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Foodborne Viruses Associated with Consumption of Shellfish

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Abstract

Seafood and shellfish have always been an important source of protein and fatty acids in the human diet and their *per capita* consumption has increased significantly across the globe in recent years. Food-borne diseases constitute a major public health hazard worldwide, and seafood is a vehicle of foodborne pathogens in 10 - 19% of these illnesses. Viruses are a major cause of shellfish-foodborne infections: between 1980 and 2012 about 359 shellfish-borne viral outbreaks were identified. The highest number of these outbreaks was reported in East Asia, followed by Europe, the USA, Oceania, Australia and Africa. The country with the greatest number of the outbreaks was Japan, with more than half of the outbreaks reported from this country. The viral pathogens responsible for outbreaks are viruses that are transmitted by the faecal-oral route, mainly Norovirus (NoV) and Hepatitis A Virus (HAV). Among shellfish, the most common vehicles for outbreaks were oysters. The factor most associated with infection is the consumption of raw or undercooked shellfish. Although there has been a considerable improvement in controlling viral foodborne pathogens, disease outbreaks due to shellfish consumption are on the increase, being a real challenge for food microbiologists and food-safety authorities. This review describes the most common viral causes of foodborne outbreaks owing to shellfish consumption, summarizes the general incidence of shellfish-related viral infections and discusses the available control options.

Keywords: *Foodborne Infections; Norovirus; Hepatitis A Virus; Hepatitis E Virus; Shellfish; Gastroenteritis; Food Safety*

Abbreviations

WHO: World Health Organization; EU: European Union; FAO: Food and Agriculture Organization; EFSA: European Food Safety Authority; CDC: Centre for Disease Control and Prevention

Introduction

Food-borne infections are an emerging, and very significant, threat to food safety and public health [1,2]. The last five decades have seen a steady growth in global seafood production and consumption, and seafood is currently the most traded food commodity in the world. According to FAO, in 2022 the world seafood production is expected to rise to above 181 million tonnes, at least 4.2% of which will come from aquaculture. According to this report, fish consumption *per capita* increased from 9.9 kg in 1960 to 19.2 kg in 2012 [2].

Asian countries play a major role in fish exportation. Fish production in Asia (both from fishing and aquaculture) has significantly grown in the last two decades and has recently accounted for around 70% of global production. In Europe, most imported seafood comes from China and Vietnam. The quantity imported from these countries to the European Union amounts to 5.3 million tonnes. In Italy, in particular, most of the imported seafood comes from Thailand, India, China and Vietnam, followed by African countries such as Tunisia, Morocco and then by North America [3].

