Environmental surveillance of norovirus and hepatitis A virus in raw oysters at seafood retails in Sinaloa, Mexico: detection of GII.P13 norovirus genotype

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Abstract

Background Norovirus (NoV) and hepatitis A virus (HAV) have emerged as the leading agents of non-bacterial acute gastroenteritis and hepatitis, respectively, primarily associated to food-borne outbreaks followed by the consumption of seafood. In Mexico, little is known about the molecular epidemiology of NoV and HAV, and a few studies have reported their presence in aquatic environments. In Sinaloa, the pleasure oyster (Crassostrea cortiziensis, Hertlein) is one of the main species consumed at seafood retails; however, information about the presence and genetic diversity of NoV and HAV is lacking. The aim of the present study was to investigate the prevalence and molecular diversity of NoV and HAV in raw pleasure oysters expended at seafood retails in Sinaloa, Mexico. Methods A total of 68 samples were collected at several seafood retails at four periods: Period 1 (October-November, 2010); Period 2 (December 2010-January 2011); Period 3 (March-April, 2011); and Period 4 (May, 2011). These oyster samples were tested for the presence of NoV and HAV by retrotranscription (RT)-nested PCR and RT-PCR, respectively. Enumeration of fecal coliforms was also conducted. In addition, an analysis of Binary Logistic Regression (BLR) was performed to determine a possible correlation of these enteric viruses with the presence of fecal coliforms and environmental temperature. Results Overall, NoV was detected in 57.3% of the 68 samples showing the highest occurrence (11/13) during Period 1 (October-November, 2010). No HAV-positive samples were detected. Fecal coliforms were more frequently detected (16/20) on Period 4 (May, 2011). A significant negative correlation between NoV and fecal coliforms was observed. A total of 28 sequences were obtained from NoV amplicons and surprisingly, phylogenetic analysis showed that all NoV sequences obtained from oysters belonged to GII.P13 genotype. Conclusions The results indicated that raw oysters expended at seafood retails are potential sources of human infection due to the presence of NoV, which interestingly were represented only by GII.P13 genotype. This is the first report confirming the presence of GII.P13 in Mexico, which may contribute with the better understanding of NoV genetic diversity and epidemiology.

Background

Norovirus (NoV) and hepatitis A virus (HAV) have emerged as important agents associated to diseases worldwide, representing the leading causes of non-bacterial acute gastroenteritis and hepatitis, respectively. NoV has been associated with outbreaks in closed settings contributing with up to 200,000 deaths per year, primarily in children with the most of deaths in developing countries [1]. In contrast, it has been estimated that 1.5 million of HAV clinical cases occur each year, where high levels of infection are associated to developing countries with poor sanitation and hygienic practices [2].

NoV is a non-enveloped virus member of the family *Caliciviridae*, which is characterized by a single-stranded positive-sense RNA genome ranging from 7.5 to 7.7 kb [3]. The NoV genome is organized in three open reading frames (ORF) in which ORF2 and ORF3 encodes the structural major and minor capsid proteins VP1 and VP2, respectively; whereas ORF1 encodes a nonstructural polyprotein that is processed by the viral protease NS6 resulting in six non-structural proteins including a RNA-dependent RNA polymerase (RdRp) [3]. Of these, VP1 and RdRp genes have been extensively used to design molecular approaches to identify and assess genetic diversity of NoV [4]. Based on DNA sequence from VP1 gene, NV is classified into seven genetic groups or genogroups (GI-GVII) [5], where GIII has only been found in cattle [6], GV in mice [7], and GVI and GVII in dogs [5]. Furthermore, each genogroup is divided into genotypes based on sequence relatedness from VP1 counting with at least 40 genotypes [5], being GII one of the most diverse consisting of 26 genotypes to date [8].

On the other hand, HAV is a single positive-stranded RNA non-enveloped virus of the genus *Hepatovirus* belonging to the family *Picornaviridae* [9]. The HAV genome size is approximately 7.5 kb and encodes a single ORF that is

translated into a polyprotein of approximately 250 kDa, that subsequently is cleaved by the viral-encoded protease into 10 mature viral proteins including four structural proteins VP4, VP2, VP3 and VP1pX, and a RdRp [9]. HAV is classified into six genotypes (I-VI) based on sequence analysis from VP1 where genotypes I-III grouped strains associated to human infection, while genotypes IV-VI infect only simians [10, 11]. Further, each genotype I and III are subdivided into A and B subtypes [9].

