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M. Alipour Amroabadi
https://orcid.org/0000-0002-7609-2701
E. Rahimi
https://orcid.org/0000-0002-3395-9280
A. Shakerian
https://orcid.org/0000-0003-4154-5456
H. Momtaz
https://orcid.org/0000-0002-5367-5672

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Incidence of hepatitis A and hepatitis E viruses and norovirus and rotavirus in fish and shrimp samples caught from the Persian Gulf

[*Incidência de vírus da hepatite A e hepatite E e norovírus e rotavírus em amostras de peixes e camarões capturados no Golfo Pérsico*]

M. *Alipour Amroabadi*[1], E. *Rahimi*[1*], A. *Shakerian*[1,2], H. *Momtaz*[3]

[1]Department of Food Hygiene, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[2]Research Center of Nutrition and Organic Products (RCNOP), Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[3]Department of Microbiology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

ABSTRACT

Foodborne viruses including hepatitis A virus (HAV), norovirus (NoV), rotavirus (RoV) and hepatitis E virus (HEV) are easily transmitted through contaminated seafoods. The current research was done to assess the incidence of RoV, NoV GI and GII,hAV and hEV in fish and shrimp samples caught from the Persian Gulf, Iran. Three-hundred and twenty fish and shrimp samples were collected. The presence of foodborne viruses were assessed by the real-time PCR. Forty-nine out of 320 (15.31%) fish and shrimp samples were positive for foodborne viruses. Distribution of hAV, NoV GI and NoV GII amongst all studied samples were 0.93%, 5.93% and 8.43%, respectively. hEV and RoV viruses were not found in studied samples. *Parastromateus niger* and *Scomberomorus commerson* fish and *Penaeus monodon* shrimp were the most frequently contaminated samples. Simultaneous incidence of hAV and NoV GI and hAV and NoV GII were 0.31% and 0.93%, respectively. Distribution of foodborne viruses in samples collected through spring, summer, autumn and winter seasons were 14.28%, 9.33%, 11.76% and 24.44%, respectively. Findings revealed that the incidence of foodborne viruses was significantly associated with seafood species and also season of sampling.

Keywords: Noroviruses, Hepatitis A virus, Seafood, Persian gulf

RESUMO

Vírus transmitidos por alimentos, incluindo hepatite A (HAV), norovírus (NoV), rotavírus (RoV) e hepatite E (HEV) são facilmente transmitidos através de frutos do mar contaminados. Esta pesquisa foi realizada para avaliar a incidência de RoV, NoV GI e GII, hAV e hEV em amostras de peixes e camarões capturadas no Golfo Pérsico, Irã. Foram coletadas 300 amostras de peixes e camarões. A presença de vírus transmitidos por alimentos foi avaliada por PCR em tempo real. Quarenta e nove das 320 amostras de peixes e camarões (15,31%) foram positivas para vírus transmitidos por alimentos. A distribuição de hAV, NoV GI e NoV GII entre as amostras estudadas foi 0,93%, 5,93% e 8,43%, respectivamente. Os vírus hEV e RoV não foram encontrados nas amostras estudadas. Os peixes Parastromateus niger e Scomberomorus commerson e o camarão Penaeus monodon foram as amostras mais frequentemente contaminadas. A incidência simultânea de hAV e NoV GI, e hAV e NoV GII foi de 0,31% e 0,93%, respectivamente. A distribuição dos vírus transmitidos por alimentos nas amostras coletadas na primavera, verão, outono e inverno foi de 14,28%, 9,33%, 11,76% e 24,44%, respectivamente. Os resultados demonstram que a incidência de vírus transmitidos por alimentos foi significativamente associada às espécies de frutos do mar e também à época da amostragem.

Palavras-chave: norovírus, vírus da hepatite A, frutos do mar, Golfo Pérsico

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*Autor para correspondência (*corresponding author*)
E-mail: ebrahimrahimi55@yahoo.com

INTRODUCTION

Fish and shrimp are popular amongst people of most regions of the world. However, transmission of viral contamination from the sea water and also contaminated staffs of fish and shrimp harvesting and production decreased their hygienic quality (Bosch *et al.*, 2018). Foodborne viruses are propagated in the human intestine and secreted in stool. They are usually transmitted by the fecal-oral route and also through the consumption of contaminated food and water. Norovirus (NoV), hepatitis A virus (HAV), hepatitis E virus (HEV), and rotavirus (RoV) are the most important foodborne viruses (Bosch *et al.*, 2018). Several outbreaks of viral foodborne diseases have been reported due to the consumption of seafood harvested from sewage-contaminated waters (Hall *et al.*, 2014; Parrón *et al.*, 2019).