In recent years viruses have been increasingly considered being the cause of food-borne disease and outbreaks [2,4,5]. A recent report published by EFSA confirmed that viruses are a threat to food safety; according to this report, 20.4% of the causative agents in food-borne outbreaks were found to be viruses [6,7]. Foods usually consumed raw, such as berries and shellfish, as well as some ready-to-eat foods, may be a vehicle for viral enteric infections. Norovirus (NoV) is believed to be the main source of non-bacterial food-borne gastroenteritis outbreaks in humans; more than 40% of cases of NoV infections are linked to food consumption. From an epidemiological point of view, another important food-borne virus is Hepatitis A (HAV). In fact, a WHO document stated that, although several kinds of viruses can be present in bivalve molluscs (BM), only NoV and HAV are responsible for human illnesses linked to the consumption of these molluscs. In addition, one EFSA opinion reported that it could not be excluded that the zoonotic Hepatitis E Virus (HEV), prevalent in pigs, might be present in sea water as a consequence of agricultural drainage and then could reach humans via shellfish consumption. Other enteric viruses such as rotavirus (RV), astrovirus (AV) and Aichi virus can also cause gastroenteritis, but their etiological role in outbreaks is not well defined. There are several production stages during which food can be contaminated. Among the factors causing food contamination are human and animal faecal contamination of the water in which shellfish grow, the use of night soil to fertilize crops, faecal contamination of the water used to wash fruit and vegetables after harvest or poor hand hygiene in infected food handlers. In fact, based on the route of contamination, food contaminated by viruses can be divided into two groups. One group, which is contaminated with enteric viruses in their environment includes BM (such as oysters, mussels and clams) and vegetables; the other group, which includes various kinds of food other than the above mentioned, comprises foods contaminated with enteric viruses, mainly during food processing or food serving by infected food handlers. Filter-feeding shellfish (e.g. BM), which constitute a large proportion of seafood, are an important vehicle for the transmission of food-borne pathogens, including the above-mentioned viruses, particularly when the shellfish grow in sewage-polluted water. As BM are filter-feeding marine organisms, they can accumulate and concentrate pathogens present in water. The global production of marine BM is over 15 million tonnes per year. Asian countries play a dominant role in marine BM production, since 85% of the world's BM comes from Asia. China is by far the largest producer of BM, followed by Japan (0.75 million tonnes per year), South Korea (0.4 million tonnes per year) and Thailand (0.23 million tonnes per year) [3]. North and South America are responsible for 9% of the global marine BM production. Most of the aquaculture production occurs in Chile (mussels and scallops), Peru (scallops), the United States (American and Pacific cupped oysters, hard clams) and Canada (mussels) [3]. Europe is responsible for 5.5% of the global production of marine BM, with an annual production of molluscs approaching 900,000 tonnes [3]. Italy is the third-biggest producer of BM in Europe, with an average production of 100,000 tonnes per year, a large part of which is concentrated in the Apulia region (South-eastern Italy) [7]. The consumption of BM in Europe is in the order of 2.6 kg *pro capita* per year, which amounts to approximately 10% of all seafood consumption [3].

In the European Union, BM are harvested from sea-production areas classified by the competent authority into 3 classes according to the load of human faecal bacterial water pollution. The *Escherichia coli* count (expressed in the Most Probable Number per 100g of BM flesh) is considered to be a marker of faecal pollution: sea water which has the lowest concentration of *E. coli* (max 230 MPN in 90% of the samples, with the rest of the samples not exceeding 4.600 MPN) is categorized as class-A; sea water which has a contamination of *E. coli* that does not exceed 4.600 MPN is categorized as class-B; sea water that exceeds 4.600 *E. coli* MPN but does not exceed 46.000 MPN is categorized as class-C (EU Reg. 627/2019). BM deriving from class-A water, are suitable for human consumption as live bivalve molluscs (LBM) without any further treatment; BM deriving from class-B and class-C water have to be submitted to a purification process in order to reduce the bacterial load (under to 230 MPN *E. coli*/100g), or else, they can be treated in plants (approved by the Competent Authority) under the specific time-temperature conditions required by law (Reg. CE 853/2004).

Viral infection in humans is more likely to occur when BM are eaten raw or undercooked, which is a large spread habit in some European countries including Italy. In fact, no data are available on outbreaks resulting from the consumption of commercially heat-treated shellfish [8], where the generally accepted time-temperature used to inactivate viruses from BM is 90°C for 90 s [8]. Viral contamination in shellfish can lead to an outbreak in the local population; the European Regulation 2073/2005 classifies food-safety criteria related to LBM placed on the market (Reg. CE 2073/2007 and [9]. However, this is based only on a bacterial indicator (e.g. *Salmonella* spp. and *Escherichia coli*), and it may not be correlated with a potential viral contamination.

Enteric viruses, like NoV and HAV, are removed slowly from the body of BM during the depuration process [10]. Some heat treatments might not be sufficient to eliminate HAV from the flesh of BM when the viral load appears high; for this reason, EFSA published a Scientific Opinion aimed at evaluating a heat treatment for eliminating HAV and NoV from LBM harvested from class-B and class-C production areas [8].