NoV and HAV are primarily transmitted via fecal-oral route, by person-to-person contact, exposition to contaminated surfaces or ingestion of contaminated food or water [12, 13] owing to the ability of these viruses to persist in the environment, in particular HAV, which is highly resistant to freezing, low pH, heating and chemical agents [9]. In this regard, food-borne outbreaks caused by NoV and HAV infections have been well documented following consumption of raw, lightly cooked or moderately treated bivalve shellfish [14-16]. One of the largest outbreaks of HAV infection occurred in China in 1988, where almost 300,000 people were infected after consumption of raw clams harvested from a contaminated area [17], while other outbreaks associated to oysters and clams have been described since 1962 in the United States [18]. In the case of NoV, numerous outbreaks have been reported around the world mainly associated to bivalve consumption [16]. Bivalves accumulate large amounts of viral particles due to they are filter feeders, thus contamination of shellfish growing or harvesting areas with human sewage represents a high-risk factor that may favor viral outbreaks and transmission [17, 19].

In Mexico, little is known about the molecular epidemiology of NoV and HAV, and there are only few studies reporting their presence in estuarine waters intended for shellfish production and recreational waters in Sinaloa [20, 21], as well as NoV detection in bell pepper during harvesting and packing [22], underlying a health risk concern, especially regarding the growing and harvesting of seafood. The pleasure oyster (*Crassostrea cortiziensis*, Hertlein)is one of the main species captured for local consumption at seafood retails; however, information about the presence and genetic diversity of NoV and HAV in pleasure oysters is lacking. Hence, the aim of the present study was to investigate the prevalence and molecular diversity of NoV and HAV in raw pleasure oysters expended at seafood retails in Sinaloa, Mexico.

Methods

Sample collection and processing

Sixty-eight pleasure oyster (*C. cortiziensis*) samples were obtained from 24 seafood retails in Culiacan city, Sinaloa, Mexico. Sampling was performed during seven months divided into four periods: Period 1 (October-November, 2010); Period 2 (December 2010-January2011); Period 3 (March-April, 2011); and Period 4 (May, 2011). Sample oysters were previously supplied at retails from six coastal locations: Altata, El Castillo, Las Puentes, Las Arenitas, El Conchal and Cospita, all located at Gulf of California (Supp. Fig. 1). At each retail, a sample of 12 oysters were collected in sterile airtight plastic bags and placed on ice, and then processed within 6 h after collection. All oysters contained in a sample were washed and striped off from their shells to obtain tissue (meat and intravalvular fluid), and then homogenized with a blender. This oyster homogenate was store at -80°C for further molecular and microbiological analysis [23].

Viral precipitation with PEG

Twenty-five grams of homogenized oyster sample was mixed with 175 ml of glycine buffer, pH 9.5 (0.1 M glycine, 0.3 M NaCl), mixed vigorously, and 30 ml were taken and clarified at 15 000 × g at 4°C for 10 min. Viral particles were

precipitated from the supernatant with 1 equal volume of 16% PEG with 0.525 M NaCl on ice for 1 h. Samples were centrifuged 10,000 × g at 4°c for 5 min and pellet was resuspended in 3 ml of nanopure water [23].

RNA extraction and RT-PCR

From precipitated viral particles, 140 µl were used for viral RNA extraction using RNA QIAamp viral RNA Mini Kit (QIAGEN) according to manufacturer's instructions. Retrotranscription was carried out using the kit Access RT-PCR System (Promega, USA) using specific primers for NoV and HAV identification (Table 1). For NoV, primers MJV12 and RegA were used to generate a 327 bp fragment for region A (RdRp, ORF1) of norovirus genome [4]; whereas for HAV, primers HEPA U and HEPA D were used to amplify a 192 bp fragment corresponding to VP1 and VP2 capsid protein interphase [24]. RT-PCR reactions were performed in 50 µl volume, containing 5 µl of RNA (NoV or HAV), 10 µl AMV/ *Tfl* 5X reaction buffer, 0.2 mM each deoxynucleoside triphosphate, 1 µM each primer, 1 mM $MgSO_4$, 5 U AVM retro-transcriptase and 5 U *Tfl* DNA polymerase. Each RT-PCR included positive and negative controls, where as positive control were used NoV particles isolated from fecal samples and the strain ATCC HM 175/18F for HAV, while negative control was ultrapure water. RT-PCR conditions for NoV were as follows: retro-transcription 30 min at 50°C, followed by 15 min at 95°C; 40 cycles consisting of denaturation 1 min at 94°C, annealing 1 min at 45°C and extension 1 min at 72°C; and a final extension of 10 min at 72°C. RT-PCR for HAV was as follows: retro-transcription 45 min at 45°C, followed by 2 min at 94°C; 40 cycles consisting of denaturation 0.5 min at 94°C, annealing 1 min at 60°C and extension 2 min at 68°C; and a final extension of 10 min at 68°C. Afterward, semi-nested PCR for NoV positive samples was performed with the GoTaq PCR Core Systems (Promega, USA) using the primers RegA and MP290 to amplify a 316 bp fragment [22], in a 50 µl volume containing 1 µl of NoV RT-PCR reaction, 10 µl of 5X GoTaq Flexi buffer, 0.2 mM each deoxynucleoside triphosphate, 1 µM each primer, 1.5 mM $MgSO_4$ and 1.25 U Taq polymerase. The amplification conditions were: initial denaturation 2 min at 95°C, 40 cycles consisting of denaturation 0.5 min at 95°C, annealing 1 min at 49°C and extension 2 min at 72°C; and a final extension of 5 min at 72°C. Amplified products were run by electrophoresis on 1% TAE agarose gel, stained with ethidium bromide and visualized with a transilluminator (Spectroline).

