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Occurrence of Hepatitis A Virus, Rotavirus and Astrovirus in *Arca noae* and Shrimp Samples Collected from Damanhur City, Egypt

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ABSTRACT
Polluted seafood is a common mean of the transmission of foodborne viruses, such as rotavirus, hepatitis A virus, and astrovirus. Eating shellfish harvested from waters polluted with sewage has been associated with several viral foodborne outbreaks. The goal of the current study was to determine the prevalence of rotavirus, hepatitis A virus, and astrovirus in shrimp and *Arca noae* samples, collected from the local market in Damanhur City, Egypt. Samples of a hundred and twenty *Arca noae* and shrimp were examined. The RT- PCR was used to detect the predefined foodborne viruses. The current data confirmed that enteric viruses were detected in eleven out of 120 samples, but did not report astrovirus in any of the collected samples. RV and HAV were detected in 2/ 60 (3.3%), 2/ 60 (3.3%) of the shrimp samples and in 4/ 60 (6.6%), 3/60 (5%) of *Arca noae* samples, respectively. This study indicates that eating *Arca noae* in a raw condition, without any preparation, poses a serious public health risk since it can spread infections to food handlers and fishermen. In contrast, shrimp are usually cooked. Our research demonstrates that *Arca noae* and shrimp are contaminated with viruses, highlighting the necessity of virological monitoring programs to guarantee the safety and quality of shellfish for human consumption, as well as serving as an invaluable surveillance tool to track new viral strains and emerging viruses.

INTRODUCTION

All aquatic animals with a shell fall under the general category of "shellfish." It is typically separated into four categories: bivalves (which include oysters, clams, mussels, and shrimp), gastropods (which include abalone, limpet, snail, and whelk), and cephalopods (which include octopus, squid, and cuttlefish).

The HAV can infect shellfish that are found in sewage-contaminated water. Bivalve shellfish filter out the saltwater in order to absorb food particles and nutrients since they are considered as filter feeders. As a result, they frequently concentrate the virus that is in the contaminated water. When a man consumes contaminated,

undercooked shellfish, he runs the risk of contracting hepatitis A. Eating bivalve shellfish, such as shrimp, oysters, and cockles, is closely linked to the outbreaks of hepatitis A.

Enteric viruses, such as hepatitis viruses (hepatitis A (HAV) and hepatitis E (HEV) and human norovirus, in fish and shellfish are a health concern worldwide. A significant number of foodborne illnesses have been reported after the ingestion of raw shellfish and fish products. Virus contamination occurs via the consumption of contaminated food, direct person-to-person contact, contact with contaminated environmental surfaces, and for hepatitis E virus, via direct contact with infected animals. Foodborne outbreak investigations have also revealed that food contamination mainly occurs in restaurants and in catering services **(Bellou *et al.*, 2013; Bosch *et al.*, 2018; Ghosh *et al.*, 2019; EFSA, 2021)**. Prevalence studies have shown that canned and processed seafood samples, such as fish fillets, and fresh or frozen fish have tested positive for norovirus in Italy and in semi-processed fishery products in Belgium **(Burkhardt & Calci 2000; EFSA, 2021; Mozgovoj *et al.*, 2022)**.

In the greater majority of the globe, consumers like eating crustaceans and fishes. However, the hygienic quality of fish and shrimp was reduced by the spread of viral infection from sea water and contaminated workers during the collection and production processes **(Bosch *et al.*, 2018)**. Foodborne viruses proliferate in the intestines of humans and are excreted in stool. They are typically spread by the fecal-oral route, as well as by consuming tainted food and drink. The four most significant foodborne viruses are rotavirus (RV), hepatitis A virus (HAV), hepatitis E virus (HEV), and norovirus (NoV) **(Bosch et al., 2018)**. Eating fish that were harvested from waters, polluted with sewage, has been connected to several viral foodborne illness outbreaks **(Hall *et al.*, 2014; Parrón *et al.*, 2019)**. The commercially edible bivalve *Arca noae* is native to the Mediterranean, Black, and Eastern Atlantic Seas. At depths of more than 100 meters, it can be found in clumps or as lone specimens attached to hard ground by byssal threads. According to studies conducted on its biology in several Mediterranean Sea sites (such as the Adriatic Sea in Tunisia), this bivalve grows slowly **(Peharda *et al.*, 2002; Morton & Peharda, 2008; Ghribi, *et al.*, 2017)**. Therefore, if contaminated food or water is consumed, the combination of high viral concentrations in ambient waters, low infective