Regarding the food-borne risk, LBV which are eaten raw (especially oysters) constitute a high risk in terms of human infections, whereas BM that are consumed eviscerated (such as scallops) or cooked (such as mussels and clams) constitute a low risk [8]. Despite several preventive measures, viral food-borne outbreaks have occurred in many countries. Most of these outbreaks were due to the consumption of raw oysters, although some outbreaks were also found to be associated with cooked oysters. Because of the short shelf life of oysters, most shellfish-borne outbreaks have been limited to a small area, even though multicounty outbreaks have also been reported [11]. NoV-associated outbreaks are quite frequent due to an increased oyster consumption; furthermore, HAV infection is the most serious viral infection linked to shellfish consumption since in that it causes debilitating disease and even death [11,12]. In the EU, the public health problem linked to the contamination of BM by enteric viruses is a very sensitive issue: in fact, according to EFSA, the most important biological hazards linked to the consumption of LBV are NoV and HAV. This review discusses the most common viral causes of these infections and summarizes the general incidence of bivalve mollusc-related viral infections worldwide.

Norovirus

Norovirus (NoV) is a major cause of acute gastroenteritis across the world; it is a highly contagious RNA non-enveloped icosahedral virus belonging to the *Caliciviridae* family and is responsible for acute gastroenteritis in children and adults [13,14]. NoV possess a 7.5 to 7.7 kb positive sense single-stranded RNA genome containing three open reading frames. It has been classified into 10 genogroups, GI-GX, of which GI, GII and GIV have been identified in humans [15]. Based on capsid gene sequences, each group is further classified into several genotypes and strains [14]; GII.4 is the leading cause of acute gastroenteritis worldwide [16]). The high viral diversity observed in NoV, leads to a more difficult implementation of a vaccine and, on the other hand, it makes a strong immunological protection difficult to achieve [17]. NoV infection leads to several blooms of acute gastroenteritis (diarrhoea and vomiting) which usually last for 2 - 4 days. Other symptoms include nausea, abdominal cramps, fever and headache. The disease is generally self-limiting and typically occurs during winter ("winter fever", "stomach flu") [18]. This infection results from eating food contaminated at source (such as LBM, berries and salad) or food contaminated by handlers during processing or serving. Food handlers infected with NoV are a potential source of infection both in sporadic cases and in outbreaks. Water can also act as source of contamination: water contaminated with NoV has caused illness which can last for up to 2 months *post* contamination. Due to its high environmental resistance, person-to-person transmission is also an important means by which the virus can spread. The wide diffusion of NoV in also due to its low infectious dose and its stability in the environment. Because human NoV cannot be cultured *in vitro*, other similar cultivable viruses have been used to establish its infectious dose for humans [19,20]. Studies carried out using these surrogates showed that the dose appears to be very low (Table 1). Regarding NoV environmental resistance, several studies reported that NoV could remain infectious in frozen food for 6 months, in refrigerated foods for 7 days and on fomites for more than a week. It has been observed that it displays stability in depurated shellfish for one week [21,22]. It can remain stable on hands and then be transferred between hands and surfaces through casual contact, which can lead to community transmission. NoV resistance to commercial disinfectants varies according to the chemical nature of the disinfectant; it is not highly resistant to chlorine disinfection. It is also sensitive to autoclaving but shows stability in waters where shellfish grow. The consumption of contaminated shellfish is responsible for around half of the human NoV outbreaks. Both genotype I and II are infectious to humans, but genotype II is more dominant [23]. NoV infections are a leading cause of non-bacterial gastroenteritis due to the consumption of raw or partially cooked shellfish, in particular oysters [23]. The closer shellfish are farmed to sewage-contaminated water, the higher the risk is of pathogen transmission to humans [24]. Among seafood, oysters are the species most often implicated in sporadic cases and outbreaks; this is due to their eating habits as filter feeders, and their ability to concentrate the virus from contaminated water [23]. However, mussels, clams, crabs, prawns, finfish, spider crabs, gooseneck barnacles and blue crabs are also sources of NoV food-borne infections [2].

