetic genotype diversity within each genogroup (Lodder & De Roda 2005). Human-related NV is genogroup I, II and IV (Vinjé *et al.* 2004). On the other hand, HAV is distributed worldwide (Kittigul *et al.* 2006) and Mexico is among the countries with a high incidence (CDC 2006). HAV has a high degree of genome conservation; seven HAV genotypes have been identified,

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four (I, II, III and VII) of human origin and the rest associated with simian species (Nainan *et al.* 2006). Studies have shown that a strains adaptation to susceptible individuals is possible while some others are well adapted to environmental settings (Van den Berg *et al.* 2005).

Large volumes of water are required to concentrate for virus detection. Adsorption-elution is the most used method (Schwab *et al.* 1995) as recommended by the Environmental Protection Agency (EPA). Cell culture and immunoassays methods are not sensitive enough for detecting all the viruses which may be present (Griffin *et al.* 2003) and its use is limited to certain groups of viruses. Polymerase chain reaction coupled to efficient concentration methods allows rapid and sensitive detection of enteric viruses in water (Huang *et al.* 2000).

Information about the presence of enteric viruses in MRWs is scarce in South America. Major efforts to improve the microbiological quality of MRWs are being made by many countries (Moce *et al.* 2005). The EPA, the European Economic Committee (EEC) and the World Health Organization (WHO) safe recreational waters guidelines use the *Enterococci* quantification as a microbial water quality index (WHO 2003). However, conclusive data remarks negative correlation between *Enterococci* and viral pathogens (APHA 1999; Griffin *et al.* 1999; Jiang *et al.* 2001; Harwood *et al.* 2005; Moce *et al.* 2005; Vantarakis *et al.* 2005). Viruses are known to persist for a longer time in MRWs (Allwood *et al.* 2003).

Since 2003, the Mexican health authorities have assessed seawater recreational quality by a monthly *Enterococci* surveillance program in main beach tourist destinations. The maximum permissible limit for *Enterococci* is 100 NMP/100 ml. However, surveillance of enteric viruses is not part of the program, thus its incidence is unknown. Therefore, the objective of the study was to evaluate HAV and NV presence and to relate with physicochemical parameters (temperature, turbidity and salinity) by Binary Logistic Regression (BRL) analysis.

MATERIALS AND METHODS

Viruses

Norovirus stool samples and HAV (ATCC-VR-1402) positive controls were kindly provided by Dr Charles P. Gerba (University of Arizona).

Study area and water sample collection

A total of 32 MRWs samples (250 l each) were collected from Altata Bay (12) and Mazatlan (20). Samples from Altata Bay were obtained between April and July of 2006, while samples from Mazatlan were collected in November 2005 and from April to July of 2006. Two sampling sites were selected from Altata Bay: site A (24° 37′ 47.11″ north latitude, 107° 55′ 45.04″ west longitude) and site B (24° 38′ 13.33″ north latitude, 107° 56′ 5.68″ west longitude) (Figure 1). These sites are constantly impacted by human and agricultural activities and wastewater discharge from at least 15 agricultural drains. Also, the Culiacan River flows towards the bay's interior, adopting estuarine characteristics. Three sites were selected in Mazatlan: site A (23° 14′ 35.42″ north latitude, 106° 27′ 15.68″ west longitude in Playa Gaviotas), site B (23° 12′ 40.56″ north latitude, 106° 25′ 18.67″ west longitude in Playa Norte) and site C (23° 11′ 4.33″ north latitude, 106° 25′ 30.33″ west longitude) (Figure 2). Contamination of Mazatlan beaches is generated by tourist activity, coastal population, and pluvial and wastewater discharge. Also, Site C is next to the Mazatlan sewage treatment plant (Figure 2).

Physicochemical parameters

Water temperature, pH, turbidity and salinity were measured at each sampling site. Water was collected 15 m

Figure 1 | Sampling sites from Altata Bay.

Figure 2 | Sampling sites from Mazatlan.

from the coastline at 30 cm depth. Temperature (degrees Celsius) and pH were measured immediately after arriving at the laboratory with portable thermometer and pH devices (Waterproof Double Junction pHTestr 3 + ; Oakton Instruments, USA). Turbidity (NTU: Nephelometric Turbidity Units) and salinity (ppt: parts per thousand) were measured at the laboratory with a turbidimeter (2100P Portable Turbidimeter; HACH Company, USA) and salinometer (HI 98203 Salintest; Hanna Instruments, USA), respectively.