NoV belongs to the family Caliciviridae and contains positive sense single-stranded RNA. NoV is a primary pathogen that causes acute viral gastroenteritis (Hardstaff *et al.*, 2018; Moore *et al.*, 2015). It has a five genogroups (GI to GV). NoV GI and NoV GII are mainly associated with severe outbreaks of viral gastroenteritis (Hardstaff *et al.*, 2018; Moore *et al.*, 2015). NoV is the leading cause of illness from contaminated food. The virus can easily contaminate food because it is very tiny and spreads easily. It only takes a very small amount of virus to make someone sick. Major transmission routes of NoV are fecal-oral through contaminated food and water and person to person contact (Hardstaff *et al.*, 2018; Moore *et al.*, 2015). RoV is a double-stranded RNA virus belonging to the family Reoviridae. RoV is classically divided into seven serogroups (A to G). RoV serogroups A, B, and C are mainly are mainly associated with severe outbreaks of viral gastroenteritis.

Group A rotavirus is a major cause of severe diarrhea in young children. It is transmitted from person to person or indirectly through contaminated food and water (Mohan *et al.*, 2014). hAV and hEV are foodborne pathogens responsible for occurrence of hepatitis in humans. Contaminated food and water are the main sources for transmission of hAV and hEV to the human population (Melgaço *et al.*, 2018; Moor *et al.*, 2018; Sánchez, 2015). hAV is a single-stranded RNA virus of the family Picornaviridae. Seafood shave has also been recognized as a source of hAV (Sánchez, 2015). hEV is also a single-stranded positive-sense RNA virus of the family hepeviridae. It is responsible for occurrence of acute hepatitis due to the consumption of water and in some cases food contaminated with stool (Melgaço *et al.*, 2018; Moor *et al.*, 2018).

The current survey was done to evaluate role of fish and shrimp in the transmission of pathogenic viruses, and the distribution of NoV, hAV, hEV and RoV in different types of fish and shrimp samples caught from the Persian Gulf, Iran.

MATERIALS AND METHODS

The study was approved by the Ethical Council of Research of the Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. From May to May 2018, a total of 320 seafood samples including fish (n= 170) and shrimp (n= 150) were randomly collected from the local harbors and retail centers, Iran. Fish samples included *Scomberomorus commerson* (S. *commerson*) (n= 50), *Otolithes ruber* (*O. ruber*) (n= 60) and *Parastromateus niger* (*P. niger*) (n= 60). Shrimp samples included *Penaeus monodon* (*P. monodon*) (n= 50), *Penaeus merguiensis* (*P. merguiensis*) (n= 50) and *Penaeus indicus* (*P. indicus*) (n= 50).

Species identification of hard-shell samples were done by an expert professor of the field of Aquaculture. Fish and shrimp samples were caught from the Boushehr port, Persian Gulf, Iran. All samples were taken from the dorsal muscles of seafoods. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately transported to the Food hygiene and Quality Control Research Center of the Islamic Azad University of Shahrekord in a cooler with ice packs and processed within 6h. All seafood samples showed normal physical characteristics including odor, color and consolidation.

A total of 1.5g of the seafood samples were homogenized with0.25M glycine 0.14M NaCl buffer (pH7.5) (Sigma, St. Louis, MO, USA). The homogenate sample was transferred to a 50ml tube and centrifuged at 10,000 × g for 30min at 4°C (Shimadzu, Japan). The primary supernatant was collected in a new tube. The pellet was resuspended in 0.25M Threoninee-0.14M NaCl

buffer (pH7.5) and was then centrifuged at 10,000 × g for 30min at 4°C (Shimadzu, Japan). The secondary supernatant was then mixed with the primary supernatant.

