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MINIREVIEW

Environmental Transmission of Human Noroviruses in Shellfish Waters

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Human noroviruses (NoV) are the most common cause of epidemic gastroenteritis following consumption of bivalve shellfish contaminated with fecal matter. NoV levels can be effectively reduced by some sewage treatment processes such as activated sludge and membrane bioreactors. However, tertiary sewage treatment and substantial sewage dilution are usually required to achieve low concentrations of virus in shellfish. Most outbreaks have been associated with shellfish harvested from waters affected by untreated sewage from, for example, storm overflows or overboard disposal of feces from boats. In coastal waters, NoV can remain in suspension or associate with organic and inorganic matter and be accumulated by shellfish. Shellfish take considerably longer to purge NoV than fecal indicator bacteria when transferred from sewage-polluted estuarine waters to uncontaminated waters. The abundance and distribution of NoV in shellfish waters are influenced by the levels of sewage treatment, proximity of shellfish beds to sewage sources, rainfall, river flows, salinity, and water temperature. Detailed site-specific information on these factors is required to design measures to control the viral risk.

In many countries, areas used for harvesting of filter-feeding bivalve shellfish for human consumption are situated in inshore coastal environments which may receive significant quantities of human fecal pollution from point-source discharges, land runoff, and overboard disposal from boats (1, 2). Over the last decades, significant investment has been made in improving sewerage infrastructure in many parts of the developed world (3, 4). However, as treatment levels have improved, contamination due to storm overflow discharges has become a more significant factor (5, 6). These impacts are likely to increase in the future in line with population growth and urban development in coastal areas and changes in rainfall patterns associated with climate change (7). Contamination of shellfish-growing waters with fecal pollution is a significant concern because of the risk of illnesses associated with such shellfish and the regulatory and market requirements for supply of safe shellfish products to consumers.

Human noroviruses (NoV) are the most frequently implicated etiological agents of sporadic cases and community outbreaks of viral gastroenteritis associated with the consumption of shellfish contaminated with fecal pollution (8, 9, 10, 11). Noroviruses belong to the family *Caliciviridae* and comprise five genogroups on the basis of sequence similarity; of these, genogroup I (GI) and genogroup II (GII) are more frequently associated with human outbreaks (12). Each genogroup is further divided into a number of genotypes on the basis of pairwise distribution (12). Although GII, particularly the GII.4 genotype, predominates in person-to-person transmission, GI strains cocirculate in human populations and are also commonly involved in shellfish-related outbreaks (13). Morphologically, NoV are nonenveloped, icosahedral viruses with a diameter of approximately 38 nm (14). The NoV genome is a 7.5-kb, positive-sense, single-stranded RNA containing three open reading frames that encode both structural and nonstructural proteins (14). The capsid protein, viral protein 1, is the most important component of the viral capsid, whereas the viral protein 2 is incorporated into the capsid in low copy numbers (14). Studies have shown that expression of human histoblood group antigens (HBGAs) is linked to susceptibility to NoV infection and that the viral capsid may have evolved from selective pressure of HBGAs (15, 16, 17). Changes in the protruding and immunogenic domain of the capsid protein sequence result in the emergence of new variants of the virus based on immunogenic evolution (18). For instance, adaptive changes in the capsid P2 domain may have caused the epidemic emergence of a novel GII.4 variant (Sydney 2012) and its replacement of New Orleans 2009 as the predominant NoV strain in circulation globally (19).

The literature contains abundant case and outbreak reports of NoV infection associated with the consumption of bivalve shellfish (20, 21, 22, 23, 24). Such outbreaks continue to occur on a regular basis worldwide (23, 24, 25) with potentially very significant associated economic costs. Batz et al. estimated that the annual cost of illness attributed to seafood contamination with NoV in the United States would be $184 million (26). However, the public health burden of NoV infection due to shellfish is probably significantly underreported as most healthy adults experience relatively mild symptoms which frequently remain undiagnosed (20, 27). NoV is the most common cause of illness in cases presenting to general practice (two consultations per 1,000 person-years) in the United Kingdom (28) and the cause of 7% of hospital admissions (all ages considered) in the United States (29). Despite increases in knowledge about NoV disease and transmission in recent years, there is very little understanding of the role of environmental transmission of the virus and what impacts disease incidence (30).

Historically, the most widely used interventions to control the

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risk of shellfish-borne disease have been the classification of production areas based on the monitoring of fecal indicator bacteria (FIB), such as *Escherichia coli*, in shellfish or their growing waters and postharvest purification treatments. However, evidence from laboratory studies has demonstrated that NoV may persist for much longer periods than FIB in actively filtering shellfish (31). The implication is that legislative standards based on FIB may not provide an accurate estimate of the risk of contamination by the pathogen and therefore may not adequately protect consumers (32, 33). Agencies responsible for protecting and promoting food safety worldwide have considered options to reduce the risk of NoV infection due to shellfish. The risk management measures considered include better environmental controls to prevent transmission pathways through to direct monitoring and control by introduction of NoV food safety standards.

Monitoring for NoV within complex environmental matrices such as sewage, freshwater, seawater, and shellfish requires detection and quantification methods that are sufficiently sensitive to detect low quantities of the virus. Since NoV cannot be cultivated, the application of sensitive molecular biological techniques, in particular, PCR, has been essential. Quantitative reverse transcription-PCR (qRT-PCR) is highly sensitive, specific, and cost-effective and has provided valuable information for risk assessment purposes (34, 35). A fully standardized PCR method is now available for detection of NoV in shellfish (36, 37). The application of such methods has provided a wealth of information about the environmental fate and distribution of NoV which would not otherwise have been available (34, 35). However, challenges remain, particularly concerning the assessment of the viability of detected virus, the application of such methods to less-well-characterized environmental matrices such as seawater, sewage, and sediments, and the potential presence in samples of substances inhibitory to the PCR which can affect sensitivity and quantitation (38).

This paper reviews available information on environmental pathways of NoV contamination of shellfish waters and identifies gaps in the knowledge required to provide improved risk management strategies.

CONTAMINATION SOURCES

Sewage discharges. Significant quantities of NoV can be introduced into the marine environment from the discharges of municipal and private wastewater treatment works (39, 40), from smaller-scale septic tanks (41, 42), and from the overflows from such systems (43). Discharges may be directly introduced into shellfish waters or into watercourses higher in the catchment. Clinical NoV strains (GI and GII) traced in environmental samples have clearly demonstrated the links between NoV in sewage effluents, freshwater, and shellfish and gastrointestinal illness (44, 45, 46).

Infected symptomatic humans can shed up to 10^9 NoV genomic copies/g feces as measured by qRT-PCR (47). NoV can also be shed asymptomatically by infected hosts for over 35 days (48). A comparison of mean NoV loads from symptomatic and asymptomatic individuals in Japan showed that GI-infected symptomatic individuals had slightly higher mean viral loads than GI-infected asymptomatic individuals, whereas GII-infected symptomatic and asymptomatic individuals had similar mean viral loads (49). Children and immunocompromised individuals usually shed virus for longer periods than healthy adults (50). However, there is heterogeneity in the duration of shedding. Milbrath et al. developed a NoV transmission model based on NoV-shedding data and concluded that long-term (105- to 136-day) shedders increase the probability of an outbreak by 33% and the severity of transmission (as measured by the attack rate) by 20% (51). Therefore, knowledge of the duration of NoV shedding can help inform NoV risk management strategies.

