CHAPTER 7

Shellfish-Associated Enteric Virus Illness: Virus Localization, Disease Outbreaks and Prevention

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1. INTRODUCTION

Enteric viruses are responsible for the majority of foodborne illnesses. These viruses include caliciviruses (classified as noroviruses and sapoviruses); picornaviruses (hepatitis A virus [HAV] and aichivirus); hepatitis E virus (HEV); astroviruses; rotaviruses; enteric adenoviruses; coronaviruses; toroviruses; and picobirnaviruses. The most frequently reported foodborne outbreaks are caused by noroviruses; formerly called the agent of winter vomiting disease, Norwalk or Norwalk-like viruses, or small round structured viruses. Hepatitis A virus is also reported as a cause of foodborne illness albeit less frequently. Children are infected in early childhood with group A rotaviruses, enteric adenoviruses, astroviruses, and caliciviruses and may develop partial immunity against them (Glass et al. 2001). Molluscan shellfish are common vehicles for virus transmission and methods are available for the detection of a wide range of viruses in shellfish as well as in the stools of infected individuals (Le Guyader et al. 2008; Iizuka et al. 2010; Richards et al. 2015; Polo et al. 2015).

Enteric viruses have undoubtedly been infecting humankind since the dawn of civilization; however, techniques to isolate and identify these viruses are still under development. With the advent of sensitive molecular methods, even non-propagable viruses may now be detected. In spite of these advances, reporting of enteric viral illnesses is poor or non-existent in many parts of the world today. Noroviruses are believed to constitute the most frequent cause of foodborne illness; however, only major outbreaks are usually recorded and accurate, quantitative assessment of the number of individuals affected is often not available. Accountability for hepatitis A and hepatitis E infections is important due to the potential seriousness of the diseases. Symptoms of illness caused by enteric viruses vary, depending on the virus and the sensitivity of the infected individuals. In healthy individuals, hepatitis A is often an asymptomatic infection with spontaneous remission, thus the true incidence of hepatitis A infection remains uncertain. The incubation period for hepatitis A is generally 15–45 days and symptoms include nausea, vomiting, anorexia, malaise, fever, jaundice, and abdominal pain usually in the upper right quadrant.
Liver damage can result from an HAV infection. Virus may be shed from infected individuals for up to 5 months (Rosenblum et al. 1991; Robertson et al. 2000). Similarly, hepatitis E can be a serious illness, but is so rare in the United States, that only a handful of cases have been recorded. In Asia and other parts of the world, outbreaks of hepatitis E are frequently encountered. The incubation period for hepatitis E is reportedly from 2 to 8 weeks and early symptoms may include vomiting, malaise, fatigue, anorexia, and low-grade fever ultimately leading to possible spleen enlargement and pain in the upper right quadrant, again from liver involvement (reviewed in Richards 2005). Clinical symptoms generally resolve within 4–8 weeks, except in pregnant women who have a 15–25% mortality rate (Mast and Krawczynski 1996).

Symptoms of human norovirus (NoV) and sapovirus include vomiting, diarrhea, nausea, abdominal cramps, chills, fever, headache, body ache, and can lead to dehydration (reviewed in Richards 2005). The incubation period for NoV illness is 1–2 days after consumption of contaminated food or water. Symptoms usually clear spontaneously after about 2 days. NoV is believed to be the most prevalent cause of foodborne illnesses in the world today. When illnesses are noted, there is seldom epidemiological follow-up to confirm the cause. Most of the illnesses are likely from drinking sewage-contaminated water or the consumption of raw or undercooked foods that are tainted by contaminated water, the hands of food-handlers, or the transfer of viruses from contaminated contact surfaces to the food. The more serious viral illnesses are from HAV and HEV, which can lead to life threatening liver disease and even death. Death is seldom a consequence of NoV and related enteric viruses, although in rare cases, death may result from severe dehydration, particularly in regions of the world where rehydration therapy is not readily available.

Other viruses of interest from a shellfish safety standpoint include rotavirus and astrovirus (reviewed in Richards 2005). Rotaviruses generally produce diarrhea, anorexia, dehydration, occasional vomiting, and dehydration, and are most commonly observed in young children who have not yet developed immunity. Most shellfish consumers would be expected to be immune to rotaviruses from normal childhood exposure. Astroviruses can also be transmitted by shellfish and often produce a mild and self-limiting illness. Symptoms include occasional vomiting, diarrhea, fever, abdominal pain and anorexia. It has a short incubation period (1–3 days) and the illness lasts for up to 4 days. The incidence of illnesses from astrovirus, NoV, sapovirus, rotavirus, and related viral pathogens is underreported partly because these viruses cause illnesses of short duration, and seldom cause mortality or serious long-term illness or disability.