Forty percent polyethylene glycol 6000 (PEG 6000) (Sigma, St. Louis, MO, USA) and 3M NaCl (Amresco, Solon, OH, USA) solution was added to the collected supernatant. The mixture was precipitated at 4°C over 3h. After precipitation, the pellet was dissolved in 0.2% Tween 80-50mM Tris-HCl (Merck, Germany), sterilized distilled water, and chloroform:isoamyl alcohol (24:1) (Sigma, St. Louis, MO,USA). The mixture was then centrifuged at 10,000 × g for 30min at 4°C (Shimadzu, Japan). The supernatant was transferred to a new tube. The remaining precipitate was dissolved in sterilized distilled water and chloroform:isoamyl alcohol (24:1). The mixture was then centrifuged another time at 10,000 × g for 30min at 4°C (Shimadzu, Japan), and the supernatant was then combined with the first supernatant. The mixture was precipitated with 40% PEG 6000 and 3M NaCl solution at 4°C over 3h. The pellet was then suspended with diethylpyrocarbonate (DEPC)-treated deionized water (Sigma, St. Louis, MO, USA).

Qualitative real-time PCR was used for detection of pathogenic viruses. The pellet suspended with DEPC-treated distilled water was used for RNA extraction using the QIAamp viral RNA mini kit (Qiagen, Valencia, CA, USA). The Reverse transcription (RT) reaction mixture (Applied Biosystems, USA) consisted of sterile diethylpyrocarbonate (DEPC)-treated water, 10X PCR Buffer, 25mM dNTPs, 50µM oligo d (T), RNase inhibitor (20U/µl), murine leukemia virus (MuLV) reverse transcriptase (50U/µl), and 5µl RNA template. RT was performed with an MJ mini cycler (Bio-Rad, CA, USA) with the following program: 42°C for 50min, 94°C for 5min, and 4°C for 5min (Tahk *et al.*, 2012). Purity (A260/A280) and concentration of extracted cDNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA).

The truth of the cDNA was assessed on a 2% agarose gel stained with ethidium bromide (0.5µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany). Two negative control reactions were performed alongside the main reactions to evaluate the accuracy of the synthesis cDNA. One PCR reaction in the presence of extracted RNA as the reaction sample and the other PCR reaction without the presence of any sample and only in the presence of water. As a rule, lack of amplification in these two reactions and amplification in the presence of cDNA indicated the validity of the synthesis. Table 1 represents the oligonucleotide primers, PCR conditions and volume of each reaction Used for detection of NoV, hAV, hEV and RoV in fish and shrimp samples.

Primers were designed to detect pathogenic viruses. First, the sequences for the desired viruses were extracted from the National Center for Biotechnology Information (NCBI) database, and then primers were designed based on the conserved regions of the different sequences for each virus using MEGA 7.0 and Oligo 7 software. PCR amplification was performed with a Thermal Cycler Dice Real-Time System (TaKaRa, Japan).

Primer application was evaluated in three different stages. In the first step, the gradient PCR reaction was performed at six different temperatures for each primer pair and the optimum binding temperature for each primer pair was selected. Secondly, PCR reactions were performed at the optimum temperature for each primer pair in the presence of positive samples (sample infected with the target virus) and negative samples (sample that did not contain the desired virus or was infected with another virus). In the third step, a standard curve was drawn for each primer pair in the presence of a positive sample. Fisher's exact test was used to analyze the correlation of virus detection rate with seafood samples. Analyses were carried out using R (Institute for Statistics and Mathematics of the WU Wien). Statistical difference was determined by P value<0.05.

Table 1. Oligonucleotide primers and probes used for detection of hAV, hEV, NoV GI, NoV GII and RoV in fish and shrimp samples

Target viruses	Oligonucleotide primer	PCR volume (25 µL)	PCR programs	PCR Product size (bp)
hAV	F: GGTAGGCTACGGGTGAAAC R: TTGCCCTAAGCACAGAGAGGT	cDNA: 100 ng Primers (10 pmol): 0.3µM of eachprimer 2X Master Mix Syder-Green: 12.5 µL	1 cycle: 95°C -- 10 min. 45 cycle: 95°C ----- 15 s 58°C ----- 35 s 72°C ----- 25 s	205
hEV	F: GGTGGTTTCTGGGGTGAC R: AGGGGTTGGTTGGATGAA	cDNA: 100 ng Primers (10 pmol): 0.3µM of eachprimer 2X Master Mix Syder-Green: 12.5 µL	1 cycle: 95°C -- 10 min. 45 cycle: 95°C ----- 15 s 55°C ----- 35 s 72°C ----- 25 s	70
NoV GI	F: CGTCCTTAGACGCCATCATCATT R: CCAGAGGAAAGTTCAGCTTATATCC	cDNA: 100 ng Primers (10 pmol): 0.3µM of eachprimer 2X Master Mix Syder-Green: 12.5 µL	1 cycle: 95°C -- 10 min. 45 cycle: 95°C ----- 15 s 58°C ----- 35 s 72°C ----- 25 s	234
NoV GII	F: CTCGACGCCATCTTCATTCAC R: GAAACAATGATACCACACTCCCAA	cDNA: 100 ng Primers (10 pmol): 0.3µM of eachprimer 2X Master Mix Syder-Green: 12.5 µL	1 cycle: 95°C -- 10 min. 45 cycle: 95°C ----- 15 s 56°C ----- 35 s 72°C ----- 25 s	249
RoV	F: CAGTGGTTGATGCTCAAGATGGA R: TCATTGTAATCATATTGAATACCCA	cDNA: 100 ng Primers (10 pmol): 0.3µM of eachprimer 2X Master Mix Syder-Green: 12.5 µL	1 cycle: 95°C -- 10 min. 45 cycle: 95°C ----- 15 s 56°C ----- 35 s 72°C ----- 25 s	131