In England, the age-adjusted community incidence of NoV-associated infectious intestinal disease is estimated to be 4.7/100 person-years (28, 52), which represents an average of 3 million disease episodes and 130,000 consultations per year (28). Untreated (screened) sewage has been found to contain mean levels of total NoV (GI plus GII) ranging from 10^2 to 10^4 genome copies/ml (53). Using data on NoV infection in susceptible volunteers to establish quantitative relationships between the ingested dose and the risk of infection, Teunis et al. (54) estimated the 50% infectious dose (ID_{50}) of NoV to be 18 viruses for nonaggregated GI NoV and indicated that the state of virus aggregation also affects the infectious dose. Clearly, therefore, crude sewage and, potentially, other sources of untreated human feces represent a very high risk for NoV contamination of the marine environment. The risk of sewage containing NoV is likely to be linked to the population size contributing to the effluent. The high rate of incidence of NoV in the community determines that, in practice, municipal sewage is inevitably contaminated irrespective of symptoms shown by infected individuals (55).

In many countries with an aging sewer infrastructure, the effects of surface runoff and the foul sewer may be combined. Designed overflows (i.e., combined-sewer overflows [CSO] and storm tank overflows) connected to such systems discharge a combination of rainwater, untreated human sewage, and, in some cases, industrial waste and debris during periods of wet weather when the treatment capacity of sewage treatment facilities is exceeded (7, 56). In addition, the sewer infrastructure may unintentionally discharge raw sewage as a result of blockages, structural and mechanical failures, or insufficient conveyance capacity. Such sanitary-sewer overflows (SSO) may present exposure pathways different from those associated with CSO discharges. In one study, levels of NoV (GI plus GII) in CSO effluents (settled storm tank overflow) averaged 1,690 copies/ml and were similar to NoV levels in screened raw influent at the works (53). Average levels of NoV in the proximity of CSO outfalls can increase 10 or more times during wet weather (43). Shellfish collected near discharging CSO outfalls may contain mean NoV levels of 10^3 PCR units/g oyster tissue (G. Greening, unpublished data). Thus, CSOs and SSOs may be a very significant source of potential NoV contamination of shellfish waters (43, 57, 58). The frequency and volume of CSO discharges are critical and are determined by the amount of rainfall in relation to the planned capacity of the wastewater system. More-frequent extreme rainfall events, as predicted by climate change modeling (59, 60), are likely to increase sewage inflows and infiltration and thus CSO discharges to coastal waters. Significant investment in stormwater storage and treatment and flow control systems will be required to reduce the impact of these discharges upon shellfisheries (7, 60).

Various studies have suggested that conventional biological sewage treatment processes are less efficient at removing NoV than at removing FIB (53, 61). Quantitative studies on NoV reduction throughout the different stages of sewage treatment are summarized in Table 1. Conventional activated sludge processes (including reductions during primary settlement) are typically

TABLE 1 Norovirus removal achieved by sewage treatment processes reported in the literature

Sewage treatment process	Log_{10} removal rate: Genogroup I	Log_{10} removal rate: Genogroup II	Reference(s)
Untreated sewage			
Settled storm sewage overflow	0.5	0.3	53
Primary treatment			
Primary settlement	0.2–0.7	0.7–0.8	63
Secondary treatment			
Waste stabilization pond	1.2 (maximum, ∼4.5)[a]	1.8 (maximum, ∼6)[a]	62
Conventional activated sludge	0.3–0.9	0.3–1.6	63, 64
Optimized activated sludge (modified Ludzack-Ettinger)	1	0.8	53
Trickling filter	0.0	0.3	63
Tertiary treatment			
Membrane bioreactor	1.5–3.3		65
UV disinfection	maximum > 0.8	0.1	53, 65
Chlorine disinfection	0.74		65

[a] Data were obtained from a graphical data display.

found to reduce NoV by 1 to 2 $\log_{10}$ units across the process. An optimized activated sludge system with tertiary ultraviolet (UV) treatment was found to reduce levels of NoV by 2 $\log_{10}$ for GI and 2.9 $\log_{10}$ for GII (53). The UV treatment stage of this process was inefficient for reducing NoV as measured by PCR (0.11$\log_{10}$) compared with *E. coli* levels (2.7 $\log_{10}$). This may reflect the inability of PCR to determine reductions in virus viability during the UV disinfection process. This possibility is supported by recent data for FRNA bacteriophage used as a surrogate for NoV during sewage treatment (58). Waste stabilization ponds are low-cost, low-maintenance, natural-treatment systems that can reduce levels of NoV GI and GII in sewage by 1.2 $\log_{10}$ and 1.8 $\log_{10}$, respectively (62). Their overall performance at reducing the virus levels is generally lower than that of activated sludge and membrane systems (62). Hewitt et al. reported variable and sporadic occurrences of NoV in sewage and treated effluent from sewage treatment works (STW) serving different-sized populations in New Zealand and reported that median concentrations of the virus in treated effluent were sometimes greater than those in the influent (66). In general, the amount of NoV associated with suspended solids appears to influence removal rates during sewage treatment. Hejkal et al. found a substantial (92%) decrease in the proportion of enteroviruses associated with solids larger than 0.3 μm in diameter from influent samples to unchlorinated effluent (67). These results suggest that viruses associated with solids, and any viruses that are secondarily adsorbed to mixed-liquor-suspended solids, would be removed during the clarification stage. Further studies are required to determine the distribution of NoV associated with solids for a range of treatment processes.

Regarding tertiary treatment, reductions of 4 $\log_{10}$ units have been reported for murine NoV (a cultivable surrogate for NoV) in collimated-beam bench-scale experiments in which the virus was exposed to ultraviolet light doses of 29 mJ/cm^2 (68). Lee et al. found that UV doses of 10, 20, and 25 mJ/cm^2 cause murine NoV1 to reduce 1, 2.8, and 3.3 $\log_{10}$, respectively (69). It should be noted that, in those UV disinfection experiments, the authors inoculated murine NoV into phosphate-buffered saline solution and that the results therefore reflect isotonic, nontoxic conditions. It has been reported that higher NoV reduction (as measured by PCR) can be achieved when titanium dioxide (TiO_2) is added to the UV disinfection process (70). However, no significant differences in NoV reduction between UV disinfection and UV disinfection plus TiO_2 treatment were found in one study (71). Membrane bioreactor technology, which combines biological-activated sludge processes and membrane filtration, has been reported to remove NoV (GI and GII) at levels ranging from 3.3 to 6.8 $\log_{10}$ units (72, 73).

From the studies reviewed, physical separation methods using membranes appear to offer the best performance for NoV removal during sewage treatment. However, it is possible that PCR-based analysis methods may underestimate the degree of virus inactivation for sewage treatment methods employing disinfection rather than physical removal (59).

Discharges from boats. Overboard discharge of untreated feces, in areas remote from point-source discharges, has been implicated in large outbreaks of NoV infection due to oyster consumption in the United States (California Department of Health Services, unpublished data) (74, 75). During an outbreak investigation carried out in Louisiana in the United States, crews from 22 (85%) of 26 oyster harvesting boats working in the area declared routine overboard disposal of feces (75).

Most large ships, including cruise ships, have some form of wastewater collection and treatment system onboard. However, discharges from such vessels may still present a significant risk, since NoV outbreaks within the closed passenger community occur relatively frequently, so effluents may be highly contaminated. For example, in 2013, the Centers for Disease Control and Prevention reported 8 cases of NoV (or presumptive NoV) infections among passengers and crew members on large (≥100-passenger) cruise vessels that participate in the Vessel Sanitation Program (http://www.cdc.gov/nceh/vsp/surv/gilist.htm#2013). An investigation into 14 laboratory-confirmed outbreaks of acute gastroenteritis that occurred on U.S. cruise ships in 2002 indicated that the characteristics persisted in successive cruises in 50% of these and that there were multiple routes of transmission in 58% of the outbreaks (76). Of the 11 outbreaks attributed to NoV, 10 were associated with GII strains and 1 with a GI strain. The virus load from shipboard treatment systems is associated with the number of persons onboard, the percentage of individuals shedding, the level at which individuals are shedding, and the volume and frequency of bowel movements (Washington State Department of Health, unpublished data). Steady-state NoV concentrations from large cruise vessel discharges to surface waters in the main basin of Puget Sound in the United States have been estimated to be 10^4 viruses/liter (J. S. Meschke and J. C. Kissel, unpublished data). Clearly, both large and small vessels can pose a significant NoV risk if they discharge fecal effluents into shellfish-growing waters.