PCR product purification and sequencing

Semi-nested PCR products were cut from agarose gels with a knife and purified using the kit Wizard SV Gel and PCR Clean-up (Promega, USA). DNA integrity was verified with a 1% agarose gel. Purified PCR products were sequenced at the Genomic Services Unit in LANGEBIO, CINVESTAV, Guanajuato, Mexico.

Nucleotide sequence accession numbers

Twenty-eight DNA sequences of NoV were obtained, submitted to GenBank database and assigned accession numbers from MK483950 to MK483977.

Phylogenetic analysis

DNA sequences were aligned with other RdRp sequences and NoV reference strains reported at GenBank using ClustalW included in MEGA 7 software [25]. The following GenBank sequences for NoV were used for alignments:

Hu/GII/Sinaloa/Mexico/M1/2007 (EU169429.1), NVO5 (EU056666.1), Hu/GII/Sinaloa/Mexico/NV24/2007 (EU884431.1), Hu/GII/Sinaloa/Mexico/NV1/2006 (EU884432.1), Hu/GII/Sinaloa/Mexico/NV20/2006 (EU884433.1), Norwalk virus (M87661.2), SRSV-KY-89/89/J (L23828.1), Hawaii calicivirus (U07611.2), Melksham (X81879.1), Minireovirus TV24 Toronto (U02030.1), Human calicivirus MX (U22498.1), SRSV-OTH-25/89/J (L23830.1), Lordsdale (X86557.1), NLV/MOH/99 (AF397156.1), Sw/NLV/Sw918/1997/JP (AB074893.1), Hu/Sommieres1203/2006/France (EF529742.1), Hu/GII.P13/6008/2010/ZAF (KC962454.1), Hu/GII.17/Wuhan/Z776/CHN/2007 (JQ751044.1), Hu/Briancon870/2004/France (EF529741.1), Hu/GII/JP/2013/GII.P17_GII.17/Saitama5309 (LC043168.1). Then, a phylogenetic tree was constructed using the Neighbor-Joining method and Kimura-2 parameter model from MEGA 7, with bootstrap analysis from 1,000 replicas, and a pairwise distance matrix was also computed using the alignment.

Fecal coliform analysis

A sample of 25 g of homogenized oyster tissue was mixed with 225 ml of PBS and homogenized with a blender. From this sample, serial dilutions of 1:100, 1:1000 and 1:10000 in PBS were performed. Subsequently, 100 µl of each dilution were inoculated in Petri dishes containing mFC broth (BD Difco, USA) by duplicated, and incubated at 45°C for 24 h. After incubation, coliform colonies were counted using a colony counter (SOL-BAT Q-20, Mexico) and results were expressed as CFU or $\log_{10}$CFU g^{-1} oyster [26].

Statistical analysis

Correlation analysis between virus and fecal coliforms presence and environmental temperature at moment of sampling was conducted with a Binary Logistic Regression (BLR) analysis in Minitab 14 software. Virus was considered as binary variables where 0 and 1 was assigned when viruses were absent or present, respectively.

Results

Presence of enteric virus and fecal coliforms in raw oysters

During the overall study period (6 months), a total of 39/68 (57.3%) oyster samples amplified region A of RdRp indicating the presence of NoV, while no HAV genetic material was detected in the raw oyster samples analyzed. Interestingly, NoV was more frequently detected from October to November, 2010 (Period 1) showing 84.6% of NoV positive samples (Table 2). Moreover, analysis of samples by month revealed that NoV was present in all sampling months, showing higher percentages (superior of 60%) of positive samples from October to March, except for December where only 33% of samples were positive for NoV (Fig. 1). Fecal coliforms as a bacterial indicator of fecal pollution were enumerated and were present in 50% (34/68) of the total oyster samples; moreover, fecal coliforms were present in a higher percent (80%) on May (Period 4) with a mean $2.5x10^5$ CFU g^{-1} ($\log_{10}$ 5.4), with variations from $2x10^2$ to $2.16x10^6$ CFU g^{-1} ($\log_{10}$ 2.3 to 6.3) (Table 2). Interestingly, higher percentages of NoV positive samples were coincident with lower occurrence of fecal coliforms when they were compared by month of sampling with the exception of March (Fig. 1), or by Periods (see Table 2), as well as when environmental temperature at moment of sampling was lower (Fig. 1). Because of this, BLR was used to estimate the probability of presence/absence of NoV in the presence of fecal coliforms or with environmental temperature. BLR analysis showed a significant negative correlation with *P* values of 0.030 for the fecal coliform presence but no association with environmental temperature