Characteristics	Description	Reference
Low infectious dose	Estimates of the infectious dose ranges from 18 to 10^3 virus particles	[19]
High shedding titre	Peak shedding ranges from 10^5 to 10^9 particles/g of stool	[65]
Prolonged shedding	Virus can be detected up to 8 weeks after symptom onset, with a median of 4 weeks; even longer durations of shedding may be detected in immunocompromised individuals	[65]
Genetic diversity	Over 30 genotypes (nine GI and 22 GII) infect humans. No long-lasting immunity. Different genotypes can infect humans over their lifetime	[66]
Environmental stability	Norovirus particles may be infectious for 2 weeks on environmental surfaces and for >2 months in water	[67]
Vomiting	Vomiting appears to be a particularly effective route of norovirus spread. Vomiting events may occur and lead to direct transmission (when in public) as well as environmental contamination from vomit droplets	[68]
Transmission through multiple routes	Noroviruses are transmitted via the faecal-oral route and vomit-oral route, and through several specific modes, including foodborne, waterborne, environmental and direct person-to-person spread.	[69]

Table 1: *Infectious dose and some characteristics that facilitate Norovirus transmission.*

The most important NoV outbreaks linked to shellfish consumption

Across the world, there have been reports of several outbreaks related to NoV infection after the consumption of contaminated seafood. It has been reported that half of the cases of food borne gastroenteritis in the US and the European Union (EU) were caused by NoV infection (Table 2). Between November 2016 and April 2017, Canada experienced two outbreaks of NoV: both outbreaks were found to be linked to oysters from British Columbia coastal waters [13]. From 2015 to 2017, the Jinan Centre for Disease Control and Prevention in China observed 15 outbreaks of acute NoV gastroenteritis [25]. In 2015 Finland also experienced an outbreak of NoV infection among lunch customers in a restaurant in Tampere [26]. Infected kitchen staff probably transmitted NoV owing to inadequate hygiene practices. In Australia (in 2014) an outbreak of NoV GII associated with the consumption of oysters was detected by RT-PCR [27]. Two separate oyster-related outbreaks of gastroenteritis were reported in Ireland in 2013-2014; in both of these outbreaks, the NoV concentration in oysters was more than 1000 genome copies/g digestive tissues, and multiple genotypes were involved [28]. In France in 2019, large concomitant NoV associated outbreaks of acute gastroenteritis were reported in several patients, the food-borne events were suspected to be linked to raw shellfish consumption [29]. In France in 2012, a NoV-linked outbreak was linked to the consumption of oysters [30]. In 2011, a NoV outbreak associated with the consumption of oysters was observed in Majorca (Spain) [31]. A NoV-induced gastroenteritis outbreak was observed in England in 2009: around 200 people were affected, and the outbreak was linked to the consumption of shellfish

[32]. The US experienced a widespread increase in acute gastroenteritis outbreaks of NoV in 2006 - 2007. During this period 257 outbreaks of NoV gastroenteritis were identified in various parts of Florida [33]. Between 2009 and 2014 eight NoV outbreaks were reported in the United States: seven of these outbreaks were due to the consumption of contaminated raw oysters, while the eighth was due to partially cooked oysters. These outbreaks were mostly correlated to NoV GII [23,34]. Between 2001 and 2008, 364 outbreaks were reported due to the consumption of various foods, among which molluscs were found to be the cause of 13% of the infections in the US. Between 2000 and 2003 around 14 outbreaks were reported in Chile, mostly due to the consumption of seafood [2]. In Australia, there have been multiple outbreaks due to various strains of NoV. On 26th and 27th December 2019, an unusually high number of food-borne outbreaks, suspected to be linked to the consumption of raw shellfish, was reported in France through the mandatory reporting surveillance system. The patients were primarily showing symptoms of acute gastroenteritis (AGE) [4,29].