In the European Union, Directive 2003/44/EC has required since 2003 that newly manufactured craft fitted with toilets shall have either holding tanks or provision to fit holding tanks (77). However, overboard discharge is not prohibited by European regulations. Some European countries (for example, France and The Netherlands) have adopted national regulations requiring the provision of land-based sewage treatment facilities for recreational vessels and other floating structures or regulations preventing overboard discharge by boats into coastal waters (http://www.rya.org.uk/infoadvice/boatingabroad/Pages/holdingtanks.aspx). In the United States, the Clean Water Act prohibits the discharge of untreated waste into U.S. territorial waters and mandates that all commercial and recreational vessels with an installed toilet be equipped with a U.S. Coast Guard-certified marine sanitation device (MSD) (http://www.law.cornell.edu/uscode/text/33/1322). However, not all MSDs are effective in removing NoV. Under the International Convention on the Prevention of Pollution by Ships (MARPOL 73/78), discharge of untreated sewage into inshore waters is prohibited but discharge of treated sewage is permitted under certain circumstances [http://www.imo.org/about/conventions/listofconventions/pages/international-convention-for-the-prevention-of-pollution-from-ships-(marpol).aspx]. Protection against NoV contamination is therefore dependent on the effectiveness of the onboard sewage treatment system. The most effective way to reduce the risks from boats and ships is clearly to ban the overboard discharge of both treated and untreated effluents in the vicinity of shellfish waters. The designation of Lynnhaven River (Virginia in the United States) as a nondischarge zone has contributed to maintaining the safety of over 30% of this river for shellfish harvesting and to the reopening of areas that had been previously closed to harvest (Virginia Department of Environmental Quality, unpublished data).

ENVIRONMENTAL RESERVOIRS

Groundwater. Studies on contaminated groundwater suggest that NoV (GI) can remain detectable for over 3 years and infectious for at least 61 days (78). Therefore, poorly maintained septic tanks or leaking sewerage infrastructures could contaminate groundwater with fecal matter which could in turn contaminate surface waters through seepage (42). Surveillance of 23 groundwater wells in Korea showed that 18% of the samples were contaminated with NoV, with 71% of these being identified as GI and 29% as GII (79). A retrospective cohort study that included structured questionnaires, microbiological investigations, and NoV genotyping also carried out in that country confirmed that the most likely cause of an outbreak of NoV gastroenteritis among visitors of a water park in Korea was associated with groundwater contaminated with NoV GI (80). NoV has been detected in United Kingdom urban catchments to depths of about 40 m in unconfined sandstone aquifers, with the seasonal pattern of prevalence coincident with the seasonality of NoV discharge to the sewerage system (81).

Sand and gravel aquifers are more likely to be contaminated than other types of aquifers (82) because the pore sizes of sand and gravel do not impede movement of the virus (83). Soil chemistry can effect migration of NoV through groundwater routes, and strains may show different characteristics (84). Binding of NoV GI.1 and GII.4 strains to silica increases with ionic strength in sodium chloride solutions at pH 8 (85). In addition to mineral content, soil organic matter (SOM) usually decreases virus attachment by competition for the same binding sites (85). SOM usually holds and retains large quantities of water. Experiments with recombinant NoV particles that are morphologically and antigenically similar to live NoV strains but lack the nucleic acid suggest that water pH significantly influences the filtration of NoV in quartz sand over a pH range of 5 to 7 (86). Higher concentrations of H^+ (lower pH) neutralize the proportion of negative charges and the exchange of cations (Ca^{2+} and Mg^{2+}) on colloids. da Silva et al. (84) found that higher concentrations of these cations increase the attachment of NoV genogroups I and II. Therefore, saline soils which contain large amounts of soluble salts, particularly calcium and magnesium, are likely to filter larger quantities of NoV. Fine sands and sandy, silty, and clay loams have lower cation exchange capacity and therefore have much less capacity to retain NoV than humus and clays.

Survival and transmission in surface waters. Human NoV are highly resistant to environmental degradation in aquatic environments, and their abundance and distribution depends significantly on the survival conditions in the receiving water (78). Several studies have reported data on the zone and duration of NoV impact of shellfish in relation to point-source pollution inputs such as sewer pipes. In New Zealand, a gradient of NoV was observed in oysters corresponding to the distance from an outfall. Total NoV levels were about 1,000 PCR units/g oyster adjacent to the outfall, decreasing to 130 PCR units/g at 10 km and 100 PCR units/g at 24 km (Greening, unpublished). In another study in Ireland, depending on the local hydrodynamic conditions, the extent of impacted area could be in excess of 4 km from the implicated discharge point (B. Doré, F. Guilfoyle, S. Keaveney, and J. Flannery, unpublished data). Following a sewage spill event, viruses (GI and GII) were detected in oysters for over 4 months (P. Scholes, G. Greening, D. Campbell, J. Sim, J. Gibbons-Davies, G. Dohnt, K. Hill, I. Kruis, P. Shoemack, and A. Davis, unpublished data). In estuarine waters receiving polluted freshwater inputs of 1.5 m^3/s on average, NoV persisted for up to 6 weeks, whereas, with lower volumes of inputs (0.96 m^3/s), NoV persisted for less than 1 month (Doré et al., unpublished). These studies demonstrated that sewage discharges may have an impact on NoV contamination at a considerable distance from the discharge point and over considerable timescales. A number of studies have failed to detect significant correlations between NoV concentrations in water (87) and shellfish (Doré et al., unpublished) and variations in salinity and temperature. This may be related to variations in physicochemical water quality and/or the fact that shellfish that are relatively close to the discharge but are outside the concentrated path of the effluent plume may not bioaccumulate viruses as much as shellfish that are further away but within the effluent plume's path (39).

Some evidence suggests that sediments, sponges (40), and plankton (88) can provide reservoirs for increased persistence of NoV, which may in turn increase the likelihood of bioaccumulation by shellfish. This raises the issue of whether NoV adsorption to organic matter is an important factor in the bioaccumulation of virus by bivalves (89).

SEASONAL AND ENVIRONMENTAL INFLUENCES

NoV (both GI and GII) is often present in sewage throughout the year, with peaks of prevalence in the winter (63, 90, 91). A winter peak of viral prevalence has also been documented in freshwater (92, 93, 94) and seawater (95) samples. This is consistent with the high incidence of NoV-associated intestinal disease (28) and the winter-biased seasonality of these infections in the United Kingdom (9). The seasonality of general-practice consultations is, however, less pronounced than that in the community (52). There is contrasting evidence on the prevalences of different NoV strains in effluent (90, 91, 96). These differences could be attributed to differential levels of resistance to removal during the treatment process (90) or to dilution effects (95) or to different patterns of strain prevalence in the community (97, 98, 99). Epidemiological evidence indicates, however, that the majority of outbreaks due to person-to-person transmission are associated with the pandemic GII.4 strain (98, 99, 100). A review of transmission routes and vehicles associated with NoV outbreaks found, however, that the distributions of this strain differ substantially according to outbreak setting, season, and hemisphere (101), and GII.4 may not therefore always be a good predictor of general NoV contamination of shellfish. It is therefore important to consider the total NoV content of shellfish in making risk management decisions.