Among the most notable foods that may contain enteric viruses are raw or undercooked molluscan shellfish (oysters, clams, mussels, and cockles). Shellfish accumulate contaminants, including enteric viruses, from their surrounding waters and bioconcentrate them within their edible tissues.
Consequently, some very large outbreaks of HAV and NoV have been reported following consumption of contaminated shellfish. Efforts to document such outbreaks have provided some light on the causes and effects of shellfish-borne disease, but do not convey the magnitude of the problem (Gerba and Goyal 1978; Richards 1985, 1987; Rippey 1994). The latest estimates from the Centers for Disease Control and Prevention (CDC) indicate that NoVs are the most common cause of acute foodborne gastroenteritis in the United States and are responsible for an estimated 5.5 million cases annually (Scallan et al. 2011; Hall et al. 2012). The vast majority of illnesses go undiagnosed and, until recently, outbreak statistics have not been systematically maintained. Improvements in monitoring, such as the development of CaliciNet in the United States in 2009, are contributing to better reporting of NoV outbreaks including the specific genogroup (genogroup I or II) and genotype responsible for the illness (Vega et al. 2011). A National Outbreak Reporting System (NORS) was also established in 2009 to collect information on NoV and other waterborne and foodborne outbreaks according to mode of transmission, etiology and setting (Manikonda et al. 2012). In 2007, a market survey of oysters in the United States was conducted by the U.S. FDA. They detected NoV in 3.9% of the oysters tested and HAV in 4.4% of the oysters (DePaola et al. 2010). Market oysters were also tested in France with NoV detected in 9% of the samples (Schaeffer et al. 2013). In stark contrast, a 2-year study of oysters from 39 commercial harvesting sites in the United Kingdom showed NoV in 76.2% (643 out of 844) of the oysters and all sites tested positive for NoV at least once (Lowther et al. 2012b). This would suggest high levels of seawater contamination in the UK. It could not be determined from the above tests what portions of the viruses in the oysters were infectious and what portions had been inactivated.

Although persons infected with NoV can develop acute vomiting and diarrhea, symptoms are usually fleeting, lasting only a day or two. Consequently, the patients do not seek medical attention because symptoms resolve rapidly and spontaneously. Those who are ill may spread the disease to family members through contamination of surfaces or by handling foods with inadequately sanitized hands. A secondary attack rate among household contacts was reported as 14% in one study (Alfano-Sobsey et al. 2012). Sick individuals often miss work for 2 or 3 days, but when they return, they may still carry the virus and be a source of infection to their workmates (White et al. 1986; Iversen et al. 1987; Haruki et al. 1991; Graham et al. 1994). A study by the CDC indicates that an estimated 5.5 million cases of foodborne NoV occur in the United States each year with 15,000 hospitalizations and 150 deaths (Scallan et al. 2011). Health care and lost productivity costs due to NoV in the United States are estimated at $2 billion annually (Batz et al. 2011).

The scientific literature contains numerous reports of disease outbreaks due to HAV and NoV in shellfish. Epidemiological linkage of an outbreak to a particular source is more difficult for some virus infections due to differences in incubation times. For instance, HAV has an incubation period of approxi-
mately 1 month and sick individuals may not be able to say with any degree of certainty where they ate or what they ate a month earlier. However, larger outbreaks are more likely to reveal the source of infection, whether it is water, food, or from a party or restaurant. Sources of NoV and sapovirus illnesses are easier to track because of the viruses’ relatively short (1–2 day) incubation period. Rotavirus causes diarrhea in infants and young children and, although it may be transmitted by foods, children often develop immunity to rotavirus at an early age. Rotavirus diarrhea may lead to dehydration and vascular collapse, particularly when rehydration therapy is not available. Although rotavirus is transmitted by the fecal-oral route, it is likely that most illnesses are from direct contact with children and fomites, rather than through the foodborne route. Astrovirus is another pathogen that has been difficult to track. Molecular diagnostic methods are now available for astroviruses, which may allow more screening of foods for the virus, especially in outbreak investigations.

2. VIRUS LOCALIZATION WITHIN SHELLFISH

Molluscan shellfish feed by filtering materials out of their surrounding water, a process referred to as bioconcentration. In this process, bivalve shellfish collect contaminants to levels much higher than in their surrounding environment. Estimates are that shellfish can bioconcentrate enteric viruses to 100 times the level in seawater. Materials filtered out of the water constitute food and include viruses, bacteria, algae, and other materials. Bioconcentration is accomplished by the initial filtration of viruses, often adsorbed to particulates, from the water by the gills. From the gills, food is diverted to the mouth where it travels to the digestive tract, which includes the stomach and digestive diverticula. A portion of the food in the digestive tract passes through the shellfish, ending up as feces, but some of the food is taken up by motile phagocytic hemocytes, which pass from the blood stream of the shellfish into and out of the lumen of the gut. It is these motile phagocytic hemocytes that are essential to carrying nutrients required to support the nutritional needs of the shellfish tissues. Viruses in environmental waters are often adsorbed to particulates, making them large enough to be filtered out by bivalves. Once within the digestive tract, viruses may be phagocytized by hemocytes where they are carried to tissues surrounding the gut, including epithelial tissues of the stomach and more distant connective tissues (Le Guyader et al. 2006b; Seamer 2007; McLeod et al. 2009; Richards et al. 2010). Hemocytes are known to contain acidic vacuoles and various digestive enzymes to degrade the complex foods into forms more readily absorbed by the cells of the mollusk. A recent study showed that the duration of virus persistence within the
hemocytes depends on the acid stability of the virus, with HAV, murine noro-virus, poliovirus, and feline calicivirus persisting for 21 days, 12 days, 1 day, and <1 day, respectively (Provost et al. 2011).