Hepatitis A virus

The Hepatitis A virus (HAV) is a non-enveloped 7.5 kb positive-stranded RNA virus of the *Picornaviridae* family, genus *hepatoviridae.* Seven HAV genotypes, named I-VII, are nowadays known [35]. Only one genotype of HAV has been identified globally. Genotypes I and III have been further classified into sub-genotypes A and B. The first documented shellfish-borne outbreak of infectious hepatitis was observed in Sweden in 1955 when 629 cases of which were found to be due to oyster consumption. Humans and several species of non-human primates are the natural reservoir of HAV [36,37]. The mode of transmission is the faecal-oral route [38] and the incubation period is 2 to 4 weeks (but sometimes as long as 45 days) [39,40]. The infection (viral hepatitis) is asymptomatic and goes unnoticed in children under 6 years of age, while older people show mild symptoms. However, the symptoms may be severe in the elderly or in immune-compromised people, and the infection may sometimes appear as fulminant hepatitis that leads to death or to emergency liver transplantation [41]. The clinical symptoms include fever, loss of appetite, malaise, diarrhoea, abdominal pain, and jaundice. The fatality rate, however, is low. HAV is extremely resistant, surviving for several days in conditions simulating the environment exposure and for several weeks in seawater [39]. Moreover, it has strong resistance to acids, being able to survive at pH 1 for 5 hours. Immunization and vaccination against HAV were recommended by the CDC advisory committee on immunization practise in 1996 (CDC, 2007). The most common route of food-borne transmission is the ingestion of faecal contaminated food (especially raw bivalve molluscs and raw vegetables) and water; in addition, poor personal hygiene and inadequate sanitation may lead to the contamination of surfaces and foods [36,39]. Like NoV, the infectious dose for HAV seems to be very low, settling around 10 - 100 viral particles.

The main reported HAV outbreaks linked to shellfish consumption

HAV infection is a global threat, with around 1.4 million cases reported worldwide each year (See table 2). Fifty percent of these cases are reported to occur in Asia. In Italy, 87 hepatitis cases were investigated in order to study the phylogenetic and epidemiological relationship among HAV strains circulating between 1997 and 2015: the most frequent risk factor was found to be the consumption of raw and undercooked shellfish [11]. In the Netherlands, two food-borne hepatitis A outbreaks in 2012 were found to be due to the consumption of mussels [42]. Between 1986 and 2012, about 359 shellfish-borne viral outbreaks were reported in various parts of the world. These infections were linked to seafood vehicles such as oysters, clams, mussels and cockles [37]. In 2007, an oyster associated HAV outbreak occurred in France due to the consumption of raw shellfish and oysters [43]. In the USA, in 2005, a multistate outbreak of HAV which infected 39 people who had eaten oysters was reported. In Spain, in 1999, 184 serologically confirmed cases of HAV were reported, and coquina clams were found to be the vehicle for transmission. In Southern Italy (in the Apulia region), two major outbreaks of HAV occurred between 1996 and 1997: the number of cases in each outbreak was 5673 and 5382 respectively. The consumption of raw shellfish was found to be the main cause of this outbreak. In Australia, in 1997, reported its first outbreak of HAV because of the consumption of oysters. In 1995 an HAV outbreak in Sweden, with 629 cases reported, was found to be due to the consumption of raw oysters [44]. In 1994 another outbreak was observed in the United States during which 26,796 cases were reported. In China, in 1988, raw clams were detected as the source of infection in Shanghai's largest epidemic of HAV; during this epidemic 292,301 cases were reported, 47 of which resulted in death. Moreover, between 1900 and 1950, several outbreaks had been reported in the United States [2].