Several studies have documented higher prevalences and levels of both NoV GI and NoV GII in commercially harvested shellfish during the winter (102, 103, 104, 105, 106, 107), although some studies have reported no substantial summer-winter seasonality with respect to the prevalence of these genogroups in shellfish (108) and shellfish-growing waters (109). This discrepancy could be due to high fluxes of sewage contamination throughout the year in the latter study sites. The observed winter peak of viral prevalence in shellfish is consistent with the higher prevalence of NoV seen in sewage and with the consequential winter seasonality of shellfish-related gastroenteritis outbreaks in temperate climates (106). In the United Kingdom, average levels of NoV in Pacific oysters (*Crassostrea gigas*) during winter months (October to March) may be as much as 17 times higher than those during the remainder of the year (110). This seasonal pattern has been observed to mirror variations in water temperature (104) and in salinity and rainfall (103). Richards et al. showed that long-term (120-day) frozen conditions do not decrease NoV RNA titers and that repeated freezing and thawing do not inactivate NoV in the environment (111). Supporting this, an investigation into an outbreak of NoV gastroenteritis in Finland suggested that the source of the virus was freshwater from a frozen river that had been contaminated with human sewage discharged 70 km upstream of the affected area 4 months earlier (112). These studies suggest that NoV contamination locked into frozen environmental water may continue to remain a risk until released by a thaw. Laboratory tests have shown that the level of NoV nucleic acid (as measured by PCR) reduces by as little as $0.03 \pm 0.01 \log_{10}$/day in environmental waters incubated at 25°C (113). Thus, virus may persist for months in both surface water and groundwater at a relatively wide range of temperatures. Significant relationships have been found between the incidence of NoV-associated gastroenteritis and rainfall in Australia (114). Similarly, Miossec et al. found that Pacific oyster samples became positive for NoV in a production area (class A under Regulation 854/2004) situated 2 to 8 km from the nearest sewage discharge when rainfall exceeded 140 mm (103). They also found that peaks in the percentage of positive samples generally coincided with peaks in rainfall and with the incidence of gastroenteritis in the community. A correlation between rainfall and general community levels of gastroenteritis was observed in Victoria, Australia (114). An average lag time of 3 months between peak average rainfall and the occurrence of NoV outbreaks in the community was observed, suggesting a possible environmental transmission route for community outbreaks. Depending on the hydrological characteristics of the catchment, NoV prevalence in polluted freshwaters may consist of several shorter peaks of various magnitudes lasting 1 to 4 days (94). There is some evidence that survival of NoV on wastewater biofilms may prolong the period of environmental persistence, particularly when the virus is not circulating in the community (115). In summary, the main factors associated with an elevated incidence or persistence of NoV contamination are the winter months, increased rainfall, and low temperatures.

ACCUMULATION AND CLEARANCE BY SHELLFISH

During filter-feeding, shellfish bioaccumulate NoV in their gills, digestive glands, and other tissues (116) within 4 to 24 h (31, 117). Pacific oysters artificially contaminated with NoV GII and transferred to tanks containing sand-filtered seawater for 10 days (water at 10 ± 2°C and containing phytoplankton) maintained fairly constant levels of NoV contamination throughout the depuration experiment (initial concentrations were 1.7×10^3 copies/g digestive tissue, with levels of 1.5×10^3 copies/g at 3 days of depuration and 1.8×10^3 copies/g at 10 days) (118). That study suggested that placing shellfish in tanks with clean water is not an effective strategy to remove NoV. However, higher rates of NoV removal have been detected when oysters are exposed to higher water temperatures. For instance, Doré et al. observed a reduction of 72% in the levels of NoV GII in oysters depurated at 17°C after 4 days and a further reduction to levels below the limit of quantitation of the assay (100 copies/g) after 6 days (32).

Many studies have detected NoV, sometimes at elevated levels, in oysters harvested from areas compliant with health status classification A (<230 most probable number [MPN] of *E. coli*/100 g) and classification B (<4,600 MPN of *E. coli*/100 g in 90% of samples) under the European system (32, 33, 110). Such contaminated shellfish may require extended relaying before they can be safely introduced into the market (32, 119). Surveillance of NoV in commercial oyster production areas in the United Kingdom, Ireland, and France detected total levels of NoV (GI plus GII) ranging from <100 genome copies/g to 10^4 genome copies/g (33). Levels of NoV GI in oysters collected from commercial beds in Georgia in the United States ranged from 10^3 to 10^8 copies/g (88). Monitoring of NoV in mussels grown in floating rafts and wild shellfish (mussels, clams, and cockles) in Galicia (Spain) detected concentrations of the virus ranging from 10^2 to 10^3copies/g for GI and from 10^1 to 10^4 copies/g for GII, with GII levels being generally higher in cultivated areas than in wild shellfish (120). Teunis et al. (54) reported a dose-response relationship in human volunteer studies between NoV GI titer (as measured by PCR genome copy numbers) and disease outcome. In susceptible volunteers, a low ingested dose may result in infection but not illness, with illness becoming more likely as the dose increases. The 50% infectious dose (ID_{50}) was reported as 18 NoV genome copies for a disaggregated inoculum, whereas the probability of an infected subject becoming ill (i.e., expressing NoV symptoms) ranged from 10% at a dose of 10^3 NoV genomes to 70% at a dose of 10^8 virus genomes. This dose-dependent outcome may help explain why asymptomatic NoV excretion has been observed in a substantial proportion (up to 16%) of healthy individuals (121) and also, since reporting of outbreaks depends on expression of clinical symptoms, why NoV levels seen in shellfish associated with outbreaks are generally higher than background environmental levels (122). Assuming that NoV detected in shellfish is viable, then this dose-response model can give an indication of the likely outcome following consumption of the NoV levels observed in shellfish surveillance studies. It can be anticipated that outcomes would range from subclinical infection to symptomatic disease depending on the dose consumed. In considering potential food standards, risk managers need to consider whether it is important to also control the risk of subclinical NoV infection.

Following a period of bioaccumulation, NoV persistence in shellfish may be facilitated by the binding of histoblood group antigens (HBGA) to digestive tissues (123). However, different NoV strains show different specificities for HBGA ligands; therefore, not all strains may be captured equally well by oysters (123). Maalouf et al. found that binding of strain GI.1 the to Pacific oyster tissues occurs more efficiently in late winter and spring, when most shellfish-related outbreaks occur in France (13). NoV recognition and binding to human HBGAs are well known (15, 124), and it has been proposed that links between shellfish carbohydrate ligands and virus strain specificity be considered for the purposes of managing viral risk (124). These specific binding patterns may help explain the difficulty in removing NoV from oysters during depuration (31).

RISK REDUCTION STRATEGIES

The risk of NoV infection may be mitigated by implementing control measures in primary production preharvest and, to a lesser extent, by postharvest purification treatments. Because most treatments are not effective in reducing NoV contamination from shellfish control measures should focus on ensuring that shellfish are harvested from areas that are not being impacted by NoV contamination. Prevention of contamination requires a high level of environmental protection, with associated regulatory standards and monitoring programs and designation of harvesting areas with appropriate water quality. However, in many parts of the developed world, shellfish farming may be situated in populated areas impacted by episodic or chronic sewage pollution events. In such cases, better understanding of the environmental risk factors for NoV contamination can assist the design of effective risk reduction strategies.