(*P*=0.398) (Table 3). The regression model explained only 5.32% for fecal coliform presence, which suggested that additional factors (physicochemical and biotic) may also influence NoV presence in addition to fecal coliforms.

Phylogenetic analysis of NoV from raw oysters

From 39 NoV positive samples, 28 amplification products of region A from RdRp gene were sequenced and compared with reported NoV sequences. It is worth noting that most of NoV genotyping is based on VP1, but recently, a dual nomenclature including RdRp genotyping has been proposed [27]. So, we conducted a phylogenetic analysis based on RdRp including complete genome reference strains and partial sequences. Surprisingly, phylogenetic analysis showed that all partial RdRp sequences obtained from oysters in the present study were clustered together with high bootstrapping (86%) with members belonging to GII.P13 and GII.17-GII.P13 genotypes (Fig. 2). Interestingly, NoV partial RdRp sequences such as M1, NVO5, NV24, NV1 and NV20, corresponding to previously unclassified RdRp sequences isolated from environmental samples of Sinaloa [20-22], were also grouped within GII.P13 genotype. Moreover, phylogenetic relationships revealed that NoV partial RdRp oyster sequences clustered with members of GII.17 genotype (which is defined by VP1 relatedness), but they were more related to Sommieres1203, ZAF, Briancon and Wuhan strains, members of GII.P13 genotype (classification based on RdRp relatedness) instead GII.17-GII.P17 strains [28]. In addition, pairwise genetic distances were consistent showing that NoV partial RdRp sequences from oysters displayed lower genetic distances ranging from 0-0.005 and 0.005-0.025 when compared with Sinaloa strains (M1, NVO5, NV24, NV1 and NV20) and GII.P13 sequences, respectively, indicating that all were closely related and belonged to the same genogroup (GII.P13); whereas genetic distances between NoV partial RdRp oyster sequences and GII.17-GII.P17 strains ranged from 0.132-0.150, indicating a higher divergence in comparison to GII.P13 genotype. For the other GII genotype members, genetic distances varied from 0.181-0.373 confirming more divergent relationships (Table 4, Supp. Material 1). Phylogenetic analysis and genetic distances strongly support that the NoV partial RdRp oyster sequences belong to the GII.P13 genotype, and to our knowledge, this constitutes the first report confirming the presence of GII.P13 genotype circulating in Northwestern Mexico.

Discussion

Prevalence of NoV and HAV in pleasure oysters

Seawater and bivalves contamination with important foodborne pathogens have been widely documented throughout the world [15, 16, 29], however, information about NoV and HAV prevalence in seafood in Mexico is scarce, thereby this work constitutes one of the first reports addressing this concern. In this regard, we investigated the prevalence of NoV and HAV in pleasure oysters expended at seafood retails in Sinaloa, Mexico, and found that only NoV was present. Our results were agreed with a previous study reporting the presence of NoV and the absence of HAV in seawater from Altata beach, which was one of the oyster harvesting sites that supplied the seafood retails visited in the present study [21]. On the other hand, HAV has been reported Mazatlán beaches, another location from Sinaloa, where NoV was more frequently detected (34.4 %) than HAV (9.4%) [21]. Other studies in Sinaloa have reported the presence of NoV and HAV in estuarine waters of Huizache Caimanero Lagunary Complex intended for shrimp cultivation as well as NoV contamination of bell pepper during harvesting and packing [20, 22]. Unfortunately, information about case-based surveillance on NoV in Mexico is limited and only few studies have been associated to NoV in traveler's diarrhea [30, 31] and clinical studies in children [32, 33]. Regarding that bivalves accumulate virus by filtering surrounding waters, the rational is that contaminated seawater that are commonly used for growing or harvesting oysters can contribute with viruses accumulation; additionally, oysters are generally eaten raw or minimally