dose required for infection, and their ability to remain stable and persist in the aquatic environment may result in adverse health outcomes. In Egypt, there is scarce information on enteric viruses' contamination of shellfish. Therefore, our study aimed to determine the detection rates of enteric viruses (RV, HAV, ASV) in *Arca noae* and shrimp samples collected from Damanhur, Behera, Egypt.

MATERIALS AND METHODS

Sampling site and processing

A total of one hundred and twenty samples were monthly collected (*Arca noae* n= 60, shrimp n= 60, (*Metapenaeus monoceros*= 30, *Penaeus semisulcatus*) for a duration of one year from January 2022 to December 2022, from five local supermarkets located in Damanhur City, Behera Governorate, Egypt. Behera Governorate is a coastal governorate in Egypt, located in the northern part of the country in the Nile Delta; its capital is Damanhur. For each sample, we purchased (1/4) kilogram and randomly selected sample from it in order to obtain about 2.5g from the digestive tract tissue and give it a code number. Viral recovery from *Arca noae* and shrimp were carried out as reported using the ISO15216-1:2017 method of **ISO 15216-1:2017** with minor modification. Each sample was obtained from the dorsal sand sack and part of dorsal tissue in shrimp and muscle of *Arca noae*. To avoid spills and cross-contamination, each sample was sealed in a separate, sterile plastic bag. It was then sent, in a cooler with ice packs to the Virology Lab, the National Research Center, where it was processed in less than 24 hours. Each sample of seafood had the usual physical characteristics, including color, consolidation, and smell. Briefly, digestive tissue was removed by dissection from each (*Arca noae*, shrimp) pooled, and finely chopped. Aliquots of 2g were placed in a 40mL polypropylene bag containing 4mL of elution buffer (Tris-HCl 100mM, glycine 50mM, 1% beef extract pH of 9.5 (Sigma-Aldrich, Canda) where they were soaked in and homogenized. The rinsing fluid was removed via strainer and centrifuged at 10,000g for 30min at 4°C to pellet the seafood particles. The pH of the decanted supernatant was adjusted to 7.2± 0.2 by the addition of 5N HCl, while the fluid was stirring constantly. The neutralized supernatant was supplemented with 10% (w/ v) polyethylene glycol (PEG) 6,000 and 0.3M NaCl (Sigma Aldrich, Canda), and was then incubated with a constant shaking at

4°C for one hour. The viruses were concentrated by centrifuging the solution at 10,000g for 30min at 4°C. The supernatant was discarded then what remained was centrifuged again at 10,000g for 5min at 4°C to compact the pellet. The pellet was then suspended in 500µL of PBS and vortexed with 500µL of chloroform: butanol, 1:1 (v/ v). Afterwards, the suspension was incubated for 5min at room temperature and centrifuged at 8,000g for 15min at 4°C. The upper aqueous phase containing viruses was directly processed by the nucleic acid extraction procedure.