The evidence summarized above indicates that NoV is removed to only a limited extent during sewage treatment processes and, consequently, that the virus is frequently detected in final effluents of STWs operating according to design conditions. Furthermore, source apportionment studies have revealed substantial site-specific variability with respect to the relative contributions of storm overflows and continuously treated discharges to the total NoV load impacting shellfish waters. A key element of any risk reduction strategy is therefore to ensure that there is sufficient dilution and dispersion of viral contamination between the sewage sources and the impacted shellfish beds. Detailed information on the location and operational performance of impacting STW, NoV concentrations in sewage effluents, flow volumes and frequencies of discharges, the occurrence and frequency of intermittent discharges (e.g., CSOs), the decay rates of NoV, and the geographical extent of the shellfish beds is required to delineate dilution areas and design monitoring programs that are representative of the spatial and temporal variability of NoV contamination in harvesting areas. Information on the main environmental factors influencing NoV contamination of shellfish (e.g., rainfall, river flows, salinity, water temperature, turbidity) is also required to ensure that the location of monitoring stations and number of samples collected are as representative as possible of the harvesting area and local environmental conditions. Routine monitoring for NoV in shellfish production areas would greatly assist understanding of whether available dilution and dispersion of potential impacting discharges are sufficient to reduce NoV contamination levels to acceptable background levels.

Currently, a sanitary survey is the main regulatory driver for collating information on pollution sources and how they impact a shellfish water. A sanitary-survey assessment can inform how and when shellfish harvesting should be suspended in the event of spills associated with sewage treatment malfunction (e.g., low disinfection performance, hydraulic overloading) or weather events triggering CSO discharges. Monitoring of NoV during and following pollution events can provide an indication of the extent of the impacted area. Consideration should be given to the worst-case scenario of contamination in the harvesting area to ensure public health protection. Effective communication between regulatory agencies and members of the shellfish industry is required to prevent contaminated shellfish being placed on the market. It is important that regulatory agencies understand that routine monitoring of FIB in shellfish or growing waters does not provide evidence of the clearance of a viral contamination risk. Following a potential pollution event, additional NoV monitoring (e.g., of shellfish, seawater, and sewage) should be carried out in the environment for the period necessary to inform on the clearance of virus from shellfish.

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The need to continue optimizing shellfish safety controls to address the NoV risk is an ongoing challenge for the shellfish industry, scientists, and regulators. Although the evidence base required to support these controls has developed considerably in recent years, further research is required to address the following concerns and goals.

- The development of effective NoV monitoring programs for commercial production areas to better understand contamination patterns and develop risk management strategies.
- The relationship between NoV removal rates and STW operational parameters and malfunctions to optimize sewage treatment processes and thus remediate shellfish waters impacted by NoV from STW discharges.
- The contribution of CSO to NoV contamination in shellfisheries to determine how that can be more effectively managed.

- The hydrographical relationships between NoV inputs and consequential impacts on shellfisheries to better model risk.
- The influence of environmental factors on the prevalence and distribution of NoV to develop pollution remediation strategies.
- Standardized methods for rapid detection and discrimination of NoV infectivity to help inform risk management of shellfisheries, particularly in relation to disinfected discharges, to ensure that harvested products are safe to eat.
- Improved effectiveness of epidemiological surveillance systems for NoV to provide insight into the burden of illness transmitted through shellfish and better manage risk.

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REFERENCES

1. **Lee R, Kay D, Wyer M, Murray L, Stapleton C.** 2010. Sanitary profiling of shellfish harvesting areas, p 121–143. *In* Rees G, Pond K, Kay D, Bartram J, Santo Domingo J. (ed), Safe management of shellfish and harvest waters. IWA Publishing, London, United Kingdom.
2. **Santo Domingo J, Edge TA.** 2010. Identification of primary sources of faecal pollution, p 51–90. *In* Rees G, Pond K, Kay D, Bartram J, Santo Domingo J. (ed), Safe management of shellfish and harvest waters. IWA Publishing, London, United Kingdom.
3. **Burian SJ, Nix SJ, Pitt RE, Durrans SR.** 2000. Urban wastewater management in the United States: past, present, and future. J. Urban Technol. **7:**33–62. http://dx.doi.org/10.1080/713684134.
4. **Sato T, Qadir M, Yamamoto S, Endo T, Zahoor A.** 2013. Global, regional, and country level need for data on wastewater generation, treatment, and use. Agric. Water Manage. **130:**1–13. http://dx.doi.org/10.1016/j.agwat.2013.08.007.
5. **Boehm AB, Grant SB, Kim JH, Mowbray SL, McGee CD, Clark CD, Foley DM, Wellman DE.** 2002. Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. Environ. Sci. Technol. **36:**3885–3892. http://dx.doi.org/10.1021/es020524u.