cooked, so the presence of pathogenic viruses such as NoV represents a potential public health risk for human infection. Physicochemical parameters such as temperature, turbidity and salinity have been reported to show a negative association with NoV presence in seawater [21, 34] and other studies have shown that NoV infections are more predominant in winter associated to low seawater temperatures [15]. In the present study, despite higher occurrence of NoV was mostly observed when environmental temperature was below 20°C, BLR analysis showed no significant association. This result could be partially explained due to the number of samples analyzed each month; in addition to further studies evaluating the seawater temperature instead environmental temperature are needed to more accurate analysis. Regarding the effect of temperature, it is thought that high temperatures may damage viral particles because of protein viral capsids and nucleic acids are more easily degraded as well as key viral enzymes required for infection are inactivated [35]. Other works have shown that oyster purges more effectively viral particles in warmer water due to heightened metabolism [36, 37]. Other factors such as UV irradiation have been reported that diminishes viral load of murine NoV and other enteric virus by DNA damage induction, although factors such as organic matter, nutrient concentration and presence of other microorganisms also alter viral stability in seawater [38]. On the other hand, BLR was also revealed that the presence of NoV was negatively correlated with the presence of fecal coliforms, consistent with previous works [22, 29, 34], although in another study no correlation was found [20], which highlights that fecal coliforms are an unreliable indicator for viral contamination and the need of tools to monitor water and seafood microbiological quality.

Despite HAV was not detected in oysters in the present study, most of HAV genotypes associated to infections have remained unknown in Mexico, and there are few studies demonstrating the presence of genotype GI subtype IB in seawater in Sinaloa [20, 21]. The genotype I is the most prevalent around the world, although the subtype IA is more common [39]. HAV prevalence is mainly related to socioeconomic conditions such as quality of food and drinking water, sanitation and density of housing [9], in addition to virus ability to survive harsh environmental conditions, which has favored HAV endemism primarily in developing countries [40]. In the case of Mexico, HAV has contributed with the most of viral hepatitis cases (84.2%) posing the country as an intermediate HAV endemicity [41]. HAV vaccination in Mexico has been implemented only in some programs in childcare units as part of the National immunization programs consisting in single-doses of HAV vaccine; however, modeling predictions for implementation of HAV universal childhood vaccination has indicated that 2-dose vaccination could reduce significantly seroprevalence in population [42]. Nonetheless, due to HAV has been detected by a serological approach, information about genetic diversity is very limited.

Phylogenetic analysis and clustering in GII.P13 genotype

Environmental surveillance is an important strategy for monitoring viruses, especially when follow-up studies of clinical cases are rarely conducted. Herein, we obtained several partial RdRp sequences from NoV positive oyster samples and surprisingly, phylogenetic analysis revealed that all of them belonged to GII.P13 strongly suggesting that only one genotype is circulating among bivalves. Moreover, NoV partial RdRp sequences obtained from oysters were genetically related to other partial RdRp sequences previously isolated from Sinaloa, where M1 was obtained from worker hands during harvesting and packing bell pepper [22]; whereas NV1, NV24 and NV20 were isolated from seawater [21], and NV05 from estuarine water [20], indicating that genetically similar strains of NoV are circulating in different environments in Sinaloa. Interestingly, other partial RdRp sequences also reported in bell pepper and aquatic environments were associated to complete genome reference strains belonging to GII.4 variant [20, 22] indicating a possible co-circulation with GII.P13 genotype in Northwestern region. In accordance with previous reports, GII.4 strains are recognized as the predominant genotype causing pandemics around the world [43]. In Mexico, little is known

about the circulating NoV strains. In fact, clinical studies in children revealed the presence of GII.1, GII.2, GII.3, GII.7 and GII.17 genotypes in the country [32, 33], while other studies have frequently reported the Hawaii, Bristol, Lordsdale and Mexico strains [44, 45].