Year	Pathogen	Number of Afflicted	Food Vehicle	Country	Reference
2016-2017	Norovirus	Two outbreaks of norovirus and acute gastroenteritis took place in Canada between November 2016 and April 2017.	Oysters	Canada	[13]
2009-2014	Norovirus	8 outbreaks	Oysters	US	[34]
2014	Norovirus	Eight cases of gastrointestinal illness in northern NSW	Oysters	New South Wales, Australia	[27]
2014	Norovirus	Two oyster-related illness outbreaks	Oysters	Ireland	[28]
2011	Norovirus	Outbreak in Majorca (Spain)	Oysters	Spain	[31]
2006-2007	Norovirus	257 outbreaks of norovirus gastroenteritis were identified in 39 of Florida's 67 counties. About 44% of outbreaks were laboratory confirmed as norovirus.	Oysters	US	[33]
From June 2015 to March 2017,	Norovirus	From June 2015 to March 2017, fifteen outbreaks of acute gastroenteritis (AGE) were reported to the Jinan Centre for Disease Control and Prevention in China.	Oysters	China	[25]
2011	Norovirus	Norovirus infections associated with frozen raw oysters - Washington, 2011, three of the seven in the party had consumed a raw oyster dish.	Oysters	US	(90) [53]
2012	Norovirus	A norovirus oyster-related outbreak in a nursing home in France, January 2012.Outbreak following a festive lunch which affected 84 (57%) residents and staff members of a nursing home in January 2012 in France	Oysters	France	[30]
2009	Norovirus	An outbreak of gastroenteritis affected at least 240 persons who had eaten at a gourmet restaurant over a period of 7 weeks in 2009 in England Diners were infected with multiple norovirus strains belonging to genogroups I and II, a pattern characteristic of molluscan shellfish-associated outbreaks	Shellfish	UK	[32]
2015	Norovirus	An Outbreak of Norovirus Infections Among Lunch Customers at a Restaurant, Tampere, Finland, 2015	Oysters	Finland	[26]
2009-2912	Norovirus	During 2009-2012, a total of 1,008 foodborne norovirus outbreaks were reported, constituting 48% of all foodborne outbreaks with a single known cause. Outbreaks were reported by 43 states and occurred year round	The most frequently named were vegetable row crops (e.g., leafy vegetables) (30%), fruits (21%), and molluscs (19%).	US	[54]

2010	Norovirus	Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. Since January 2010, 334 cases in 65 clusters were reported from five European countries: the United Kingdom, Norway, France, Sweden and Denmark	Oysters	United Kingdom, Norway, France, Sweden and Denmark,	[55]
2020	Norovirus	**Large concomitant outbreaks of acute gastroenteritis emergency visits in adults and food-borne events suspected to be linked to raw shellfish, France, December 2019 to January 2020.**	Linked to the consumption of raw shellfish	France	[4]
1997-2015	Hepatitis A virus	**Hepatitis A virus strains circulating during 1997-2015 in Campania, a Southern Italy region with periodic outbreaks**	The most frequent risk factor was the consumption of raw/undercooked shellfish (75/87, 86.2%)	Italy	[11]
, 2012	Hepatitis A virus	**International linkage of two food-borne hepatitis A clusters through traceback of mussels, the Netherlands, 2012**	Mussels	Netherlands,	[42]
1999	Hepatitis A virus	One hundred eighty-four serologically confirmed cases of hepatitis A were reported in eastern Spain in 1999. A matched case-control study implicated imported coquina clams complying with European Union shellfish standards as the source of infection;	Clams	Spain	[56]
2007	Hepatitis A virus	Following the notification of nine hepatitis A cases clustered in the Cotes d Armor district in northwestern France, epidemiological, environmental and microbiological investigations were set up in order to identify the source and vehicle of contamination and implement control measures. In total, 111 cases were identified in the outbreak	**Oysters**	France	[43]
2004	Hepatitis A virus	A large outbreak of hepatitis A virus (HAV) infection occurred in 2004 in Campania, a region of southern Italy, with 882 cases reported between 1 January and 1 August.	Shellfish	Italy	[57]
1997-2015,	Hepatitis A virus	87 hepatitis A cases were investigated. The most frequent risk factor was the consuming raw/undercooked shellfish (75/87, 86.2%)	Shellfish	Italy	[11]
2005	Hepatitis A virus	The 39-oyster consumption-related cases of hepatitis A reported in 2005 represent the first large outbreak of hepatitis A associated with shellfish consumption in the United States in >15 years	Shellfish	US	[58]
1960-1989	Hepatitis A virus	Numerous hepatitis A outbreaks were linked to the consumption of raw molluscan shellfish in the United States between 1960 and 1989. at least 10 clusters of hepatitis A illnesses, totaling 39 persons, occurred in four states among restaurant patrons who ate oysters. Epidemiologic data indicated that oysters were the source of the outbreak	Oysters	US	[59]