6. **Stapleton CM, Wyer MD, Crowther J, McDonald AT, Kay D, Greaves J, Wither A, Watkins J, Francis C, Humphrey N, Bradford M.** 2008. Quantitative catchment profiling to apportion faecal indicator organism budgets for the Ribble system, the UK's sentinel drainage basin for Water Framework Directive research. J. Environ. Manage. **87:**535–550. http://dx.doi.org/10.1016/j.jenvman.2006.11.035.
7. **Rose JB, Epstein PR, Lipp EK, Sherman BH, Bernard SM, Patz JA.** 2001. Climate variability and change in the United States: potential impacts on water- and foodborne diseases caused by microbiologic agents. Environ. Health Perspect. **109**(Suppl 2)**:**211–221. http://dx.doi.org/10.2307/3435011.
8. **Lipp EK, Rose JB.** 1997. The role of seafood in foodborne diseases in the United States of America. Rev. Sci. Tech. Off. Int. Epiz. **16:**620–640.
9. **Lees DN.** 2000. Viruses and bivalve shellfish. Int. J. Food Microbiol. **59:**81–116. http://dx.doi.org/10.1016/S0168-1605(00)00248-8.
10. **Lee RJ, Younger AD.** 2002. Developing microbiological risk assessment for shellfish purification. Int. Biodeter. Biodegr. **50:**177–183. http://dx.doi.org/10.1016/S0964-8305(02)00084-7.
11. **Butt AA, Aldridge KE, Sanders CV.** 2004. Infections related to the ingestion of seafood. Part I: viral and bacterial infections. Lancet Infect. Dis. **4:**201–212. http://dx.doi.org/10.1016/S1473-3099(04)00969-7.
12. **Zheng D-P, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS.** 2006. Norovirus classification and proposed strain nomenclature. Virology **346:**312–323. http://dx.doi.org/10.1016/j.virol.2005.11.015.
13. **Maalouf H, Zakhour M, Le Pendu J, Le Saux J-C, Atmar RL, Le Guyader FS.** 2010. Norovirus genogroups I and II in oysters: tissue distribution and seasonal variations. Appl. Environ. Microbiol. **76:**5621–5630. http://dx.doi.org/10.1128/AEM.00148-10.
14. **Donaldson EF, Lindesmith LC, LoBue AD, Baric RS.** 2010. Viral shape-shifting: norovirus evasion of the human immune system. Nat. Rev. Microbiol. **8:**231–241. http://dx.doi.org/10.1038/nrmicro2296.
15. **Shirato H.** 2011. Norovirus and histo-blood group antigens. Jpn. J. Infect. Dis. **64:**95–103.
16. **Lindesmith LC, Donaldson EF, LoBue AD, Cannon JL, Zheng D-P, Vinje J, Baric RS.** 2008. Mechanisms of GII.4 norovirus persistence in human populations. PLoS Med. **5:**e31. http://dx.doi.org/10.1371/journal.pmed.0050031.
17. **Huang P, Farkas T, Zhong W, Tan M, Thornton S, Morrow AL, Jiang X.** 2005. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. J. Virol. **79:**6714–6722. http://dx.doi.org/10.1128/JVI.79.11.6714-6722.2005.
18. **Lindesmith LC, Donaldson E, Baric R.** 2011. Norovirus GII.4 strain antigenic variation. J. Virol. **85:**231–242. http://dx.doi.org/10.1128/JVI.01364-10.
19. **Eden J-S, Hewitt J, Lim KL, Boni MF, Merif J, Greening G, Ratcliff RM, Holmes EC, Tanaka MM, Rawlinson WD, White PA.** 2014. The emergence and evolution of the novel epidemic norovirus GII.4 variant Sydney 2012. Virology **450–451:**106–113.
20. **Sugieda M, Nakajima K, Nakajima S.** 1996. Outbreaks of Norwalk-like virus-associated gastroenteritis traced to shellfish: coexistence of two genotypes in one specimen. Epidemiol. Infect. **116:**339–346. http://dx.doi.org/10.1017/S0950268800052663.
21. **Prato R, Lopalco PL, Chironna M, Barbuti G, Germinario C, Quarto M.** 2004. Norovirus gastroenteritis general outbreak associated with raw shellfish consumption in South Italy. BMC Infect. Dis. **4:**37. http://dx.doi.org/10.1186/1471-2334-4-37.
22. **Webby RJ, Carville KS, Kirk MD, Greening G, Ratcliff RM, Crerar SK, Dempsey K, Sarna M, Stafford R, Patel M, Hall G.** 2007. Internationally distributed frozen oyster meat causing multiple outbreaks of norovirus infection in Australia. Clin. Infect. Dis. **44:**1026–1031. http://dx.doi.org/10.1086/512807.
23. **Smith AJ, McCarthy N, Saldana L, Ihekweazu C, McPhedran K, Adak GK, Iturriza-Gómara M, Bickler G, O'Moore É.** 2012. A large foodborne outbreak of norovirus in diners at a restaurant in England between January and February 2009. Epidemiol. Infect. **140:**1695–1701. http://dx.doi.org/10.1017/S0950268811002305.
24. **Westrell T, Dusch V, Ethelberg S, Harris J, Hjertqvist M, Jourdan-da Silva N, Koller A, Lenglet A, Lisby M, Vold L.** 2010. Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. Euro Surveill. **15:**pii=19524. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19524.
25. **Iwamoto M, Ayers T, Mahon BE, Swerdlow DL.** 2010. Epidemiology of seafood-associated infections in the United States. Clin. Microbiol. Rev. **23:**399–411. http://dx.doi.org/10.1128/CMR.00059-09.
26. **Batz MB, Hoffmann S, Morris JG, Jr.** 2011. Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida, Gainesville, FL.
27. **Tam CC, Rodrigues L, O'Brien SJ.** 2003. The study of infectious intestinal disease in England: what risk factors for presentation to general practice tell us about potential for selection bias in case-control studies of reported cases of diarrhoea. Int. J. Epidemiol. **32:**99–105. http://dx.doi.org/10.1093/ije/dyg007.
28. **Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, Gray JJ, Letley LH, Rait G, Tompkins DS, O'Brien SJ.** 2012. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut **61:**69–77. http://dx.doi.org/10.1136/gut.2011.238386.
29. **Lopman BA, Hall AJ, Curns AT, Parashar UD.** 2011. Increasing rates of gastroenteritis hospital discharges in US adults and the contribution of norovirus, 1996–2007. Clin. Infect. Dis. **52:**466–474. http://dx.doi.org/10.1093/cid/ciq163.
30. **Lopman B, Gastañaduy P, Park GW, Hall AJ, Parashar U, Vinjé J.**