We suspect that partial RdRp sequences obtained from oysters corresponding to RdRp associated to GII.17 (genotype designated by VP1 relatedness) due to genetic distance was lower with GII.17-GII.P13 and GII.17-GII.P17 strains in comparison to other GII genotypes. At the same time, GII.P13 oyster sequences were more related to GII.17-GII.P13 strains based on genetic distance (Table 4). Interestingly, GII.P13 genotype has been only associated to GII.17 strains, in which several GII.P13 strains have been isolated from healthy children and acute gastroenteritis patients from France, China and Cameroon [28, 46, 47]. It is well known that recombination is a driving force in norovirus evolution due to the possibility of recombination events when different NoV strains co-circulates or co-infects a host, favoring the emergence of new recombinant strains [48]. In fact, the ORF1-ORF2 overlap has been demonstrated to be a recombinant hotspot, which may give raise the emergence of novel variants [49]. The detection of GII.P13 NoV genotype in oysters implies a health concern due to GII.17-GII.P13 strains have been isolated from stools of gastroenteritis patients in Europe and Asia [28, 50]. In fact, GII.17 variants have replaced the predominant GII.4 genotypes in geographical areas such as China and Japan [28]. Although GII.P13 genotype has been only associated to GII.17, we cannot dismiss that GII.P13 sequences of the present study could be derived from a distinct VP1 genotype due to recombinational events on the ORF1-ORF2 overlap [28]; however, we cannot evaluate this possibility due to our GII.P13 sequences are lacking of the ORF1-ORF2 overlap, so further studies sequencing this region or complete genomes are needed to evaluate this possibility. With the emergence of novel genotype variants, it has been proposed to reconsider RdRp and VP1 sequences for genotyping, as well as data of clinical symptoms, immune status and blood group of host in order to establish a better study and monitoring of newly emerged virus [27, 28]. So far, this study is the first report confirming the presence of GII.P13 in Northwestern Mexico, highlighting the importance of molecular epidemiology surveillance to understand the distribution, evolution and the emergence of novel genotypes.

Conclusions

The research conducted in the present study showed that raw oysters expended at seafood retails are potential sources of human infection due to the presence of NoV. Interestingly, the results demonstrated the prevalence of GII.P13 genotype which could locally co-circulate with GII.4 strains according with previous reports [22]. Importantly, this study constitutes the first report confirming the presence of GII.P13 genotype in Mexico, providing new insights for the better understanding of NoV genetic diversity and epidemiology.

Abbreviations

BLR, binary logistic regression; FC, fecal coliforms; HAV, hepatitis A virus; NoV, norovirus; ORF, open reading frames; RdRp, RNA-dependent RNA polymerase; RT-PCR, retrotranscription-polymerase chain reaction

Declarations

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Availability of data and materials

Part of analyzed data in the present study is included in this article. Additional data are provided as Supplemental Figure 1 and Supplemental Material 1. All sequences obtained are available in GenBank Database.

Authors´ contributions

CV analyzed the data and wrote de manuscript. LA contributed to phylogenetic analysis and editing of the manuscript. AMC collected and processed oyster samples, and performed RT-PCR and fecal coliform enumeration. BGG provided the oyster samples, made important intellectual contributions and edited the manuscript. JLF conceived and designed the study. All authors read and approved final manuscript.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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Tables

Table 1 Primers used for norovirus (NoV) and hepatitis A virus (HAV) detection in raw oysters.

Virus	Name	Sequence[a]	Size (bp)	References
NoV	MJV12	5’-TAYCAYTATGATGCHGAYTA-3’	327	[4]
	RegA	5’-CTCRTCATCICCATARAAIGA-3’		[4]
	MP290	5’-GAYTACTCYCSITGGGAYTC-3’	316	[22]
HAV	HEPA U	5’-CAGCACATCAGAAAGGTGAG-3’	192	[24]
	HEPA D	5’-CTCCAGAATCATCTCCAAC-3’		[24]

[a]Degenerate primers: Y=C or T; R=A or G; H=A, T or C; I=Inosine; S=C or G.

Table 2 Enterovirus detection and fecal coliform enumeration in raw oysters collected at seafood retails from 2010 to 2011.

Period	Sampling months	Enteric viruses		Fecal coliforms		
		NoV positive/tested (%[a])	HVA positive/tested (%[a])	Positive samples/Total (%[a])	CFU (log_{10}) range g^{-1}	Mean CFU (log_{10}) g^{-1}
1	October-November 2010	11/13 (84.6)	0/13 (0)	2/13 (15.3)	$3.3x10^4$-$1.5x10^5$ (4.52-5.18)	$1.4x10^4$ (4.1)
2	December2010-January 2011	7/11 (63.6)	0/11 (0)	5/11 (45.4)	$6.7x10^3$ -$7x10^4$ (3.83-4.85)	$1.4x10^4$ (4.1)
3	March-April 2011	13/24 (54.1)	0/24 (0)	11/24 (45.8)	$1x10^3$ - $2x10^4$ (3-4.3)	$2.7x10^3$ (3.4)
4	May 2011	8/20 (40)	0/20 (0)	16/20 (80)	$2x10^2$ - $2.16x10^6$ (2.3-6.3)	$2.5x10^5$ (5.4)
Total		**39/68 (57.3)**	**0/68 (0)**	**34/68 (50)**		

[a] Percentage of positive samples.

CFU, colony forming units.

Table 3 Binary logistic regression (BLR) to predict the presence of NoV using fecal coliform presence and environmental temperature as indicators in raw oysters.