Filtration, elution and concentration

MRWs samples were concentrated by the EPA adsorption-elution method (EPA 1984, 1995, 2001) and *Standard Methods for the Examination of Water and Wastewater* (APHA 1999). Water samples were pH adjusted to 3.5 ± 0.1 with 6 N HCl before filtration. A negatively charged filter cartridge (DFN 0.45-10UN; Filterite; Pall Corporation) with 0.45 μm pore size was used to perform virus adsorption from water samples. Flow rate was adjusted to 19 l/min with a pump system (Honda, WX10). Samples were stored at 4°C until further processing. Virus elution was performed by adding 3% beef extract (Bioxon) solution (pH 9.0 ± 0.1) to the filter and soaking for 10 min. The eluent was washed off the filter with a positive pressure (0.4 kg/cm^2). The elution step was repeated once (Huang *et al.* 2000). Eluates were subsequently concentrated by organic flocculation and the pH was adjusted (3.5 ± 0.1, 1 N HCl), then they were stirred for 30 min and centrifuged for 20 min at 2,450 × *g*. The precipitate was dissolved with 30 ml of 0.15 M Na_2PO_4 (Fermont) (pH 9.0–9.5) to a final pH of 7.0–7.5. Concentrated samples were stored at −20°C until RNA extraction.

Extraction of viral RNA

Concentrated samples were subjected to RNA extraction using QIAmp viral RNA mini kit (Qiagen, USA). The extracted RNA was used directly for RT-PCR analysis.

Reverse transcription polymerase chain reaction (RT-PCR)

The NV and HAV primer pair selected in this study were the same as previously described by Griffin *et al.* (1999) and Vinjé *et al.* (2004), respectively (Table 1). RT-PCRs were sequentially carried out in one reaction tube with a Qiagen OneStep RT-PCR kit (Qiagen) for NV and Access RT-PCR System (Promega, USA) for HAV. RT-PCR for NV was performed in 50-μl volume, containing 29.75 μl of sample RNA, 1 × buffer 5 ×, 400 μM (each) deoxynucleoside triphosphate, 2 μl of enzyme mix, 10 U RNase inhibitor and 0.6 μM of each primer. The Thermocycler program consisted in one cycle of reverse transcription for 30 min at 50°C, followed by 15 min at 95°C; 40 cycles of 1 min at 94°C, 1 min at 45°C, extension for 1 min at 72°C and final extension for 10 min at 72°C. RT-PCR for HAV was performed in 50-μl volume, containing 34.25 μl of sample RNA, 1 × buffer 5 ×, 200 μM (each) deoxynucleoside triphosphate, 0.5 U of reverse-transcriptase enzyme AMV, 0.5 U Polymerase Tfl DNA, 1 mM $MgSO_4$, 10 U RNase inhibitor and 1 μM of each primer. Viral RNA was reverse transcribed for 45 min at 45°C, followed by 2 min at 94°C to activate DNA taq polymerase; 40 cycles of 0.5 min at 94°C, 1 min at 60°C, extension for 2 min at 68°C and a final extension for 7 min at 68°C. Negative control was composed of RT-PCR mixture and nuclease-free water. Amplified products were analysed by electrophoresis on 1% agarose

Table 1 | Oligonucleotide sequences used for detection of norovirus and hepatitis A virus

Virus	Primer	Sequence (5′–3′)	Amplicon size and target	References
NV	MJV12	TAYCAYTATGATGCHGAYTA	327 bp Region A	Vinjé *et al.* (2004)
	REGA	CTCRTCATCICCATARAAIGA		
HAV	HEPA U	CAGCACATCAGAAAGGTGAG	192 bp VP1 and VP2 capsid protein interphase	Griffin *et al.* (1999)
	HEPA D	CTCCAGAATCATCTCCAAC		

gel stained with ethidium bromide and visualized by UV light with a transiluminator (Spectroline). A positive reaction control and a negative reaction control (ultra-pure water) were included with each series of tests. All standard precautions as well as strict good laboratory practices were followed in order to prevent any PCR cross contamination. The pre-PCR manipulations (RNA isolation and PCR setup) were performed in a clean room that was physically isolated from the post-PCR processing area; dedicated pipettes and reagents were used for each location.