Nucleic acid extraction and viral detection

A Qiagen Viral RNA Extraction Kit (QIAGEN, Germany) was used to extract viral RNA from 140ul of concentrated samples according to the manufacturer's instructions. The RNA was either immediately kept at -20 or subject directly to cDNA synthesis to convert RNA to DNA to be ready for PCR assay. To create cDNA, 5µl of extracted RNA and 2µl of a particular reverse primer for each virus were combined. The RNA template's helices were opened by heating it to 65°C for 5 minutes, and then the mixture was cooled on ice for 2 minutes to prevent the development of secondary structures. Then we added 2µl of dNTP, 0.5µl of maxima reverse transcriptase (Thermo scientific (200U/ µl, Korea), 5µl of 5x RT buffer, 0.5µl RNase inhibitor, 10µl DEPC-treated water to form 25µl reaction mixtures. After heating the mixture to 30°C for 30 minutes, 42°C for an hour, and 95°C for five minutes to stop the enzyme from operating; it was cooled on ice.

The two rounds of PCR that made up the polymerase chain reaction used to detect HAV were as follows: in the first round, we used 3ul of synthesized cDNA, 12.5ul of Dream Taq PCR master mix, 1ul of forward primer A-F1, 1 ul of A-R1 reverse primer, and 7.5ul of DEPEC water to obtain a reaction mixture of 25ul, with a temperature condition of 5 min at 95c, followed by 40 cycles of denaturation of 94c for 45 sec, annealing at 55c for 45 sec, and an extension of 72c for 1min, with a final extension at 72c for 10 min. The obtained PCR product was then subjected to a second round of PCR conditions with another two specific primers, 1ul of the PCR product of the first round and 12.5ul of Dream Taq master mix, 1ul of forward primer A-F2, and 1ul of reverse primer A-R2 and 9.5ul of DEPEC water were used, and the PCR conditions were as follows: 35 cycles of 94c for 30 seconds, 62c for 45 seconds, and 72c for 1 minute, with a

final extension time of 72c for 5 minutes. In case of ASV, forty cycles of amplification (948C, 1min, 508C, 1min, and 728C, 1min) were performed with the primer mentioned in Table (1). While, in case of RV, the PCR program included a 9-min denaturation step at 95°C and 40 cycles of amplification for 1min at 94°C, for 1min at 50°C, and for 1min at 72°C, followed by a final elongation step of 7min at 72°C. In order to confirm the rotaviral nature of the 186-bp, we used primers VP6F and VP6R.PCR products of 10µl were mixed with 2µl loading dye and analyzed by electrophoresis (General biosystem, Germany) using 1.5% agarose gel (electrophoresis grade, iNtRoN, Cat.No.32033) containing 0.5µg ethidium bromide. The PCR product was run at 100V for 30min in submarine electrophoresis (model: HB1214, RATED: 0-150V,0-100ma), and visualized under transilluminator (SPECTROLINE, MODEL-TM-312A), electrophoresis power supply (CONSORT,3000v-300Ma, E833) comparing the low-grade DNA ladder (NORGEN, cat# 11400, Canada).

Table 1. Oligonucleotide primers sequences used for detection of rotavirus, astrovirus and hepatitis A virus in bivalve and shrimp samples

Virus	PCR round	**Primer**	**Sequence**	**Band size**	**Reference**
HAV	1st	A-F1	5'-CTATTCAGATTGCAAATTAYAAT-3'	391bp	**(Villar *et al.*, 2006)**
		A-R1	5'-AAYTTCATYATTTCATGCTCCT-3'		
	2nd	A-F2	5'-TATTTGTCTGTYACAGAACAATCAG-3'	244bp	
		A-R2	5'-AGGRGGTGGAAGYACTTCATTTGA-3'		
ASV		Mon269	CAACTCAGGAAACAGGGTGT	448bp	**(Stacy, *et al.*, 2009)**
		Mon270	TCAGATGCATTGTCATTGGT		
RV		VP6-3	(5_-GCTTTAAAACGAAGTCTTCAAC-3	186-bp	**(Villena *et al.*, 2003)**
		Vp6-4	(5_-GGTAAATTACCAATTCCTCCAG-3		

RESULTS

Over the 12-month study period, a total of 120 seafood samples were tested for the presence of ASV, HAV, RV. This included 60 shrimp samples and 60 *Arca noae* samples collected from five local markets in Damanhur City. The applied method failed to amplify astrovirus in the studied *Arca noae* and shrimp samples.