RESULTS

A total of 320 seafood samples were studied for presence of NoV, hAV, hEV and RoV. Applied method failed to amplify hEV and RoV in studied fish and shrimp samples. Real-time PCR amplification curves are shown in Figure 1. Additionally, real-time PCR melting curves are shown in Figure 2.

Table 2 represents the distribution of NoV, hAV, hEV and RoV in fish and shrimp samples. Forty-nine out of 320 (15.31%) fish and shrimp samples were positive for viruses. Incidence of hAV, NoV GI and NoV GII amongst all studied samples was 0.93%, 5.93% and 8.43%, respectively. Applied method failed to detect hEV and RoV in studied samples. *P. niger* had the highest incidence of NoV GII (8.33%), while S. *commerson* had the highest incidence of hAV (2%) and NoV GI (6%) amongst all studied fish species. *P. monodon* had the highest incidence of hAV (2%), NoV GI (12%) and NoV GII (18%) amongst all studied shrimp species. Simultaneous incidence of hAV and NoV GI was 0.31%. Simultaneous incidence of hAV and NoV GII was 0.93%. Statistically significant difference was found between type of samples and incidence of pathogenic viruses ($P<0.05$).

Table 3 represents the seasonal distribution of hAV, NoV GI and NoV GII in fish and shrimp samples. Incidence of pathogenic viruses in all studied samples collected through spring, summer, autumn and winter seasons was 14.28%, 9.33%, 11.76% and 24.44%, respectively. Furthermore, incidence of pathogenic viruses in raw fish samples collected through spring, summer, autumn and winter seasons was 12.50%, 6%, 6% and 20%, respectively. Moreover, incidence of pathogenic viruses in raw shrimp

samples collected through spring, summer, autumn and winter seasons was 16.66%, 11.42%, 20% and 28%, respectively. Statistically significant differences were found between season of sampling and incidence of pathogenic viruses (P<0.05).