Cloning and sequencing of RT-PCR products

Three and two positive samples for NV and HAV, respectively, were cloned prior to sequencing. RT-PCR products were cloned and transformed using TOPO TA Cloning for sequencing kit (Invitrogen, USA) according to the manufacturer's instructions; after transformation at least ten positive colonies were selected and cultured for 24 h at 37°C in LB medium, containing 50 μg/ml ampicillin. Plasmids were isolated using the Wizard Plus SV Minipreps DNA Purification System (Promega), eluted in 100 μl of water, quantified by absorbance with a biophotometer (Biophotometer 6131; Eppendorf, USA) and sent to the molecular biology department of the Cellular Physiology Institute, National Autonomous University of Mexico (UNAM) for sequencing analysis.

Phylogenetic analysis

Nucleotide sequences were BLAST searched and strain similarities were identified based on GenBank database entries. Multiple sequence alignments were generated using the BioEdit program. Distances were calculated by the Jukes–Cantor method and neighbour-joining trees were constructed using MEGA version 3.1 as previously described by Kumar *et al.* (2004).

Nucleotide sequence accession numbers

The sequences reported in this study have been deposited into GenBank under the following accession numbers: EU884429 to EU884433.

Statistical analysis

A Binary Logistic Regression model was used to determine if temperature, turbidity and salinity predict the probability of NV and HAV occurrence in marine waters. The model used was:

$$P(Y=1)=\frac{1}{1+e^{-(\beta_0+\beta_1 x_1+\beta_2 x_2+\cdots+\beta_k x_k)}}$$

where Y is the dependent variable (viruses), $P(Y=1)$ is the predicted probability that the dependent variable is a positive response, β_0 through β_k are the k regression coefficients and x_1 through x_k are the independent variables (environmental parameters). Viruses were treated as a binary variables; a score of 0 or 1 was assigned when viruses were absent or present, respectively. The 92% confidence level was estimated.

RESULTS AND DISCUSSION

Presence of HAV and NV in MRWs

A total of 32 MRWs samples were collected during the course of the study, 12 from Altata and 20 from Mazatlan. RT-PCR virus data were reported as presence/absence. A total of 18/32 (56.3%) samples were positive. Both viruses were detected in 6.25% of the samples. Four (22.2%)

positive samples were detected in Altata and 14 (77.8%) were detected in Mazatlan. NV was detected in 15/32 (46.9%) MRWs, from which 4/32 (12.5%) were detected in Altata and 11/32 (34.4%) in Mazatlan. NV was more frequently isolated in site B (Altata and Mazatlan). Both beaches are constantly impacted by non-treated agricultural and domestic wastewater. HAV was detected in 3/32 (9.4%) MRWs samples from Mazatlan in sites A and B. No MRWs samples were positive for HAV in Altata beach. Both viruses were more frequently detected in the spring (Table 2). Previous studies performed worldwide have reported the presence of Enterovirus, poliovirus, reovirus, Adenovirus, HAV and Norwalk viruses in MRWs (Muscillo *et al.* 1999; Aulicino *et al.* 2000; Pianetti *et al.* 2000; Pina 2001; Katayama *et al.* 2002; Vantarakis *et al.* 2005). Presence of pathogenic viruses in MRWs poses a potential public health risk. Diverse epidemiological studies have demonstrated that viral infections can be acquired while bathing in contaminated water (Pina 2001; Griffin *et al.* 2003). Since 2003, the Mexican government established an *Enterococci* surveillance program to assess water quality in main tourist destinations. During the present study the level of *Enterococci* reported by Mexican health authorities never exceeded the maximum permissible limits (100 NMP/100 ml).

The present study demonstrated, for the first time, HAV and NV presence in MRWs of the northwestern state of Sinaloa. NV was more frequently detected than HAV in MRWs samples. In a previous study Enterovirus (79%), HAV (63%) and NV (10%) were detected in coastal water samples with similar temperature and salinity conditions (29–33°C and 27–34 ppt) as the ones described in the present study (25–38°C and 23–35 ppt) (Griffin *et al.* 1999). In Japan, Katayama *et al.* (2002) analysed seawater samples from two beaches showing NV presence and positively correlated with contaminated water coming from river and wastewater discharge from urban population.