Studies on enteric virus retention by shellfish have shown that viruses can persist for periods significantly longer than bacterial indicator organisms, like *Escherichia coli* and fecal coliforms (Cook and Ellender 1986; Power and Collins 1989, 1990). Once within the tissues surrounding the gut, virus elimination by simple passage through the intestines via the feces is no longer an option. Early work on virus localization in the Pacific oyster (*Crassostrea gigas*) was performed in New Zealand using cricket paralysis virus (an insect picornavirus) (Hay and Scotti 1986); the virus was found not only in the lumen of the stomach but also in the stomach epithelium and digestive diverticula following a period of virus uptake. Similar results were observed with human pathogenic viruses. For instance, NoVs were detected in the lumen of the gut of Pacific oysters after bioaccumulation of virus-contaminated seawater (Le Guyader et al. 2006b). Norovirus was also detected in the lumen of the stomach and in epithelial tissues surrounding the stomach and digestive diverticula of Pacific oysters after viruses were bioconcentrated (McLeod et al. 2009; Seamer 2007; Richards et al. 2010). Another study showed the localization of HAV in basal cells of the ciliated epithelium of the stomach and in the hepatopancreas of Eastern oysters (*Crassostrea virginica*) (Romalde et al. 1994). Poliovirus was also shown to have a similar fate upon bioconcentration in oysters (Richards et al. 2010). Thus, the persistence of viruses in contaminated shellfish appears to be related to the sequestration of viruses from the lumen of the gut to tissues surrounding the gut and to hemocytes which may retain viable virus for extended periods.

Shellfish depuration is a commercial process which is widely used worldwide to purge microbes and other contaminants from shellfish (reviewed by Richards 1988). It involves the purging of microbes and other materials from bivalve shellfish by placing them in tanks of clean seawater, often recirculated and disinfected by means of ultraviolet light, ozone, or other means. Depuration is practiced widely throughout Europe, New Zealand and parts of Australia. In the United States, “approved” shellfish growing waters are widespread; thus, depuration is only occasionally practiced. The depuration process is usually performed for about 3 days, although in some places, like New South Wales, Australia, only 36 h of depuration are required. The overall success of the depuration process is determined by reductions in bacterial counts, often using fecal coliform bacteria as indicators. The translocation of viruses from the lumen of the digestive tract to tissues surrounding the tract, and the overall resilience of viruses to the effects of various digestive processes within the hemocytes renders depuration of shellfish relatively ineffective from the context of virus removal.
3. CASE STUDIES

Since reporting of viral illnesses and their association with a particular food are inadequate at best due to poor reporting practices, this section will not attempt to tabulate and list outbreaks by country or food source. Instead, the focus will be on highlighting specific, shellfish-related outbreaks by known shellfish-borne viral pathogens in countries around the globe and to indicate sources of contamination, when known.

3.1. Hepatitis A Virus

The United States has experienced numerous outbreaks of hepatitis A associated with shellfish. Major reported outbreaks date back to 1961 with 459 cases in New Jersey and New York from the consumption of clams; 372 cases in Pennsylvania, Connecticut, and Rhode Island in 1964 from clams; and 293 cases in Georgia, Missouri, New Mexico, Oklahoma, and Texas in 1973 from oysters from Louisiana (reviewed in Richards 1985). Oysters associated with the 1973 outbreaks were consumed raw, but were reportedly obtained from waters that met the bacterial standards of the National Shellfish Sanitation Program (Portnoy et al. 1975; Mackowiak et al. 1976). Flooding of polluted Mississippi River water into oyster growing areas occurred 2 months earlier and may have been responsible for the outbreaks (Portnoy et al. 1975; Mackowiak et al. 1976).

A multistate outbreak of hepatitis A was attributed to the consumption of raw oysters from Florida (Desenclos et al. 1991). The attack rate was calculated at 19 persons per 10,000 dozen oysters consumed in restaurants.

The largest outbreak of hepatitis A on record occurred in and around Shanghai, China, from January through March, 1988. Over 293,000 individuals became ill after eating clams harvested from recently opened mud flats outside of Shanghai (Xu et al. 1992) with 47 deaths reported (Cooksley 2000). Most of the cases were associated with direct consumption of the clams, rather than from person-to-person transmission. Since the incubation period to develop hepatitis A is around 30 days, many people had eaten the clams before any illnesses were apparent. During this same period, factory workers in Shanghai also developed hepatitis A after eating raw and cooked clams (Wang et al. 1990; Halliday et al. 1991; Tang et al. 1991). Since thorough cooking is known to inactivate enteric viruses, it appears that the clams were not fully cooked or were re-contaminated after cooking. Between 1976 and 1985, there were 109 cases of hepatitis A reported in Japan and 11 % were believed to be from consuming raw shellfish (Kiyosawa et al. 1987; Konno et al. 1983). Another study reported 225 cases of hepatitis A in Japan with raw oysters being the likely vehicle for infection (Fujiyama et al. 1985).