2012. Environmental transmission of norovirus gastroenteritis. Curr. Opin. Virol. **2**:96–102. http://dx.doi.org/10.1016/j.coviro.2011.11.005.

31. **Schwab KJ, Neill FH, Estes MK, Metcalf TG, Atmar RL.** 1998. Distribution of Norwalk virus within shellfish following bioaccumulation and subsequent depuration by detection using RT-PCR. J. Food Prot. **61:** 1674–1680.
32. **Doré B, Keaveney S, Flannery J, Rajko-Nenow P.** 2010. Management of health risks associated with oysters harvested from a norovirus contaminated area, Ireland, February–March 2010. Euro Surveill. **15:**pii=19567. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19567.
33. **EFSA Panel on Biological Hazards (BIOHAZ).** 2012. Scientific opinion on norovirus (NoV) in oysters: methods, limits and control options. EFSA J. **10:**2500. http://dx.doi.org/10.2903/j.efsa.2012.2500.
34. **Wyn-Jones AP, Sellwood J.** 2001. Enteric viruses in the aquatic environment. J. Appl. Microbiol. **91:**945–962. http://dx.doi.org/10.1046/j.1365-2672.2001.01470.x.
35. **Girones R, Ferrús MA, Alonso JL, Rodriguez-Manzano J, Calgua B, de Abreu Corrêa A, Hundesa A, Carratala A, Bofill-Mas S.** 2010. Molecular detection of pathogens in water—the pros and cons of molecular techniques. Water Res. **44:**4325–4339. http://dx.doi.org/10.1016/j.watres.2010.06.030.
36. **Lees D, CENWG6 TAG4 Working Group.** 2010. International standardisation of a method for detection of human pathogenic viruses in molluscan shellfish. Food Environ. Virol. **2:**146–155. http://dx.doi.org/10.1007/s12560-010-9042-5.
37. **International Organization for Standardization.** 2013. Microbiology of food and animal feed—horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR—part 1: method for quantification. ISO/TS 15216–1:2013. ISO, Geneva, Switzerland.
38. **Bosch A, Pintó RM, Le Guyader FS.** 2009. Viral contaminants of molluscan shellfish: detection and characterisation. *In* Shumway SE, Rodrick GE (ed), Shellfish safety and quality. Woodhead Publishing Ltd., Cambridge, United Kingdom.
39. **Goblick GN, Anbarchian JM, Woods J, Burkhardt W, III, Calci K.** 2011. Evaluating the dilution of wastewater treatment plant effluent and viral impacts on shellfish growing areas in Mobile Bay, Alabama. J. Shellfish Res. **30:**979–987. http://dx.doi.org/10.2983/035.030.0341.
40. **Futch JC, Griffin DW, Banks K, Lipp EK.** 2011. Evaluation of sewage source and fate on southeast Florida coastal reefs. Mar. Pollut. Bull. **62:** 2308–2316. http://dx.doi.org/10.1016/j.marpolbul.2011.08.046.
41. **Cook A, Lowther J, Price-Hayward M, Lee R.** 2009. Spatial and temporal pattern of norovirus contamination in a Pacific oyster fishery, p 81–87. *In* Lassus P (ed), Proceedings of the 7th International Conference on Molluscan Shellfish Safety, Nantes, France, 14 to 19 June 2009.
42. **Borchardt MA, Bradbury KR, Alexander EC, Jr, Kolberg RJ, Alexander SC, Archer JR, Braatz LA, Forest BM, Green JA, Spencer SK.** 2011. Norovirus outbreak caused by a new septic system in a dolomite aquifer. Ground Water **49:**85–97.
43. **Rodríguez RA, Gundy PM, Rijal GK, Gerba CP.** 2012. The impact of combined sewage overflows on the viral contamination of receiving waters. Food Environ. Virol. **4:**34–40. http://dx.doi.org/10.1007/s12560-011-9076-3.
44. **Ueki Y, Sano D, Watanabe T, Akiyama K, Omura T.** 2005. Norovirus pathway in water environment estimated by genetic analysis of strains from patients of gastroenteritis, sewage, treated wastewater, river water and oysters. Water Res. **39:**4271–4280. http://dx.doi.org/10.1016/j.watres.2005.06.035.
45. **Nenonen NP, Hannoun C, Horal P, Hernroth B, Bergström T.** 2008. Tracing of norovirus outbreak strains in mussels collected near sewage effluents. Appl. Environ. Microbiol. **74:**2544–2549. http://dx.doi.org/10.1128/AEM.02477-07.
46. **Wall R, Dymond N, Bell A, Thornley C, Buik H, Cumming D, Petersen N.** 2011. Two New Zealand outbreaks of norovirus gastroenteritis linked to commercially farmed oysters. N. Z. Med. J. **124:**63–71.
47. **Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, Graham DY.** 2008. Norwalk virus shedding after experimental human infection. Emerg. Infect. Dis. **14:**1553–1557. http://dx.doi.org/10.3201/eid1410.080117.
48. **Leon J, McDaniels M, Lyon GM, Abdulhafid G, Dowd M, Etienne K, Liu P, Schwabb K, Moe CL.** 2008. Norovirus human infectivity, immunology, and persistence in groundwater. J. Fed. Am. Soc. Exp. Biol. **26:** 856.31.
49. **Ozawa K, Oka T, Takeda N, Hansman GS.** 2007. Norovirus infections in symptomatic and asymptomatic food handlers in Japan. J. Clin. Microbiol. **45:**3996–4005. http://dx.doi.org/10.1128/JCM.01516-07.
50. **Furuya D, Kuribayashi K, Hosono Y, Tsuji N, Furuya M, Miyazaki K, Watanabe N.** 2011. Age, viral copy number, and immunosuppressive therapy affect the duration of norovirus RNA excretion in patients diagnosed with norovirus infection. Jpn. J. Infect. Dis. **64:**104–108.
51. **Milbrath MO, Spicknall IH, Zelner JL, Moe CL, Eisenberg JN.** 2013. Heterogeneity in norovirus shedding duration affects community risk. Epidemiol. Infect. **141:**1572–1584. http://dx.doi.org/10.1017/S0950268813000496.
52. **Phillips G, Tam CC, Conti S, Rodrigues LC, Brown D, Iturriza-Gomara M, Gray J, Lopman B.** 2010. Community incidence of norovirus-associated infectious intestinal disease in England: improved estimates using viral load for norovirus diagnosis. Am. J. Epidemiol. **171:** 1014–1022. http://dx.doi.org/10.1093/aje/kwq021.
53. **Campos CJA, Avant J, Lowther J, Till D, Lees D.** 2013. Levels of norovirus and *E. coli* in untreated, biologically treated and UV-disinfected sewage effluent discharged to a shellfish water. J. Water Res. Prot. **5:**978–982. http://dx.doi.org/10.4236/jwarp.2013.510101.
54. **Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL.** 2008. Norwalk virus: how infectious is it? J. Med. Virol. **80:**1468–1476. http://dx.doi.org/10.1002/jmv.21237.
55. **Iwai M, Hasegawa S, Obara M, Nakamura K, Horimoto E, Takizawa T, Kurata T, Sogen S-I, Shiraki K.** 2009. Continuous presence of noroviruses and sapoviruses in raw sewage reflects infections among inhabitants of Toyama, Japan (2006 to 2008). Appl. Environ. Microbiol. **75:**1264–1270. http://dx.doi.org/10.1128/AEM.01166-08.
56. **Passerat J, Outtara NK, Mouchel J-M, Rocher V, Servais P.** 2011. Impact of an intense combined sewer overflow event on the microbiological water quality of the Seine River. Water Res. **45:**893–903. http://dx.doi.org/10.1016/j.watres.2010.09.024.
57. **Hata A, Katayama H, Kojima K, Sano S, Kasuga I, Kitajima M, Furumai H.** 2014. Effects of rainfall events on the occurence and detection efficiency of viruses in river water impacted by combined sewer overflows. Sci. Total Environ. **468–469:**757–763. http://dx.doi.org/10.1016/j.scitotenv.2013.08.093.
58. **Flannery J, Keaveney S, Rajko-Nenow P, O'Flaherty V, Doré W.** 2013. Norovirus and FRNA bacteriophage determined by RT-qPCR and infectious FRNA bacteriophage in wastewater and oysters. Water Res. **47:** 5222–5231. http://dx.doi.org/10.1016/j.watres.2013.06.008.
59. **Hunter PR.** 2003. Climate change and waterborne and vector-borne disease. J. Appl. Microbiol. **94:**37S–46S.
60. **Baker-Austin C, Campos CJA, Turner A, Higman W, Lees D.** 2013. Impacts of climate change on human health. MCCIP Sci. Rev. **2013:**257–262.
61. **Ottoson J, Hansen A, Björlenius B, Norder H, Stenström TA.** 2006. Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. Water Res. **40:**1449–1457. http://dx.doi.org/10.1016/j.watres.2006.01.039.
62. **da Silva AK, Le Saux J-C, Parnaudeau S, Pommepuy M, Elimelech M, Le Guyader FS.** 2007. Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviours of genogroups I and II. Appl. Environ. Microbiol. **73:** 7891–7897. http://dx.doi.org/10.1128/AEM.01428-07.
63. **Nordgren J, Matussek A, Mattsson A, Svensson L, Lindgren P-E.** 2009. Prevalence of norovirus and factors influencing virus concentrations during one year in a full-scale wastewater treatment plant. Water Res. **43:**1117–1125. http://dx.doi.org/10.1016/j.watres.2008.11.053.
64. **Flannery J, Keaveney S, Rajko-Nenow P, O'Flaherty V, Doré W.** 2012. Concentration of norovirus during wastewater treatment and its impact on oyster contamination. Appl. Environ. Microbiol. **78:**3400–3406. http://dx.doi.org/10.1128/AEM.07569-11.
65. **Francy DS, Stelzer EA, Bushon RN, Brady AMG, Williston AG, Riddell KR, Borchardt MA, Spencer SK, Gellner TM.** 2012. Comparative effectiveness of membrane bioreactors, conventional secondary treatment, and chlorine and UV disinfection to remove microorganisms from municipal wastewaters. Water Res. **46:**4164–4178. http://dx.doi.org/10.1016/j.watres.2012.04.044.
66. **Hewitt J, Leonard M, Greening GE, Lewis GD.** 2011. Influence of wastewater treatment process and the population size on human virus profiles in wastewater. Water Res. **45:**6267–6276. http://dx.doi.org/10.1016/j.watres.2011.09.029.
67. **Hejkal TW, Wellings FM, Lewis AL, LaRock PA.** 1981. Distribution of

viruses associated with particles in wastewater. Appl. Environ. Microbiol. **41:**628–634.