Table 2 | Occurrence of viruses in marine recreational water

Sampling site	Season	No. of positive samples NV*	HAV†
Altata Bay	Spring	4	0
	Summer	0	0
Mazatlan	Autumn	2	2
	Spring	5	0
	Summer	4	1
Total		15	3

*NV, norovirus.
†HAV, hepatitis A virus.

Different factors may influence viral presence in Altata and Mazatlan beaches. Altata receives wastewater discharge from at least 15 agricultural drains. Also, the Culiacan River flows towards the bay's interior, adopting estuarine characteristics. Mazatlan beaches are also polluted by tourist activities, pluvial discharge, coastal population and a sewage treatment plant, which discharges directly into the ocean.

Phylogenetic analysis of NV and HAV from MRW

Three (NV1, NV19, NV20) of the fifteen positive NV samples were sequenced. Nucleotide sequences were compared (327 bp) with sequences previously reported at the GenBank and showed association with NV. The isolates in the present study were similar (69.1–81.8% identity) to the strains Hawai, Lordsdale, Melksham, Bristol, Arg320 and Mexico (Accession numbers: U07611, X86557, X81879, X76716, AF190817 and U22498, respectively) isolated in the United States, the United Kingdom, Argentina and Mexico, respectively (Figure 3), indicating that NV strains detected in MRW samples were closely related to genogroup II (Figure 3). The strains did not show strong identity with representative genotype strains, which can indicate the probability of finding new genotypes. Nucleotide diversity among these amplicons exceeded in 10% of samples. Approximately 40 to 50% diversity was noted between some animal calicivirus strains (Jena and Murine Calicivirus) and our strains. A phylogenetic tree constructed by the neighbour-joining method is shown in Figure 3.

Positive NV in MRWs samples belong to genogroup II, hence it is important to consider the health risk to bathers (I, II and IV) (Vinjé *et al.* 2004). Furthermore, studies have reported that NV strains from genogroup II are widely distributed and have been associated with recent outbreaks (Parshionikar *et al.* 2003). Nishida *et al.* (2003) analysed oysters from a Japanese beach, detecting NV strains classified within genogroups I and II. Lodder & De Roda (2005) detected F-specific and somatic phages, Rotavirus,