In 1997, 467 cases of hepatitis A occurred in New South Wales, Australia, from the consumption of oysters harvested from Wallis Lake (Conaty et al. 2000). One person died and a class action suit was filed on behalf of the victim and those who became ill. Before marketing, the government of New South Wales requires that all shellfish be subjected to the commercial process of depuration.
Depuration has been shown to be effective in eliminating many bacterial pathogens and spoilage organisms from molluscan shellfish, but does not completely eliminate enteric viruses such as HAV and NoVs (Richards 1988; Richards et al. 2010). Long-term relaying may be a better alternative to naturally purging viruses from shellfish. Relaying is when shellfish are removed from marginally polluted growing areas and replanted into clean waters for an extended period, often ≥10 days, to more extensively purge contaminants (reviewed in Richards 1988). This duration provides safer shellfish but some viruses (e.g., HAV) have been shown to persist in a viable state for up to 3 weeks in oysters (Kingsley and Richards 2003).

Europe too has had its share of hepatitis A outbreaks associated with contaminated shellfish. Outbreaks of hepatitis A associated with the consumption of oysters, cockles, and mussels have been reported in England, Wales, and Ireland (Maguire et al. 1992; O'Mahony et al. 1983; and Polakoff 1990). An outbreak of hepatitis A from imported clams, with secondary spread to a public school, was reported in Italy (Leoni et al. 1998). The total cost of one outbreak of hepatitis A involving 5889 cases in Italy was estimated at $24 million while costs to each sick individual were estimated at $662 (Lucioni et al. 1998). Raw mussels and clams were the apparent vehicles of transmission for an outbreak of HAV in Italy and a dose-response relationship was observed between illness and the amount of shellfish consumed (Mele et al. 1989). Spain experienced HAV outbreaks in 1999 with 184 cases from the consumption of clams meeting European Union standards (Sanchez et al. 2002). Clams imported from Peru led to 183 cases of hepatitis A in Spain and the virus was detected in 75% of the clam samples tested (Bosch et al. 2001). A survey of South American imports showed the presence of HAV in 4 of 17 lots of mollusks (Romalde et al. 2001). The outbreak of hepatitis A associated with imported frozen clams led to a call for improved risk assessment to prevent such outbreaks (Pintó et al. 2009).

3.2. Noroviruses

A review of the early literature indicates 6049 documented cases of shellfish-associated gastroenteritis in the United States between 1934 and 1984 (reviewed by Richards 1987). Since no bacterial pathogens were associated with these illnesses and symptomology was consistent with NoV illness, it seems likely that NoVs were the causative agents. One outbreak involved 472 cases of gastroenteritis from the consumption of Louisiana oysters. This outbreak resulted in 25% of Louisiana's one-quarter million acres of shellfish beds being closed, an estimated loss to the industry of $5.5 million, and disruption of harvesting for 500 licensed oystermen (Richards 1985). Some outbreaks were small, such as the one in Florida in 1980 involving only six individuals who ate raw oysters (Gunn et al. 1982). In another case, oysters from a defined area in Louisiana were associated with outbreaks of NoV illness in at least five states: Louisiana, Maryland, Mississippi, North Carolina,
and Florida (Centers for Disease Control 1993). Although these oysters were distributed throughout the United States, outbreaks were identified only in these five states. Identification of the source of contaminated shellfish was facilitated by tags (labels) that had been placed on sacks of oysters indicating, among other things, the location of harvest. Shellfish tagging is commonly required by regulators in order to facilitate shellfish tracking in the event of an outbreak.

The worst period on record for NoV outbreaks in the United States was in 1982–1983 when New York experienced numerous outbreaks associated with raw and steamed clams (Centers for Disease Control 1982; New York State Department of Health 1983) and from oysters (Morse et al. 1986). At least 441 people developed acute gastroenteritis and eight of these individuals subsequently developed hepatitis A as well. Ten outbreaks during the summer were attributed to the illegal harvesting of oysters by an unlicensed digger in polluted waters that were closed to shellfishing along the Massachusetts coast (Morse et al. 1986). Other contaminated shellfish were obtained from Rhode Island waters. Another series of outbreaks in the winter was from clams harvested in New York waters. Negative publicity and the lack of confidence in the safety of local shellfish prompted shellfish dealers to obtain clams depurated in England. Unfortunately, these clams led to over 2,000 illnesses in 14 separate outbreaks in New York and New Jersey over a 3-month period (Richards 1985). These clams, served at a picnic, were responsible for over 1100 cases of NoV illness in one outbreak. The U.S. Food and Drug Administration investigated the outbreaks and concluded that depuration was poorly monitored in plants from which the shellfish were obtained (Food and Drug Administration 1983). Indeed, depuration itself may contaminate shellfish if the waters used for depuration are compromised, as may have occurred in an outbreak of hepatitis A involving 111 individuals in France (Guillois-Bécel et al. 2009).