The monthly rates of identification differed slightly between viruses. Table (2) represents the distribution of ASV, HAV and RV in *Arca noae* and shrimp samples. Detection rates ranging from 3.3 to 6.6%. Eleven out of the 120 (9.1%) shrimp and *Arca noae* were tested positive for viruses (9.1%).

The incidence of HAV was 3.3, 6.6% in shrimp and *Arca noae*, respectively, and incidence of RV was 3.3, 5% for shrimp and *Arca noae*, respectively. Astrovirus was not found in the examined samples using the applied approach. Shrimp had a 3.3% simultaneous incidence of HAV and RV. Higher detection rate was observed in cooler months during December and January.

Table 2. The monthly collected number of samples in relation to hepatitis A, rotavirus, and astrovirus RT-PCR in shrimp and *Arca noae* samples

Month/ no. of sample	Shrimp RT-PCR			*Arca noae* RT-PCR		
	HAV	RV	ASV	HAV	RV	ASV
January /5	1	1	0	1	2	0
February/5	0	0	0	0	0	0
March/5	0	0	0	0	0	0
April/5	0	0	0	0	0	0
May/5	0	0	0	1	0	0
June/5	0	0	0	0	0	0
July/5	0	0	0	0	0	0
August/5	0	0	0	0	0	0
September/5	0	0	0	1	0	0
October/5	0	0	0	0	0	0
November/5	1	0	0	0	0	0
December/5	0	1	0	1	1	0
Total	2/60	2/60	0/60	4/60	3/60	0/60
percentage	3.3%	3.3%	0%	6.6%	5%	0%

Table 3. Seasonal variation of different viruses detected in shrimp and *Arca noae*

Season	Number of positive sample in shrimp/ collected samples	% of positivity	Number of positive samples in *Arca noae*/ collected samples	% of positivity
Winter	**4/15**	**26.66%**	**5/15**	**33.33%**
Spring	**O/15**	**0.00%**	**0/15**	**0.00%**
Summer	**0/15**	**0.00%**	**0/15**	**0.00%**
Autumn	**0/15**	**0.00%**	**1/15**	**6.66%**

DISCUSSION

In the present study, we investigated the incidence and diversity of enteric viruses in bivalve shellfish (shrimp and *Arca noae*) samples collected from five local market in Damanhur City, Egypt, between January 2022 and December 2022. Overall, 9.1% of shellfish samples were positive for one or more viruses. This is the first study that report prevalence of foodborne viruses in *Arca noae* and shrimp samples in Damanhur, Egypt. Compared to our results, other studies detected RVA with similar frequency in the bivalve samples (varying from 0 to 8.3%) in Brazil, Thailand, Italy, South Korea and Singapore **(Rigotto *et al.*, 2010; Torok *et al.*, 2018; Fusco *et al.*, 2019, Purpari *et al.*, 2019; Tan *et al.*, 2021)**. Human enteric viruses are mostly concentrated in the stomach and digestive diverticula of shellfish **(Schwab *et al.*, 1998)**. Thus, we made the decision to target these tissues specifically for examination **(Atmar *et al.*, 1995**).

Fusco *et al.* (2019) reported that the incidence of HAV, and RV amongst the shellfish samples collected from the gulf of Naples, Italy from 2015 to 2017 were 8.90, 9.00%, respectively, which is similar to our finding of foodborne viruses. **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 higher than our detection rate. **Purpari *et al.* (2019)** reported that the incidence of HAV, RV in seafood samples collected from Italy were 13.00, 0%, respectively.