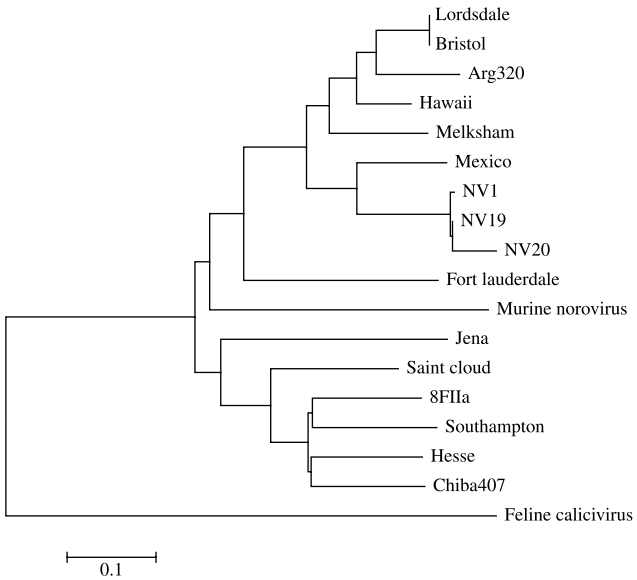

Figure 3 | Phylogenetic tree constructed based on a portion of the amplified region of RNA polymerase gene of 3 NV isolates (N1, N19, N20) and 15 reference strains available at the GenBank. Distances were calculated by the Jukes-Cantor method and the tree was plotted by using the neighbour-joining method. GenBank accession numbers for the reference strains were: Hu/NLV/Norwalk/8FIIa/1968/JP, M87661; Hu/NLV/Southampton/1991/UK, L07418; Hu/NLV/Hesse/1997/DE, AF093797; Hu/NLV/Chiba407/1987/JP, AB022679; Hu/NLV/Hawaii/1971/US, U07611; Hu/NLV/Lordsdale/1993/UK, X86557; Hu/NLV/Melksham/1968/UK, X81879; Hu/NLV/Bristol/1993/UK, X76716; Hu/NLV/Mexico/1989/MX, U22498; Hu/NLV/Arg320/1995/AR, AF190817; Hu/NLV/Fort Lauderdale/560/1998/US, AF414426; Hu/NLV/SaintCloud/624/1998/US, AF414427; Jena (bovine enteric calicivirus), AJ11099; Murine Calicivirus, AR862950.

reovirus, Enterovirus and NV genogroup II strain in samples from river water and treated and untreated wastewater in the Netherlands. Results from the present study showed a greater identity with the Mexican strain (81.8% with NV1 and NV19 and 75.7% with NV20). The Mexico strain was isolated from the faeces of Mexican children (Jiang *et al.* 1995).

In order to characterize HAV strains detected in MRWs samples, two of three positive samples (HAV1 and HAV2) were sequenced at the VP1-VP2 capsid protein interphase. Comparative analysis with other isolates sequences previously reported at the GenBank indicates the presence of HAV strains in MRW samples. The isolated HAV strains were similar to strains MBB, LA1 and HAF-203 (accession numbers: M20273, AF314208 and AF268396, respectively) from the United States, China and Brazil, respectively. These strains belong to genotype I, sub genotype B with 94.2–99.4%. This sub genotype is one of the most infective to humans (I, II, III and VII) (Costa *et al.* 2003). Diverse studies have shown that the IA sub genotype is the most predominant genotype worldwide (Villar *et al.* 2006), including in Mexico (Nainan *et al.* 2006). However, the present study demonstrated the occurrence of genotype I and sub genotype B, which coincide with Hernandez Morga *et al.* (2009).

Nucleotide diversity among these amplicons was 2%. Sequences comparison of HAV isolates from MRWs samples (HAV1 and HAV2) and HM-175/18f (ATCC VR-1402) control strain showed identities of 99.4% and 98.9%, respectively. The constructed phylogenetic tree is shown in Figure 4.

Worldwide, the most abundant HAV genotypes are I and III (Nainan *et al.* 2006). HAV strains isolated from MRWs samples (HAV1 and HAV2) were compared with control strain HM-175/18f (ATCC VR-1402) and showed

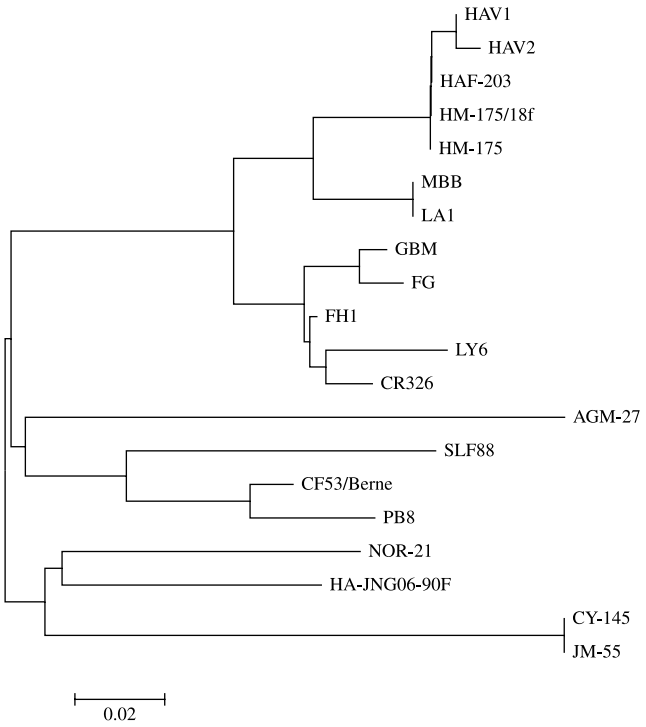

Figure 4 | Phylogenetic tree constructed based on VP1-VP2 capsid protein interphase of 2 HAV (HAV1, HAV2) isolates and 18 reference strains available in GenBank. Distances were calculated by the Jukes-Cantor method and the tree was plotted by using the neighbour-joining method. Reference strains and GenBank accession numbers were as follows: FH1, AB020567; LY6, AF485328; GBM, X75215; CR-326, M10033; FG, X83302; HM-175, M14707; HM-175/18f, M59808; MBB, M20273; LA1, AF314208; HAF-203, AF268396; CF53/Berne, AY644676; PB8, DQ141214; NOR-20, AJ299463; HA-JNG06-90F, AB258387; CY-145, M59286; AGM-27, D00924; SLF88, AY644670.