An outbreak of NoV gastroenteritis occurred in 1983 in Rochester, New York. A survey indicated that 84 (43 %) of 196 people interviewed had NoV-like symptoms after eating “cooked” clams served at a clambake. The clams were harvested off the coast of Massachusetts from waters known to be contaminated by untreated municipal sewage (Truman et al. 1987). This outbreak may have been avoided if the clams had been fully cooked or if the shellfish had been obtained from waters meeting the standards of the National Shellfish Sanitation Program. Several other NoV outbreaks in the United States have been associated with cooked oysters (Kirkland et al. 1996; McDonnell et al. 1997). In an outbreak of NoV gastroenteritis that affected 129 individuals in Florida in 1995, sick individuals had eaten raw, cooked, and what were reported to be thoroughly cooked oysters (McDonnell et al. 1997). Those who ate the so called thoroughly cooked oysters made a subjective judgment on the degree to which their shellfish had been cooked; it is unlikely that thoroughly cooked oysters would cause illness unless they were re-contaminated after cooking, perhaps by dirty gloves used during shucking, by use of contaminated shucking knives, or by placing cooked product on unsani-
tized tables or on NoV-contaminated ice. There was speculation that the source of the NoV contamination was the overboard dumping of sewage in the oyster harvesting area (McDonnell et al. 1997). This is not the first instance when overboard disposal of feces or vomit led to contaminated shellfish beds followed by outbreaks of illness. Kohn et al. (1995) conducted a survey of crew members from oyster harvesting boats and learned that 85% of the boats disposed of sewage overboard. Although this is against regulations, monitoring for compliance is very difficult. Berg et al. (2000) also reported the overboard disposal of sewage by oyster harvesters in Louisiana as the likely source of contaminated oysters in at least two outbreaks. New Zealand experienced a number of oyster-associated outbreaks of NoV illness and overboard disposal of sewage from recreational boats was suggested as a likely source of contamination (Simons et al. 2001). Likewise, an outbreak of oyster-associated NoV gastroenteritis in Canada was suspected to be from contamination by an ill harvester (McIntyre et al. 2012).

A study by the CDC (Hall et al. 2012) gave a breakdown of foodborne NoV outbreaks in the United States from 2001 to 2008; a total of 2,922 outbreaks were reported of which 13% were attributable to shellfish, 16% to fruits and nuts, and 33% to leafy vegetables (Hall et al. 2012). A comprehensive review of world literature from 1980 to 2012 showed that shellfish were responsible for an estimated 359 viral outbreaks of which 83.7% and 12.8% were ascribed to NoV and HAV, respectively, with oysters causing an estimated 58.4% of the illnesses (Bellou et al. 2013).

Other countries have also experienced shellfish-associated NoV outbreaks. For example, a widespread outbreak of NoV illness involving over 2,000 people occurred in Australia in 1978 and was subsequently linked to oyster consumption (Murphy et al. 1979; Grohmann et al. 1980). Another outbreak in Australia affected 25 of 28 people who ate raw oysters at a hotel (Linco and Grohmann 1980). In response to these outbreaks, in 1981, the government of New South Wales, Australia, implemented regulation requiring that all shellfish be subjected to depuration (Ayers 1991). A study was commissioned to determine whether depurated oysters from two sites in Australia would cause illness in human volunteers (Grohmann et al. 1981). Depurated oysters from one site produced NoV illness in 52 people but those from the second site did not. Oysters were also the presumptive vehicle of NoV transmission to residents of New South Wales and Queensland in a 1996 outbreak involving 97 cases (Stafford et al. 1997). More recently, an outbreak involving 306 cases of NoV illness were reported from oysters in Tasmania (Lodo et al. 2014).

In Japan, both oysters and clams have been associated with NoV outbreaks. A study of 80 outbreaks of acute gastroenteritis from 1984 to 1987 revealed that 53 were associated with the consumption of oysters (Sekine et al. 1989). Another study reported five outbreaks of NoV illness from eating raw oysters (Otsu 1999). In a review of NoV outbreaks in Okayama, Japan, over a 5 month period, 9 of 46 outbreaks (20%) were attributed to shellfish (Hamano et al. 2005). A study involving 286 fecal specimens from 88 oyster-associated out-
breaks of illness in Japan showed that NoV was associated with 85 out of 88 (96.6 %) of the outbreaks, and 197 of 286 (68.9 %) of the fecal specimens analyzed were positive for NoV (Iritani et al. 2014). Clams imported from China caused 22 cases of NoV gastroenteritis and four cases of hepatitis A in Japan (Furuta et al. 2003). Chinese clams imported into the United States and served in a restaurant were associated with five cases of NoV illness in New York (Kingsley et al. 2002b). Regulations required that the clams be cooked before import to the United States. Although these clams were labeled as cooked, they had the appearance of raw product. Molecular analyses detected both NoV and HAV in the clams, although no hepatitis A cases were reported. Extremely high levels of fecal coliforms were also detected in the clams (Kingsley et al. 2002b).

NoV outbreaks in Europe have also been reported. Cockles were linked to an early outbreak of NoV (Appleton and Pereira 1977). Mussels were responsible for an outbreak at a national convention in the United Kingdom and a dose response relationship was noted (Gray and Evans 1993). English oysters that had been depurated and served at a birthday party caused nine cases of NoV gastroenteritis (Ang 1998). Oysters from France were associated with NoV illness in 127 French and 200 Italian consumers (Le Guyader et al. 2006a). Contamination of the oysters was associated with a heavy rain event. Lowther et al. (2012a) reported a dose-response relationship between NoV contamination level and infection risk. Oysters implicated in an outbreak contained significantly higher levels of NoV RNA than oysters taken from commercial production areas where outbreaks were not observed.