In the present study among all 120 seafood samples, HAV was detected in only 6 samples: 4 shrimp and 2 *Arca noae*, accounting for 3.3 and 6.6%. Our results were

extremely similar to the incidence of HAV that was documented in South Korea, Japan, and the United Kingdom **(Formiga-Cruz *et al*., 2002; Hansman *et al*., 2008; Seo *et al*., 2014)**. In contrast, Brazil, Portugal, and Italy have reported higher incidence rates of HAV in seafood samples (22.00– 36.00%) **(Croci *et al*., 2000; Coelho *et al*., 2003; Mesquita *et al*., 2011)**. A lower detection rate has been reported in Iran's fish and shrimp diet due to the detection of HAV in only 0.93% of samples.

According to **Hansman *et al.* (2008)**, 42.00% of seafood samples taken from Japan have RV. Moreover, according to **Mohan *et al.* (2014)**, 2.50% of the seafood samples taken from India had RV. A survey carried out in China revealed similar results, with 3.33% of the seafood samples testing positive for RV **(Kou *et al.*, 2005)**. A research carried out in France found an incidence as high as 52.00% **(Le Guyader *et al*., 2000)**.

Frequent untreated sewage discharge into coastal waters has a detrimental impact on the marine ecosystem and may be a contributing factor in foodborne or waterborne illness epidemics. Lastly, our research shows that shellfish biomonitoring can be used as a virological surveillance method to detect and track new viral variations.

CONCLUSION AND FUTURE PERSPECTIVES

Due to the limitations of RT-PCR and the absence of cell culture method, foodborne virus viability could not be established. It is crucial to consume shellfish safely since they are a significant marine food source and major carriers of enteric viruses. However, by monitoring foodborne viruses in *Arca noae* and shrimp samples, public health may be promoted, and viral food poisoning may be prevented. Preventing epidemics of enteric viruses caused by shellfish will largely depend on the development of quick, precise, and field-applicable technologies for detecting these viruses. Continued monitoring of molecular epidemiological data and continued research on the bioaccumulation mechanisms of different enteric viruses in shellfish will help in the development of specific drugs for the treatment of acute gastroenteritis caused by viral infection.

- **Ethical Approval and Consent to participate:** Not applicable
- **Consent for publication:** Not applicable**.**

- **Availability of data and materials:** All data generated or analyzed during this study are included in this published article.

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REFERENCES

Atmar, R. L.; Neill, F. H.; Romalde, J. L. and Guyader, F. L. (1995). Detection of Norwalk virus and hepatitis A virus in shellfish tissues with the PCR. Appl. Environ. Microbiol., 61**:**3014– 3018.

Bellou, M.; Kokkinos, P. and Vantarakis, A. (2013). Shellfish-Borne Viral Outbreaks: A Systematic Review. Food Environ. Virol., 5**:** 13–23. [CrossRef] [PubMed].

Bosch, A.; Gkogka, E. and Le Guyader, F.S. (2018). Foodborne Viruses: Detection, Risk Assessment, And Control Options in Food Processing. Int. J. Food. Microbiol., 285**:**110-128.

Bosch, A.; Gkogka, E.; Le Guyader, F.S.; Loisy-Hamon, F.; Lee, A.; van Lieshout, L.; Marthi, B.; Myrmel, M.; Sansom, A. and Schultz, A.C. (2018). Foodborne Viruses: Detection, Risk Assessment, and Control Options in Food Processing. Int. J. Food Microbiol., 285: 110–128. [CrossRef] [PubMed].

Burkhardt, W. and Calci, K.R. (2006). Selective Accumulation May Account for Shellfish-Associated Viral Illness. Appl. Environ. Microbiol., 66 **:** 1375–1378. [CrossRef] [PubMed].

Coelho, C.; Heinert, A.; Simões, C. and Barardi, C. (2003). Hepatitis A Virus Detection in Oysters (Crassostrea Gigas) In Santa Catarina State, Brazil, By Reverse Transcription–Polymerase Chain Reaction. J. Food. Prot., 66**:** 507-511.