identities of 99.4 and 98.9%, respectively. Comparison of nucleotide sequences from different HAV strains allowed determination of viral geographic origin. HAV strains analysed came from different countries: Australia, the United States, China, Brazil, Peru, North Africa, Europe and Japan (Costa *et al.* 2003).

Predictive relationship between physicochemical parameters and viruses

Binary Logistic Regression (BLR) was used to estimate the risk of presence/absence of NV and HAV in MRWs under different conditions of temperature, turbidity and salinity. Data obtained on enteric viruses were converted to binary data and the relationship between physicochemical parameters and presence/absence of each pathogen was assessed. The range of physicochemical parameters from study sites are summarized in Table 3. During spring, water temperature ranged from 25 to 31°C in Altata beach and 25 to 29°C in Mazatlan, while in summer the range was from 32 to 38°C and 30 to 36°C in Altata beach and Mazatlan, respectively. Turbidity values ranged from 1.46–5.00 in Altata beach (mean turbidity = 3.27 NTU) and 1.56–5.10 in Mazatlan (mean turbidity = 2.44 NTU). Salinity was similar in Altata beach (mean salinity = 30.65 ppt) and in Mazatlan (mean salinity = 30.45 ppt). The pH values ranged from 7.92 to 8.18 in Altata beach and 7.91 to 8.20 in Mazatlan; pH values were not selected for risk prediction due to low variability.

BLR analysis was used to evaluate the predictive capability of physicochemical parameters in determining NV and HAV presence/absence. Analysis of results showed that the presence of NV was correlated with temperature (OR = 0.66; $p = 0.02$), turbidity (OR = 0.41; $p = 0.08$) and salinity (OR = 0.64; $p = 0.05$). There was no correlation between HAV and physicochemical parameters. An increase of one unit in temperature, turbidity and salinity diminishes NV risk presence in seawater. These results are illustrated in Table 4.

NV occurrence

Estimated logistic regression parameters for temperature, turbidity and salinity were $\beta_1 = -0.42$, $\beta_2 = -0.88$, $\beta_3 = -0.44$, respectively. p-values for temperature, turbidity and salinity were 0.017, 0.080 and 0.048, with an odds ratio of 0.66, 0.41 and 0.64, respectively. There was a significant relationship between physicochemical parameters and NV presence in MRWs. LRA analysis showed that as temperature, turbidity and salinity increase the NV risk presence in water diminishes. The viral particles stability is likely to be affected by temperature within seasons, days and even hours (Pina 2001). The present study showed NV presence in samples collected during autumn, spring and summer. HAV was detected in samples collected during autumn and summer. Samples collected in the spring showed a higher frequency of NV, followed by summer and autumn. This behaviour may be attributed to water temperature, since low temperatures favour the occurrence and stability of viruses. Water temperatures in the autumn were between 25 and 26.5°C, in the spring between 25 and 31°C and in the summer between 30 and 38°C. Studies conducted in eight countries showed NV presence throughout the year. Studies conducted in the United Kingdom and Australia reported

Table 3 | Range of physicochemical parameters from Altata Bay and Mazatlan

		Physicochemical parameters			
Beach	**Site**	**Temperature (°C)†**	**Turbidity (NTU)‡**	**Salinity (ppt)§**	**pH**
Altata Bay	A	26–38 (29.86)	1.46–8.00 (3.21)	27.50–33.50 (30.52)	7.92–8.11 (8.02)
	B	25–38 (29.36)	2.20–5.00 (3.00)	23.00–35.00 (30.79)	7.98–8.18 (8.09)
Mazatlan	A	25–31 (27.87)	1.56–3.89 (2.41)	25.00–33.50 (31.19)	8.06–8.20 (8.13)
	B	26–31 (28.71)	1.10–5.00 (2.40)	25.00–35.00 (30.21)	7.94–8.10 (8.02)
	C	26–36 (30.17)	1.46–5.10 (2.53)	28.00–32.50 (29.87)	7.91–8.12 (8.03)

†°C, centigrade.
‡NTU, nefelometric units.
§Parts per thousand.