3.3. **Hepatitis E Virus**

Like other enteric viruses, infection with HEV occurs via the fecal-oral route. It is a major cause of epidemic as well as sporadic viral hepatitis in endemic regions of Asia, the Indian subcontinent, Africa, and the Americas (Arankalle et al. 1994; Balayan 1997; Velazquez et al. 1990; Clayson et al. 1997). Hepatitis E is less frequently detected in Europe and only a handful of cases have been reported in the United States. In some developing countries, HEV may account for over 50 % of acute viral hepatitis cases (Balayan 1997; Clayson et al. 1997). Like HAV, HEV normally causes an acute, self-limiting disease with a low mortality rate; however, during pregnancy mortality rates between 15 and 25 % have been reported (Mast and Krawczynski 1996). Epidemiological studies have shown that transmission of HEV occurs predominately by ingestion of contaminated water (Arankalle et al. 1994; Balayan 1997), with low incidence of person-to-person or foodborne transmission established to date. Shellfish consumption was considered a risk factor for sporadic cases of hepatitis E in Eastern Sicily (Cacopardo et al. 1997) and undercooked cockles and muscles were associated with HEV infection in India (Tomar 1998). Epidemiological follow-up is difficult with this virus because of a 15–60 day incubation period and the sporadic distribution of illnesses. To date, no large outbreaks of shellfish-associated hepatitis E have been reported although it
should be considered a potential emerging pathogen which may in time pose a more serious threat in the United States and other countries. A study of 286 shellfish samples collected from environmental sites impacted by pigs, wild boars and human waste in France failed to show the presence of HEV (Grodski et al. 2014), even though HEV is known to be zoonotically associated with pigs. In Scotland, 36 of 39 mussels (92%) from the West Coast and 5 of 9 (55%) mussels from the East Coast were reportedly positive for HEV, primarily genotype 3, at levels between 3.7 and 5.2 log_{10} RT-PCR units per ml (Crossan et al. 2012). Genotype 3 HEV was also detected in 2 out of 32 packages (1.6%) of freshwater bivalves (Corbicula japonica) obtained from a fish market in Japan (Li et al. 2007). The source of the contamination was speculated to be from wildlife, possibly deer and wild boar.

4. DISEASE PREVENTION

4.1. Routine Monitoring and Regulations

The United States and the European Union (EU) have implemented criteria for the harvesting and processing of molluscan shellfish. Under the guidelines of the National Shellfish Sanitation Program (Anon. 2011), shellfish harvesting in the United States has been historically based on water quality criteria derived from sanitary surveys of shellfish growing water. The surveys are based on the levels of total or fecal coliforms in the water and are determined during periodic water sampling and testing. Water testing has served the country well since its implementation in 1925 (Frost 1925). Sanitary surveys were originally undertaken to reduce the incidence of typhoid fever among shellfish consumers and a successful outcome was achieved. Today, shellfish growing waters are classified as approved, conditionally approved, restricted, conditionally restricted, or prohibited, depending on the level of coliform contamination.

According to the National Shellfish Sanitation Program guide (Anon. 2011), shellfish obtained from waters with a most probable number (MPN) of fecal coliforms <14/100 ml are classified as approved for shellfish harvesting and direct sale. Shellfish waters are classified as restricted if the fecal coliform levels are under 88/100 ml, while shellfish are prohibited from harvest when the waters have an MPN >88 fecal coliforms/100 ml. Since water classification is an ongoing process and the history of a site can be determined by an examination of past data, some areas with intermittent contamination may be classified as conditionally approved and conditionally restricted. Such waters come under a management plan and shellfish are permitted to be harvested for direct sale or for depuration/relaying when the criteria of the plan are met. Shellfish from restricted areas can be harvested only if they are subjected to depuration or relaying before they enter the marketplace. Shellfish from prohibited areas may never be harvested or marketed.
In contrast, the EU follows Council Directive 91/492/EEC (Anon. 1991), which regulates shellfish based on the levels of fecal coliforms or *E. coli* in the shellfish meats, rather than in the shellfish growing waters. Under this system, shellfish meats are classified in one of four categories: A, B, C, or D, as shown in Table 7.1. The number of fecal coliforms and *E. coli* are determined by MPN and the results are reported per 100 g of shellfish meat. The differences between the US standard, which is based on water quality criteria, and the EU standard, which is based directly on shellfish quality criteria, have led to some disagreement between government regulators of the two regions. However, both standards contribute significantly to the reduction of shellfish-borne illness from bacterial contaminants although their effectiveness in reducing viral illnesses remains unknown. There are many shellfish growing waters in the US that are perceived to be clean enough for direct harvest and sale of shellfish, whereas, shellfish meats are seldom clean enough in the EU for direct shellfish harvest and sale. As a consequence, most shellfish in the EU must be depurated or relayed before they are marketed. In contrast, depuration is seldom required in the US. Regardless of which standard is used, the levels of fecal coliforms are not a good indicator of the virological quality of shellfish, because enteric viruses persist longer than coliforms within shellfish tissues and they depurate poorly. Therefore, reliance on coliforms as a predictive index for virus presence is not very effective. Only when coliform levels are high do the standards prevent the direct sale of potentially virus-laden shellfish. Unfortunately, viruses tend to be more resilient to the effects of sewage treatment processes and environmental stressors than coliforms and hence water containing low or negligible levels of indicator bacteria may still contain high levels of enteric viruses.