Croci, L.; Medici, D. and Scalfaro, C. (2000) Determination of Enteroviruses, Hepatitis A Virus, Bacteriophages and Escherichia Coli in Adriatic Sea Mussels. J. Appl. Microbiol., 88**:**293-298.

European Food Safety Authority; European Centre for Disease Prevention and Control. (2021). The European Union One Health 2020 Zoonoses Report. EFSA J., 19**:** 324. [CrossRef].

Formiga-Cruz, M.; Tofino-Quesada, G. and Bofill-Mas, S. (2002). Distribution Of human Virus Contamination In Shellfish from Different Growing Areas In Greece, Spain, Sweden, And The United Kingdom. Appl. Environ. Microbiol., 68**:**5990-5998.

Fusco, G.; Anastasio, A. and Kingsley, D.H. (2019). Detection Of hepatitis A Virus and Other Enteric Viruses in Shellfish collected In the Gulf of Naples, Italy. Int. J. Environ. Publ. Health., 16**:**2588.

Ghosh, S.K.; Lekshmi, M.; Das, O.; Kumar, S. and Nayak, B.B. (2019). Occurrence of Human Enteric Adenoviruses in Fresh Tropical Seafood from Retail Markets and Landing Centers. J. Food Sci., 84**:** 2256–2260. [CrossRef] [PubMed]
Ghosh, S.K.; Lekshmi, M.; Das, O.; Kumar, S. and Nayak, B.B. (2019). Occurrence of Human Enteric Adenoviruses in Fresh Tropical Seafood from Retail Markets and Landing Centers. J. Food Sci., 84**:** 2256–2260. [CrossRef] [PubMed]
Ghribi, F.; Bejaoui, S.; Rabeh, I.; Aouini, F.; Chetoui, I. and El Cafsi, M. (2017). Effects of Culinary Methods on Nutritional Characteristics of the Edible Shellfish Noah's Ark (*Arca noae* L., 1758) from Tunisian Coasts. J. Aquat. Food Prod. Technol., 26**:** 1324–1336. [**Google Scholar**] [**CrossRef**]

Hall, A.J.; Wikswo, M.E.; Pringle, K.; Gould, L.H. and Parashar, U.D. (2013). Vital Signs: Foodborne Norovirus Outbreaks-United States, 2009-2012. Mmwr. Morb. Mortal. Wkly Rep., 63**:**491- 2014.

Hansman, G.S.; Oka, T.; Li, T.C.; Nishio, O.; Noda, M. and Takeda, N.(2008). Detection Of human Enteric Viruses in Japanese Clams. J. Food. Prot., 71**:**1689-1695.

ISO 15216-1. (2017). Microbiology of the Food Chain–Horizontal Method for Determination of Hepatitis A Virus and Norovirus Using Real-Time RT-PCR—Part1: Method for Quantification. International Organization for Standardization: Geneva, Switzerland.

Kou, X.; Wu, Q.; Zhang, J. and Fan, H. (2005). Study On Rotavirus Detection with Single-Tube Semi nested Rt-Pcr Method in Shellfish. *Acta.* Microbiol. Sinica., 45**:** 401-404.

Le Guyader, F.; Haugarreau, L.; Miossec, L.; Dubois, E. and Pommepuy, M. (2000). Three-Year Study To Assess human Enteric Viruses In Shellfish. Appl. Environ. Microbiol., 66**:** 3241-3248.

Mesquita, J.R.; Vaz, L.; Cerqueira, S.; _Castilho, F.; _Santos, R.; Monteiro, S.; Manso, F.C.; Romalde, J. and Nascimento. M.S.J. (2011). Norovirus, Hepatitis A Virus and Enterovirus Presence In Shellfish forming quality harvesting Areas In Portugal. Food Microbiol., 28**:**936-941.