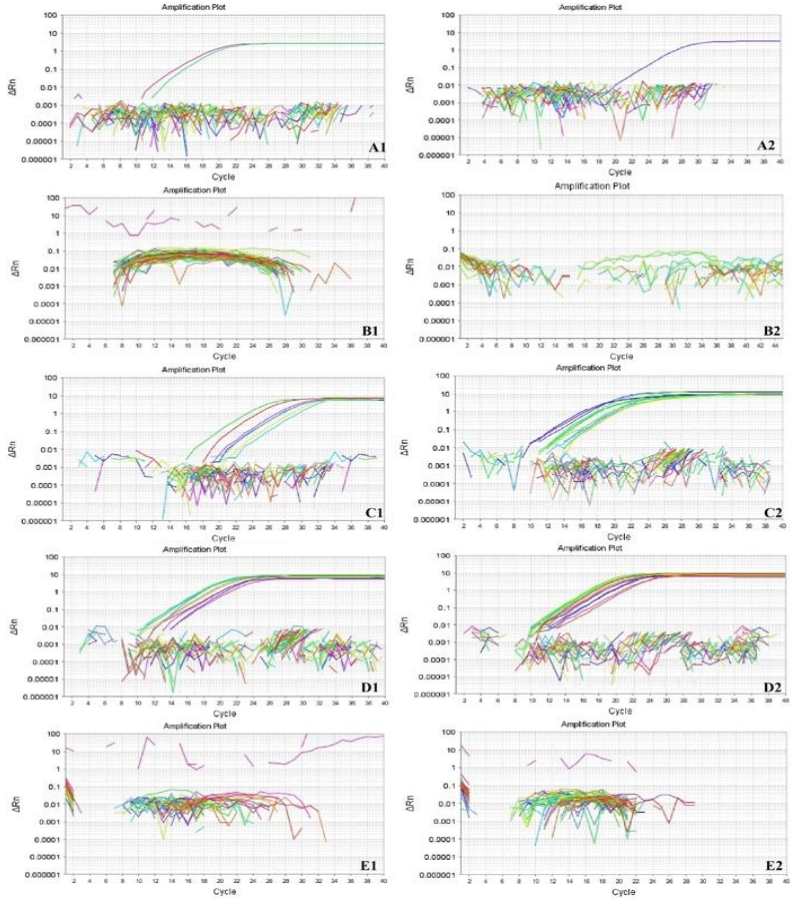

Figure 1. Samples of amplification cycles of studied pathogenic viruses in fish and shrimp samples. A1:hAV in fish samples, A2:hAV in shrimp samples, B1:hEV in fish samples, B2:hEV in shrimp samples, C1: NoV GI in fish samples, C2: NoV GI in shrimp samples, D1: NoV GII in fish samples, D2: NoV GII in shrimp samples, E1: RoV in fish samples, E2: RoV in shrimp samples.

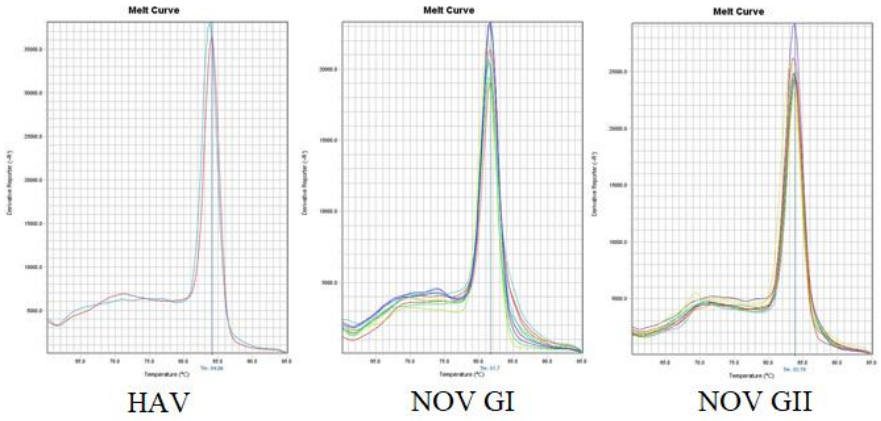

Figure 2. Samples of melting cycles of studied pathogenic viruses in fish and shrimp samples.

Table 2. Distribution of hAV, hEV, NoV GI, NoV GII and RoV in different species of fish and shrimp samples

Type of samples		N. samples collected	N. samples positive for viruses (%)						
			HAV	HEV	NoV GI	NoV GII	RoV	HAV + NoV GI	HAV + NoV GII
Fish	S. *commerson*	50	1(2)	-	3(6)	3(6)	-	-	1(2)
	O. ruber	60	-	-	1(1.66)	2(3.33)	-	-	-
	P. niger	60	1(1.66)	-	3(5)	5(8.33)	-	1(1.66)	1(1.66)
	Total	170	2(1.17)	-	7(4.11)	10(5.88)	-	1(0.58)	2(1.17)
Shrimp	*P. monodon*	50	1(2)	-	6(12)	9(18)	-	1(2)	1(2)
	P. merguiensis	50	-	-	3(6)	3(6)	-	-	-
	P. indicus	50	-	-	3(6)	5(10)	-	-	-
	Total	150	1(0.66)	-	12(8.00)	17(11.33)	-	-	1(0.66)
Total		320	3(0.93)	-	19(5.93)	27(8.43)	-	1(0.31)	3(0.93)

Table 3. Seasonal distribution of hepatitis A and hepatitis E viruses and noroviruses and rotavirus in fish and shrimp samples

Types of samples (N positive samples)	N samples collected in each season				N (%) samples positive in each season			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Fish (19)	40	40	50	40	5(12.50)*	3 (6)	3(6)	8(20)
Shrimp (30)	30	35	35	50	5(16.66)	4(11.42)	7(20)	14(28)
Total (49)	70	75	85	90	10(14.28)	7(9.33)	10(11.76)	22(24.44)

*Positive samples were obtained from all detected viruses.