Shellfish growing waters are often impacted by the disposal of sewage from commercial and recreational vessels (Kohn et al. 1995; McDonnell et al. 1997; Simons et al. 2001) leading to sporadic contamination events that are difficult to assess by either the US or EU methods. In a Florida outbreak, the attack rate for HAV in seafood establishments was estimated to be 1.9 per 1,000 dozen oysters eaten (Desenclos et al. 1991). Such low-level contamination would likely miss detection using the EU meat standard, because of the low

| Classification          | Fecal coliform limit | *E. coli* limit |
|-------------------------|----------------------|-----------------|
| A—sell without processing | <300 MPN/100 g       | <230 MPN/100 g  |
| B—depurate or relay     | <6,000 MPN/100 g     | <4,600 MPN/100 g|
| C—prolonged relay       | <60,000 MPN/100 g    | NA              |
| D—prohibited            | >60,000 MPN/100 g    | NA              |

NA: not applicable

Table 7.1 Council directives for the production and marketing of shellfish according to European Union standards based on fecal coliform or *E. coli* levels in the meats (Anon. 1991)
number of samples tested, the likely randomness of contamination, and the lack of correlation between coliforms and enteric viruses within the meats. In contrast, the utility of the water standard is also limited by the lack of correlation between coliforms and viruses, the generally lower numbers of coliforms (and viruses) in the water compared to the meats, and the lack of homogeneity of the water due to tides, winds, currents, and point source contamination events. One benefit of the water standard is that over many years of monitoring, the history of a particular water body becomes known such that predictions may be made for areas that are likely to be hot spots for fecal contamination versus those that are more likely to be less problematic.

4.2. Enhanced Monitoring and Enforcement

Monitoring of shellfish and their harvesting areas coupled with enforcement of regulations are both essential to shellfish safety. A number of areas are in need of better monitoring and enforcement if outbreaks are to be curtailed. Tighter enforcement of laws against dumping waste in shellfish harvesting areas would reduce the incidence of enteric virus illness. An area in need of enhanced monitoring is the illegal practice of harvesting shellfish from closed areas, a practice called poaching or bootlegging. Some outbreaks have been attributed to the sale and consumption of poached or bootlegged shellfish (Morse et al. 1986; Desenclos et al. 1991). Typically, the penalties for those who perpetrate such crimes have been relatively small. According to US and EU guidelines, all lots of shellfish must contain tags (US) or health marks (EU), which label the lot with information that allows the shellfish to be tracked to their source. This is important in outbreak investigations as health authorities seek epidemiological evidence to curb the spread of disease. Enhanced monitoring of tags and health marks would serve as a deterrent against poachers.

Tighter enforcement of import laws are needed to restrict the importation of tainted shellfish. Shellfish exported from China, England, Ireland, Peru, and many other countries have been apparent vehicles of enteric virus illness. Exporting countries are required to subscribe to the standards in place for the receiving country. Transactions are often sealed with a memorandum of understanding (MOU) between the exporting and importing nations. Failure to comply with the MOU would impose dire consequences upon the exporting country, including the withdrawal of the MOU in cases which show wanton disregard for the requirements of the agreement. Harvesters, processors, and shippers should meet criteria deemed necessary to ensure the safety of their merchandise. Hazard analysis critical control point (HACCP) plans should be in place to monitor factors that are important in ensuring shellfish safety. Practices to restrict the presence of fecal pollution in shellfish will likely reduce the incidence of bacterial and enteric viral illness in shellfish consumers, although direct measure of the benefits in regard to possible viral loads is difficult to ascertain.
4.3. Improved Sewage Treatment

Another intervention to reduce virus levels in shellfish would be to improve upon sewage treatment plants and septic systems, particularly in coastal regions near rivers, lakes, and shellfish-growing areas. Adequate monitoring and maintenance of treatment facilities are important to reduce viral loads emitted into the environment. The US routinely chlorinates effluent wastewater and this practice has some penetrating effects on particulate matter that contains potential pathogens. After treatment, the chlorine may be inactivated with sodium thiosulfate. In contrast, the EU often uses ultraviolet irradiation to treat sewage effluent. The lack of penetrating ability, particularly in turbid water or in water containing particulate matter, and the lack of any residual properties imparted by the UV would be expected to allow some viruses and bacteria to escape inactivation. The technology is available to eliminate or substantially reduce enteric viruses from sewage; however, few if any engineers have designed sewage treatment facilities with virus reduction in mind. Treatment plant maintenance and operation should be tightly controlled so that the facility works at its optimal efficiency. Controls should be in place to prevent or reduce accidental releases of untreated sewage during flooding events.