2001	Norovirus and HAV	A party of 57 people dined together in a restaurant in Hamamatsu City on December 11, 2001. The next day, 22 of them developed symptoms of acute gastroenteritis, such as diarrhea, vomiting, and fever. G	Purple Washington clams	Japan	[60]
1999	Hepatitis A virus	**An important hepatitis A outbreak in eastern Spain in September 1999**	Shellfish	Spain	[61]
2001	Hepatitis A virus	An outbreak of hepatitis A, affecting 183 people, occurred in Valencia (Spain). Epidemiological evidence pointed to an association of the outbreak with consumption of Coquina clams (Donax sp), imported frozen from Peru.	Consumption of Coquina clams (*Donax* sp), imported frozen from Peru.	Valencia (Spain).	[62]
1997	Hepatitis A virus	Between 22 January and 4 April 1997, 467 hepatitis A cases were reported to the New South Wales Health Department, Australia	Oysters	New South Wales, Australia	[63]
1996	Hepatitis A virus	In total, 5889 cases of hepatitis A virus infection were reported during an outbreak in Puglia, a region of Southern Italy, in 1996.	The primary cause of the outbreak was consumption of contaminated food (raw shellfish, vegetables, etc.), with a contributory risk factor of person-to-person transmission	Italy	[64]

Table 2: *Some outbreaks associated with NoV and HAV.*

Hepatitis E virus: A challenge for mollusc consumption?

Food-borne Hepatitis E infections are becoming a problem in some European and non-European countries [45,46]. The Hepatitis E virus (HEV) includes genotypes originating only from humans (HEV-1 and HEV-2), as well as zoonotic genotypes originating from animals, especially pigs (HEV-3 and HEV-4). This infection is generally self-limiting or asymptomatic, but it can result in a severe and sometimes lethal illness, especially in immune compromised [40] and pregnant women (Genotypes 1 and 2) [47]. As HEV is an enteric virus, it is shed in faeces and could thus be present in sea water as a consequence of wastewater discharge from human or zootechnical sewage and it may accumulate in LBV: the consumption of raw seafood and oysters is considered a risk factor for the development of this infections [48]. However, to date, there are no specific guidelines on the reduction of the HEV load in LBM, and the procedures that are known to be useful in lowering the risk to public health in cases of other food-borne viruses should also be applied in cases of HEV.

Conclusion

Shellfish-borne diseases are contributors to the global disease burden and to mortality-rates. Acquiring a better understanding of the impact that shellfish consumption has on food-borne diseases is an important step towards finding ways to mitigate the risks. Shellfish-borne outbreaks can be lethal, particularly where public health measures are inadequate. It could be a problem in areas where there has been a deterioration in water quality. It is important to assess the impact of these outbreaks on public health to enable future predictions, aid, policy actions and to improve the ability to adapt and to handle the infections. Nowadays there is little evidence which supports the growth of NoV and HAV *in vitro*, and so their detection in foodstuff mostly depends on molecular techniques such as RT-PCR [49]. Sequence analysis of the detected strains allows to conduct epidemiological studies on these food-borne viruses [50-52]. In fact, in countries with a relevant seafood production, it has become increasingly important to gather information on virus contamination in shellfish. At a time when shellfish production is predicted to increase both the frequency and intensity of shellfish-borne outbreaks in many areas, it is vital, to a public health perspective, to understand and reduce the impact of these events., It is important to point out that the commonly used treatments on the market for LBM (short-term purification and relaying) are considered effective against contamination by terrestrial bacteria (e.g. *E. coli* and *Salmonella* spp.) but have failed to reduce the number of viruses in oysters. Thus, wastewater surveillance, coupled with a strictly adherence with GMP in the LBM chain, could be widely used for preventing outbreaks. Furthermore, it is imperative to study the molecular epidemiology of these viruses, also using Next Generation Sequencing, and to monitor the emergence of new and more aggressive strains.

Author Contributions

N.G. and P.A., guided and edited the whole manuscript, A.A. wrote the manuscript, C.M.C., T.A., B.D., P.F. and I.A. edited the manuscript. All the authors revised and approved the final manuscript.

Conflict of Interest

There is no conflict of interest among the authors.

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