Mohan, V.; Rawat, S. and Lokesh, K. (2014). Prevalence Of Rotavirus in Shellfish from Southern Kerala. Vet. World., 7**:**821-824.

Morton, B. and Peharda, M. (2008). The biology and functional morphology of *Arca noae* (Bivalvia: Arcidae) from the Adriatic Sea, Croatia, with a discussion on the evolution of the bivalve mantle margin. Acta Zool., 89**:** 19–28. [**Google Scholar**] [**CrossRef**]

Mozgovoj, M.; Miño, S.; Barbieri, E.S.; Tort, F.L.; Victoria-Montero, M.; Frydman, C.; Cap, M.; Baron, P.J.; Colina, R. and Matthijnssens, J. (2022) GII.4 Human Norovirus and G8P[1] Bovine-like Rotavirus in Oysters (Crassostrea Gigas) from Argentina. Int. J. Food Microbiol., 365**:** 109553. [CrossRef].

Parrón, I.; Álvarez, J.; Jané, M.; Razquin, E.; Guix, S.; Camps,K. and Domínguez, À. (2019). A Foodborne Norovirus Outbreak In A Nursing Home And Spread To Staff And Their Household Contacts. Epidemiol. Infect., 147**:** E225.

Peharda, M.; Richardson, C.A.; Onofri, V.; Bratoš, A. and Crnčević, **M**. (2002). Age and growth of the bivalve *Arca noae* L. in the Croatian Adriatic Sea. J. Molluscan Stud., 68**:** 307–310. [**Google Scholar**] [**CrossRef**][**Green Version**].

Purpari, G.; Macaluso, G.; Di Bella, S.; Gucciardi, F.and Mira, F. (2019) Molecular Characterization of Human Enteric Viruses in Food,Water Samples, and Surface Swabs in Sicily. Int. J. Infect. Dis., 80**:** 66–72. [CrossRef] [PubMed].

Rigotto, C.; Victoria, M.; Moresco, V.; Kolesnikovas, C.K. and Corrêa, A. A. (2010). Assessment of Adenovirus, Hepatitis A Virus and Rotavirus Presence in Environmental Samples in Florianopolis, South Brazil. J. Appl. Microbiol., 109**:** 1979–1987. [CrossRef] [PubMed.

Schwab, K. J., F. H. Neill, M. K. Estes, T. G. Metcalf, and R. L. Atmar. (1998). Distribution of Norwalk virus within shellfish following bioaccumulation and subsequent depuration by detection using RT-PCR. J. Food Prot., **61:**1674– 1680.

Seo, D.J.; Lee, M.H. and Son, N.R. (2014). Seasonal and Regional Prevalence of Norovirus, Hepatitis A Virus, Hepatitis E Virus, And Rotavirus in Shellfish Harvested from South Korea. Food. Control., 41**:**178-184.

Stacy, R.; Finkbeiner, Binh -Minh Le, Lori, R and Holtz, G. A. (2009). Detection of Newly Described Astrovirus N1LB1 in Stool Samples No3: Dol: 10.3201/ eid 1503.081213.

Tan, M.T.H.; Ho, S.X.; Chu, J.J.H. and Li, D. (2021). Application of Virome Capture Sequencing in Shellfish Sold at Retail Level in Singapore.Lett. Appl. Microbiol., 73**:**486–494. [CrossRef].

Torok, V.; Hodgson, K.; McLeod, C.; Tan, J.; Malhi, N.and Turnbull, A. (2018). National Survey of Foodborne Viruses in Australian Oystersat Production. Food Microbiol., 69**:** 196–203. [CrossRef]

Villena C, El-Senousy.M. W, F. Abad. X, and Pinto.L. (2003). Group A Rotavirus in Sewage Samples from Barcelona and Cairo: Emergence of Unusual Genotypes Applied. Envi. Micr., 76**:** 3919–3923.