4.4. Analytical Techniques

Monitoring for viruses in water or shellfish is encouraged using molecular methods, namely reverse transcription-polymerase chain reaction (RT-PCR). New RT-PCR protocols continue to be developed along with improved methods to extract the viruses from water and shellfish (reviewed in Richards et al. 2015). Unfortunately, such methods are limited in their practical application because they fail to differentiate infectious from non-infectious viruses (Richards 1999). Direct assays for infectious viruses would be desirable; however, wild-type HAV, HEV, NoVs, sapoviruses and astroviruses have been either difficult or impossible to propagate or to assay in common laboratory animals. Recently, a method was described for the propagation of NoV in mice but it needs further validation (Taube et al. 2013). Even more recently, Jones et al. (2014) published a promising study reporting the successful propagation of NoV in a human lymphoblastoid B cell line. NoV replication occurred in the presence of enteric bacteria that express histo blood group antigens where viral genome copy numbers increased up to 25-fold by 5 days post infection. Viral structural and non-structural proteins also increased. They also reported a nearly 600-fold increase in viral genomes in a co-culture of human lymphoblastoma B cells and HT-29 intestinal epithelial cells after 3 days (Jones et al. 2014). Validation of their procedures is likely underway. Another technique which offers some promise for identifying infectious NoV is based on the binding of infectious NoVs to porcine mucin followed by quantitative RT-PCR (Dancho et al. 2012). NoV that was inactivated by heat, ultraviolet light or high pressure processing failed to bind to porcine mucin.
The porcine mucin binding assay coupled with quantitative RT-PCR may be a breakthrough in the search for a method to identify infectious NoVs in extracts obtained from shellfish and other foods. Many strains of wild-type HAV can be difficult to propagate in cell culture, and cell culture systems for HEV remain elusive. New virus propagation assays are needed to adequately assess shellfish safety from a virological perspective.

4.5. Processing Strategies
As mentioned in section 2.0, shellfish depuration is only minimally effective in reducing enteric viruses due to virus migration from the gut to tissues surrounding the gut. Long-term relay of shellfish from marginally contaminated waters to clean waters is more likely to render shellfish safer (Richards 1988); however, the exact duration required for relay is uncertain and is dependent on many factors including seawater temperature, shellfish species, level of initial contamination, virus type, etc. Intervention strategies to reduce or eliminate enteric virus contamination in shellfish should be implemented on multiple fronts and lessons from previous outbreaks should be heeded. Perhaps the simplest intervention available to consumers is cooking. In most outbreaks, raw or lightly cooked mollusks appear to be the primary vehicles of infection. Alternative processing strategies, like irradiation and high hydrostatic pressure processing, have been proposed. The high levels of irradiation required to inactivate enteric viruses impart undesirable flavor characteristics to shellfish meats. On the other hand, high hydrostatic pressure processing for 5 min was shown effective in inactivating 7-log10 of HAV and feline calicivirus, a surrogate for NoVs (Kingsley et al. 2002a). High pressure inactivates viruses by denaturation of capsid proteins (Kingsley et al. 2002a) and sanitizes the shellfish from bacterial pathogens and spoilage organisms as well. Human NoV was successfully inactivated in oysters using high pressure processing, as determined through clinical trials using pressure-treated oysters (Leon et al. 2011).

4.6. Disease Reporting and Epidemiological Follow-Up
Improved reporting and epidemiological follow-up are needed to understand the magnitude of enteric virus outbreaks and to stop outbreaks once they occur. Such reporting has been effective in Italy where 35 participating local health units link incidence notification with serology and follow-up questionnaires in their surveillance for HAV (Mele et al. 1986, 1997). In a survey of ten EU countries, eight had national databases for hepatitis A statistics (Lopman et al. 2002). Likewise, the CDC has maintained statistics on reported cases of hepatitis A in the US. Although some countries maintain statistics on the number of cases of hepatitis A reported, few determine the source of the illness due to the high cost for epidemiological follow-up. NoV illness is not a notifiable disease in most countries, meaning that there are no formal, mandated systems requiring that illnesses be reported.
4.7. **Hygienic Practices**

Most outbreaks of shellfish-associated viral illness appear to be from shellfish contaminated within their natural environment. However, some cases, particularly those involving cooked shellfish, may actually be from product contamination by shuckers, handlers, or fomites. The contamination of foods by unsanitized hands of food handlers has led to numerous outbreaks of hepatitis A and NoV (Richards 2001). Better enforcement of hand washing practices may prevent some potential outbreaks from becoming a reality. Likewise, sanitary standards generally applied in the food industry should be enforced in the shellfish industry, especially on harvesting boats, in processing plants, transport facilities, and restaurants. Better education and monitoring of food handlers are needed to ensure compliance with food sanitation requirements.

5. **SUMMARY**

Numerous outbreaks of shellfish-borne enteric virus illness have been reported worldwide. Most notable among the outbreaks are those caused by NoV and HAV. Lessons learned from outbreak investigations indicate that most outbreaks are preventable. Anthropogenic sources of contamination will continue to invade shellfish growing waters. Shellfish, by their very nature, will continue to bioconcentrate these contaminants, including enteric viruses. There is no quick fix for enteric virus contamination of shellfish; however, vigilance on behalf of the industry, regulatory agencies, and the consumer could substantially reduce the incidence of illness. Enhanced monitoring in all areas of shellfish production, harvesting, distribution, and processing would help to reduce viral illnesses. Pollution abatement and improved hygienic practices on behalf of the industry and consumers are needed. Improved analytical techniques for the detection of enteric viruses in shellfish will lead to enhanced shellfish safety and better protection for the consumer and the industry. Better reporting and epidemiological follow-up of outbreaks are keys to reducing the transmission of foodborne viral infections. It is anticipated that recent advances in analytical techniques, particularly for NoV, will lead to better monitoring capabilities for food and water and a reduction in the incidence of enteric virus illness among shellfish consumers.

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Shellfish-Associated Enteric Virus Illness…

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