DISCUSSION

To our knowledge, this is the first report of the incidence of foodborne viruses in fish and shrimp samples caught from the Persian Gulf, Iran. Compared with the detection rates of viruses, NoV GI (5.93%) and NoV GII (8.43%) were the significantly prevalent pathogens in fish and shrimp samples caught from the Persian Gulf, Iran. Incidence of hAV (0.93%) was entirely low amongst the studied samples. High incidence of NoV GII was also reported in different previously published investigations conducted on South Korea (Park *et al.*, 2012) and Italy (La Bella *et al.*, 2017). Seo *et al.* (2014) reported that the incidence of NoV GI, NoV GII and hAV amongst the shellfish samples collected from local market in the Kyounggi area, South Korea were 5.90%, 21.70% and 0.70%, respectively.

The used TaqMan Probe Real-Time PCR failed to detect hEV and RoV in studied samples which was similar to our findings. Fusco *et al.* (2019) reported that the incidence ofhAV, NoV GI, NoV GII, RoV and hEV amongst the shellfish samples collected from the gulf of Naples, Italy from 2015 to 2017 were 8.90%, 10.80%, 39.70%, 9.00% and 0%, respectively which showed the higher incidence of foodborne viruses than our findings. Mesquita *et al.* (2011) reported that 34 (69.00%) out of the 49 shellfish batches collected from the Portugal was contaminated by at least one of the NoV, hAV and EV. They showed that simultaneous incidence of NoV/HAV, NoV/enterovirus (EV) and EV/HAV, was 6.00%, 8.00% and 8.00%, respectively.

Purpari *et al.* (2019) reported that the incidence of hAV, NoV GI, NoV GII, RoV and hEV in seafood samples collected from Italy were 13.00%, 10.20%, 5.60%, 0% and 0.90%, respectively. High contamination rate of NoV in seafood was reported in 45.00% and 54.00% in Spain and in 38.00% and 37.00% in Thailand and Portugal, respectively (Diez-Valcarce *et al.*, 2012; Kittigul *et al.*, 2011; Mesquita *et al.*, 2011; Vilariño *et al.*, 2009).

Among all 320 seafood samples, hAV was detected in only 2 fish (1.17%) and one shrimp (0.66%) sample. Our finding was very close to the incidence of hAV reported in the United Kingdom, South Korea and Japan (Formiga-Cruz *et al.*, 2002; Hansman *et al.*, 2008; Seo *et al.*, 2014). Higher incidence rate of hAV in seafood samples (22.00-36.00%) was reported Brazil, Portugal and Italy (Coelho *et al.*, 2003; Croci *et al.*, 2000; Mesquita *et al.*, 2011). Because hAV was detected in only 0.93% of fish and shrimp samples, it has been suggested that the possibility of transmission of hAV through the consumption of fish and shrimp in Iran may be very low.

Fascinatingly, hEV and RoV have not been detected in fish and shrimp samples collected from the Persian Gulf, Iran. hEV is a significant

infection amongst the European countries, and over the last 10 years more than 21,000 acute clinical cases with28 fatalities have been notified with an overall 10-fold increase in reported hEV cases; the majority (80%) of cases were reported from France, Germany and the UK (Hazards *et al.*, 2017). However, as infection in humans is not notifiable in all Member States, and surveillance differs between countries, the number of reported cases is not comparable and the true number of cases would probably be higher (Hazards *et al.*, 2017).

Similar to findings of the present study, hEV contamination was not found in seafood samples in Finland, South Korea and Thailand (Diez-Valcarce *et al.*, 2012; Seo *et al.*, 2014). The applied method failed to detect hEV and RoV in studied samples. The probable reason for this finding is the absence of these two pathogenic viruses in a sea water and also amongst the studied samples. Additionally, staffs of the fishing centers were negative for these two pathogenic viruses. Differences in the diet of studied samples, distance of living from the beach, depth of their lives and finally their route of maintenance are probable factors affecting differences in the incidence of different viruses in diverse samples.

RoV has been documented as a scarce reason for causing seafood-associated disease. This may be due to low survival in the marine environment or poor diagnostics. Rotaviruses is often associated with infant diarrhea but may not be considered in adults. Furthermore, adults may excrete only low quantities of the virus which could be missed under the electron microscope. Moreover, the concentration of RoV shedded in stool samples is relatively low (3×10^5-5×10^{11} viral particles/g of stool) (Mohan *et al.*, 2014).