Fecal coliforms	Presence	Temperature (°C)
Regression coefficient	-1.112	-0.0700
Constant	0.875	1.87
P-value	0.030	0.398
OR	0.3289	0.9324

OR, Odds ratios

Table 4 Genetic distances at nucleotide sequence level of RdRp sequences obtained in this study with other reported NoV sequences.

RdRp sequences[a]	nt divergence
Sinaloa sequences (M1, NV05, NV24, NV1, NV20)	0-0.005
GII.17-GII.P13	0.005-0.025
GII.17-GII.P17	0.132-0.150
GII[b]	0.181-0.373
GI	0.511-0.531

[a]All strain sequences were included in the phylogenetic analysis of this work.

[b]All GII.17 and Sinaloa sequences were not included

nt, nucleotide.

Additional Files Legend

Supp. Fig. 1 Map of the oyster harvesting sites where oysters were collected and then transported at seafood retails: Altata, El Castillo, Las Puentes, Las Arenitas, El Conchal and Cospita, Sinaloa, México, during 2010-2011.

Supplemental Material 1 Pairwise distance matrix computed from RdRp partial sequences obtained in the present study and reported sequences.

Figures

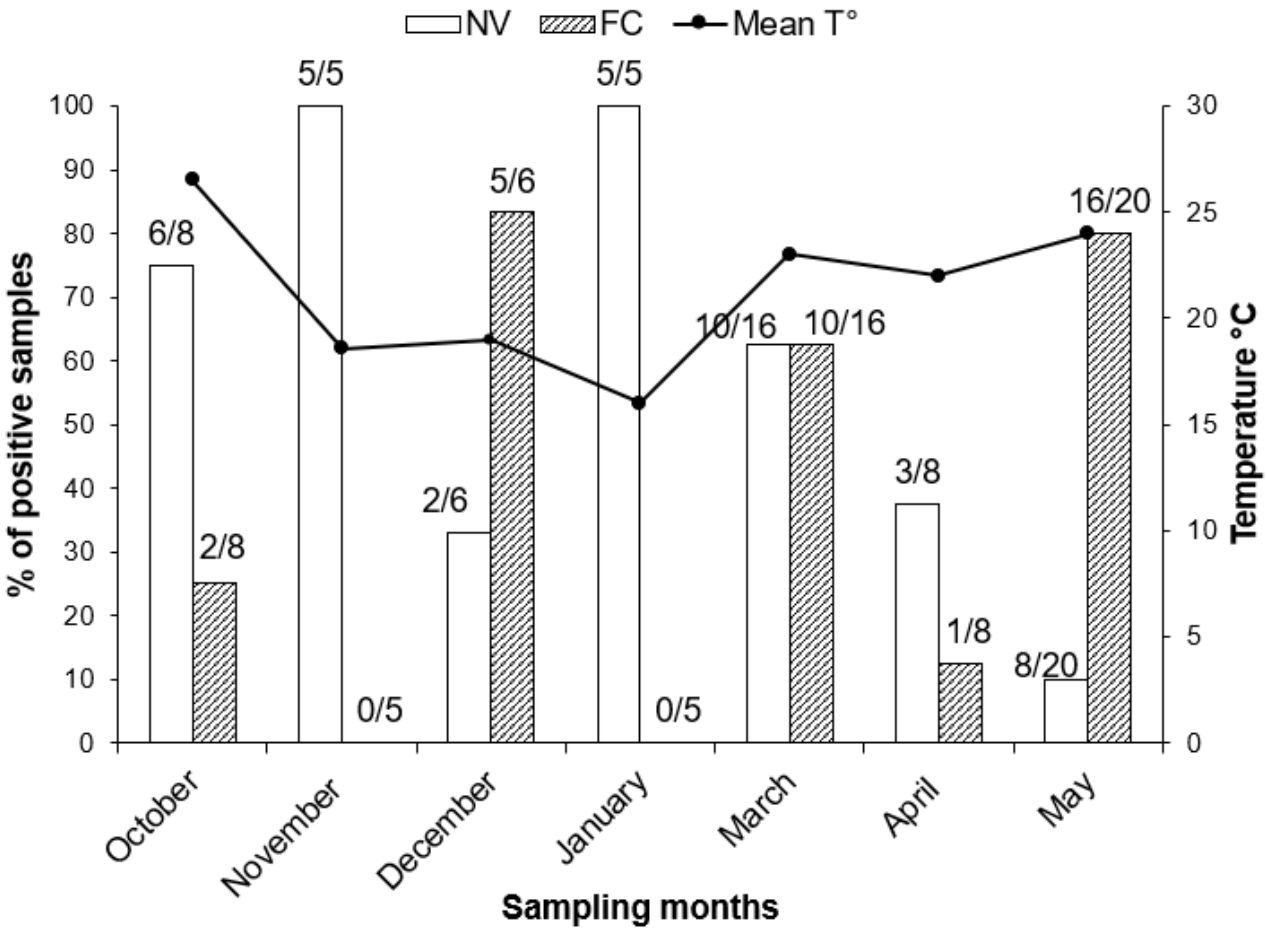

Figure 1

Distribution of percentage of NoV positive oyster samples over seven months of sampling. Data labels on columns correspond to the number of positive samples/total number of samples. Mean temperature represents the environmental temperature at seafood retails when oysters were collected. FC, fecal coliforms. NoV, norovirus.