Table 4 | Logistic regression analysis describing correlation between enteric viruses and physicochemical parameters

Enteric viruses	Physicochemical parameters	Logistic regression parameter RC*,†	OR‡,†	P-value†
NV	T§	−0.42	0.17	0.02
	Tr‖	−0.88	0.51	0.08
	S¶	−0.44	0.22	0.05
HAV	T	−0.19	0.20	0.34
	Tr	0.02	0.47	0.96
	S	−0.32	0.23	0.17

*RC, regression coefficient; †OR, odds ratio; ‡$p < 0.08$; §T, temperature; ‖Tr, turbidity; ¶S, salinity.

NV occurrence in the spring and summer (Mounts *et al.* 2000). As previously described, MRWs temperature plays a critical role in viral infectivity (Lo *et al.* 1976). The occurrence of NV in the water at 25°C is higher than at 38°C. Allwood *et al.* (2003) analysed inoculated potable water with Feline Calicivirus (FCV) at 4, 25 and 37°C, during 28 days. They found that an increment in temperature diminished the time required for 90% viral reduction at 7.3, 5.2 and 2 days, respectively.

Results obtained in the present study stated that NV presence can be greater at temperatures of 25–31°C (autumn and spring) than 30–38°C (summer). Thus, the probability of detecting NV increases as temperature diminishes, remaining stable for longer periods of time. Data obtained in this study coincide with those of Lo *et al.* (1976), who determined that temperature (4, 15, 25, 37°C) and salinity (10, 20, 34 ppt) have an effect in poliovirus 1, echovirus 6 and coxsackievirus B5 survival in seawater, showing that the higher the temperature, the faster the viruses lost infectivity. High temperatures cause protein denaturation of viral capsids and breaking of phosphodiester links of viral nucleic acids, inhibiting the adsorption of viruses in their hosts and inactivating enzymes required for their replication (Bhattacharya *et al.* 2004).

NV occurrence in MRWs was greater at a turbidity of 1.1 NTU than at 8 NTU. Laboratory studies have demonstrated that turbidity in seawater is responsible for inactivation, removal and reduction of viruses in an aquatic environment (Patti *et al.* 1987; Maier *et al.* 2000; Bongiorni *et al.* 2005). Noble & Fuhrman (1997) demonstrated that infectivity of viruses in coastal water was diminished by the presence of heat labile substances (enzymes) and microorganisms. NV was greater at a salinity of 35 ppt than at 23 ppt. It has been reported that solutions with a salinity of 1 M concentration are able to increase protein solubility (Badui 1999). Saline concentration in this study varied from 1.66–2.53 M/l NaCl.

HAV occurrence

Estimated logistic regression parameters for temperature, turbidity and salinity were $\beta_1 = -0.19$, $\beta_2 = -0.02$, $\beta_3 = -0.32$, respectively. p-values for temperature, turbidity and salinity were 0.34, 0.96 and 0.17 with an odds ratio of 0.83, 1.02 and 0.72, respectively. The environmental parameters did not appear to predict the occurrence of HAV. There was no significant relationship between environmental parameters (temperature, turbidity and salinity) and HAV presence in MRWs ($p > 0.08$). The sampling period might have an influence on the lack of predictor capabilities between viruses and physicochemical parameters and sample size was small (32 samples). Vantarakis *et al.* (2005) analysed Enterovirus, Adenovirus and HAV presence in recreational seawater. Statistical analysis showed that physicochemical parameters did not have significant correlation in virus presence.

CONCLUSIONS

This study is the first in Mexico demonstrating the occurrence of NV and HAV in MRWs in Mexico. The physical chemical quality of the marine waters was found to be related to the occurrence of NV, but not HAV. HAV is known to be one of the most stable enteric viruses in the environment and this may account for the lack of relationships observed in this study, as well as possibly the number of samples collected.

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