Reversely, the infective dose of RoV is relatively low (10-100 virus particles) which makes it a significant foodborne pathogen (Mohan *et al.*, 2014). RoV can contaminate seafood growing areas from a number of sources including septic tank leakages, boat discharges, overflows and spills from sewage treatment plants seepage from sewage reticulation networks and accidental contamination after heavy rainfall (Mohan *et al.*, 2014). Similar to findings of the present study, Vilariño *et al.* (2009) reported that RoV had the least incidence amongst all foodborne viruses detected in seafood samples caught from the Spain. Unlike the findings of the present investigation, Hansman *et al.* (2008) reported that the incidence of RoV in seafood samples caught from Japan was 42.00%. Mohan *et al.* (2014) reported that the incidence of Rov in seafood samples collected from India was 2.50%. Similar findings were found in a survey conducted in China where in 3.33% of the seafood samples were positive for RoV (Kou *et al.*, 2005). Incidence as high as 52.00% has been reported in a study conducted in France (Le Guyader *et al.*, 2000).

Findings of the present study showed higher contamination rate of foodborne viruses in fish and shrimp samples collected through the winter season. In numerous investigations, seasonal variation of foodborne viruses has been clarified by the fact that viruses are less effectively removed from seafoods in winter and could survive better in colder seasons than in warmer (La Bella *et al.*, 2017). Many researches have reported a seasonal distribution of NoV detection in seafood samples mostly in the colder months (Le Guyader *et al.*, 2000; Lowther *et al.*, 2012; Suffredini *et al.*, 2012), thus suggesting seasonality for these virus infections. NoV illnesses related to seafood consumption present a seasonal pattern, generally showing a peak incidence during the wintertime.

This seasonality could be attributed to several factors, including increased stability of viruses at low water temperature, reduced solar inactivation, and selective bioaccumulation of these pathogens by seafoods (Suffredini *et al.*, 2012). Similarly, Seo *et al.* (2014) reported that the incidence of NoV GI, NoV GII and hAV amongst the seafood samples collected through summer and winter seasons were 6.80% and 5.60%, 13.60% and 25.00% and 0.00% and 0.90%, respectively. Furthermore, Fusco *et al.* (2019) stated that hAV-positive seafood samples displayed a seasonal pattern, with samples only testing positive between December and April (colder months).

However, further investigations are required to find more epidemiological properties of foodborne viruses in seafood samples. High incidence of diverse foodborne pathogens other than pathogenic viruses has been stated in different kinds of food samples, particularly

foods with animal origins (Abdolmaleki *et al*., 2019a, 2019b; Atapoor *et al*., 2014; Dehkordi 2011a, 2011b; Dehkordi *et al*., 2012a, 2012b, 2012c;, 2013; Dehkordi *et al*., 2014a, 2014b; Dehkordi and Rafsanjani 2013; Ghorbani *et al*., 2016; Hasanpour Dehkordi *et al*., 2017; Hemmatinezhad *et al*., 2015; Mashak *et al*., 2020a, 2020b; Momtaz *et al*., 2012, 2013; Mousavi *et al*., 2014; Momtaz *et al*., 2015; Nejat *et al*., 2015; Rahi *et al*., 2020; Rahimi *et al*., 2014; Ranjbar *et al*., 2017, 2018a; 2018b, 2018c, 2019a, 2019b, 2020;; Safarpoor Dehkordi *et al*., 2013, 2017, 2018). Thus, principles of food safety should be observed to decrease the outbreaks of several foodborne diseases.

CONCLUSIONS

NoV, particularly NoV GII is the most prevalent foodborne virus in fish and shrimp samples caught from the Persian Gulf, Iran. In keeping with this, hAV and NoV GI should be considered as other important foodborne viruses in the present study. Findings revealed that the incidence of foodborne viruses was significantly associated with seafood species and also seasonal variations. *P. niger* and S. *commerson* fish and *P. monodon* shrimp were the most commonly contaminated species. Higher contamination rate was detected in winter season. Although the viability of foodborne viruses could not be confirmed due to the limitation of real-time RT-PCR and the lack of cultivation techniques, monitoring of foodborne viruses in fish and shrimp samples may contribute to the prevention of viral food poisoning and the promotion of public health.

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