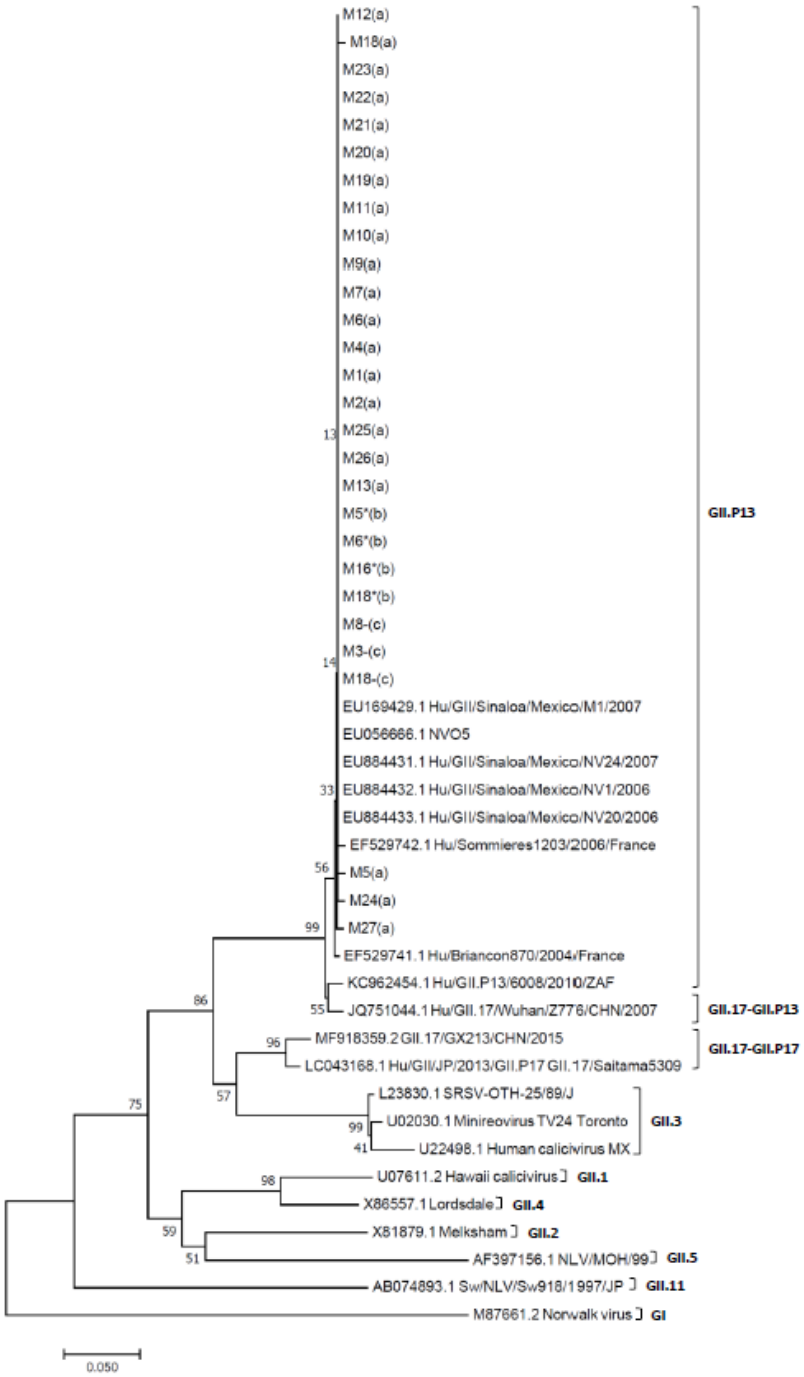

Figure 2

Phylogenetic tree based on RdRp gene of 28 NoV partial sequences obtained from raw oyster from seafood retails. Reference sequences obtained from GenBank are indicated by the accession numbers preceding the strain name. Nomenclature corresponding to VP1 classification is presented, and only in cases where genotyping based on RdRp is available, it is indicated with a capital P followed by the genotype designation, for example, GII.P13. Bar indicates genetic distance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suppmat1.pdf
- SuppFig1.pptx