68. **Park GW, Linden KG, Sobsey MD.** 2011. Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. Lett. Appl. Microbiol. **52:**162–167. http://dx.doi.org/10.1111/j.1472-765X.2010.02982.x.
69. **Lee J, Zoh K, Ko G.** 2008. Inactivation and UV disinfection of murine norovirus with TiO_2 under various environmental conditions. Appl. Environ. Microbiol. **74:**2111–2117. http://dx.doi.org/10.1128/AEM.02442-07.
70. **Kato T, Tohma H, Miki O, Shibata T, Tamura M.** 2005. Degradation of norovirus in sewage treatment water by photocatalytic ultraviolet disinfection. Nippon Steel Tech. Rep. **92:**41–44.
71. **Sano D, Ueki Y, Watanabe T, Omura T.** 2006. Membrane separation of indigenous noroviruses from sewage sludge and treated wastewater. Water Sci. Technol. **54:**77–82.
72. **Sima LC, Schaeffer J, Le Saux J-C, Parnaudeau S, Elimelech M, Le Guyader FS.** 2011. Calicivirus removal in a membrane bioreactor wastewater treatment plant. Appl. Environ. Microbiol. **77:**5170–5177. http://dx.doi.org/10.1128/AEM.00583-11.
73. **Simmons FJ, Kuo DH-W, Xagoraraki I.** 2011. Removal of human enteric viruses by a full-scale membrane bioreactor during municipal wastewater processing. Water Res. **45:**2739–2750. http://dx.doi.org/10.1016/j.watres.2011.02.001.
74. **Berg DE, Kohn MA, Farley TA, McFarland LM.** 2000. Multi-state outbreaks of acute gastroenteritis traced to fecal-contaminated oysters harvested in Louisiana. J. Infect. Dis. **181**(Suppl 2):S381–S386. http://dx.doi.org/10.1086/315581.
75. **Kohn MA, Farley TA, Ando T, Curtis M, Wilson SA, Jin Q, Monroe SS, Baron RC, McFarland LM, Glass RI.** 1995. An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters. Implications for maintaining safe oyster beds. JAMA **273:**466–471. http://dx.doi.org/10.1001/jama.1995.03520300040034.
76. **Widdowson MA, Cramer EH, Hadley L, Bresee JS, Beard RS, Bulens SN, Charles M, Chege W, Isakbaeva E, Wright JG, Mintz E, Forney D, Massey J, Glass RI, Monroe SS.** 2004. Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus—United States, 2002. J. Infect. Dis. **190:**27–36. http://dx.doi.org/10.1086/420888.
77. **European Communities.** 2003. Directive 2003/44/EC of the European Parliament and of the Council of 16 June 2003 amending Directive 94/25/EC on the approximation of the laws, regulations and administrative provisions of the Member States relating to recreational craft. Off. J. Eur. Union **L214, 26.8.2003:**18–35.
78. **Seitz SR, Leon JS, Schwab KJ, Lyon GM, Dowd M, MsDaniels M, Adbulhafid G, Fernandez ML, Lindesmith LC, Baric RS, Moe CL.** 2011. Norovirus infectivity in humans and persistence in water. Appl. Environ. Microbiol. **77:**6884–6888. http://dx.doi.org/10.1128/AEM.05806-11.
79. **Jung JH, Yoo CH, Koo ES, Kim HM, Na Y, Jheong WH, Jeong YS.** 2011. Occurrence of norovirus and other enteric viruses in untreated groundwaters of Korea. J. Water Health **9:**544–555. http://dx.doi.org/10.2166/wh.2011.142.
80. **Koh S-J, Cho HG, Kim BH, Choi BY.** 2011. An outbreak of gastroenteritis caused by norovirus-contaminated groundwater at a waterpark in Korea. J. Korean Med. Sci. **26:**28–32. http://dx.doi.org/10.3346/jkms.2011.26.1.28.
81. **Powell KL, Taylor RG, Cronin AA, Barrett MH, Pedley S, Sellwood J, Lerner DN.** 2003. Microbial contamination of two urban sandstone aquifers in the UK. Water Res. **37:**339–352. http://dx.doi.org/10.1016/S0043-1354(02)00280-4.
82. **Abbaszadegan M, LeChevalier M, Gerba C.** 2003. Occurrence of viruses in US groundwaters. J. Am. Water Works Assoc. **95:**107–120.
83. **Borchardt MA, Haas NL, Hunt RJ.** 2004. Vulnerability of drinking-water wells in La Crosse, Wisconsin, to enteric-virus contamination from surface water contributions. Appl. Environ. Microbiol. **70:**5937–5946. http://dx.doi.org/10.1128/AEM.70.10.5937-5946.2004.
84. **da Silva AK, Kavanagh OV, Estes MK, Elimelech M.** 2011. Adsorption and aggregation properties of norovirus GI and GII virus-like particles demonstrate differing responses to solution chemistry. Water Sci. Technol. **45:**520–526. http://dx.doi.org/10.1021/es102368d.
85. **Redman JA, Grant SB, Olson TM.** 1997. Filtration of recombinant Norwalk virus particles and bacteriophage MS2 in quartz sand: importance of electrostatic interactions. Environ. Sci. Technol. **31:**3378–3383. http://dx.doi.org/10.1021/es961071u.
86. **Hernroth BE, Conden-Hansson A-C, Rehnstram-Holm A-S, Girones R, Allard AK.** 2002. Environmental factors influencing human pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: the first Scandinavian report. Appl. Environ. Microbiol. **68:**4523–4533. http://dx.doi.org/10.1128/AEM.68.9.4523-4533.2002.
87. **Hernandez-Morga J, Leon-Felix J, Peraza-Garay F, Gil-Salas BG, Chaidez C.** 2009. Detection and characterization of hepatitis A virus and norovirus in estuarine water samples using ultrafiltration—RT-PCR integrated methods. J. Appl. Microbiol. **106:**1579–1590. http://dx.doi.org/10.1111/j.1365-2672.2008.04125.x.
88. **Gentry J, Vinjé J, Guadagnoli D, Lipp EK.** 2009. Norovirus distribution within an estuarine environment. Appl. Environ. Microbiol. **75:**5474–5480. http://dx.doi.org/10.1128/AEM.00111-09.
89. **Katayama H, Haramoto E, Oguma K, Yamashita H, Tajima A, Nakajima H, Ohgaki S.** 2008. One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. Water Res. **42:**1441–1448. http://dx.doi.org/10.1016/j.watres.2007.10.029.
90. **Victoria M, Guimarães FR, Fumian TM, Ferreira FFM, Vieira CB, Shubo T, Gagliardi JP, Miagostovich MP.** 2010. One year monitoring of norovirus in a sewage treatment plant in Rio de Janeiro, Brazil. J. Water Health **8:**158–165. http://dx.doi.org/10.2166/wh.2009.012.
91. **La Rosa G, Pourshaban M, Iaconelli M, Muscillo M.** 2010. Quantitative real-time PCR of enteric viruses in influent and effluent samples from wastewater treatment plants in Italy. Ann. Ist. Super. Sanità **46:**266–273. http://dx.doi.org/10.4415/ANN_10_03_07.
92. **Haramoto E, Katayama H, Oguma K, Ohgaki S.** 2005. Application of cation-coated filter method to detection of noroviruses, enteroviruses, adenoviruses, and torque teno viruses in the Tamagawa River in Japan. Appl. Environ. Microbiol. **71:**2403–2411. http://dx.doi.org/10.1128/AEM.71.5.2403-2411.2005.
93. **Pérez-Sautu U, Sano D, Guix S, Kasimir G, Pintó R, Bosch A.** 2012. Human norovirus occurrence and diversity in the Llobregat river catchment, Spain. Environ. Microbiol. **14:**494–502. http://dx.doi.org/10.1111/j.1462-2920.2011.02642.x.
94. **Westrell T, Teunis P, van den Berg H, Lodder W, Ketelaars H, Stenström TA, de Roda Husman AM.** 2006. Short- and long-term variations of norovirus concentrations in the Meuse river during a 2-year study period. Water Res. **40:**2613–2620. http://dx.doi.org/10.1016/j.watres.2006.05.019.
95. **Katayama H, Tanaka A, Otaki M, Ohgaki S.** 2004. Determination of naturally occurring noroviruses in coastal seawater by alkaline elution after acid rinse using negatively charged membrane. Water Sci. Technol. Water Supply **4:**73–77.
96. **Cheng H-WA, Lucy FE, Broaders MA, Mastitsky SE, Chen C-H, Murray A.** 2012. Municipal wastewater treatment plants as pathogen removal systems and as contamination source of noroviruses and *Enterococcus faecalis*. J. Water Health **10:**380–389. http://dx.doi.org/10.2166/wh.2012.138.
97. **Lysén M, Thorhagen M, Brytting M, Hjertqvist M, Andersson Y, Hedlund K-O.** 2009. Genetic diversity among food-borne and waterborne norovirus strains causing outbreaks in Sweden. J. Clin. Microbiol. **47:**2411–2418. http://dx.doi.org/10.1128/JCM.02168-08.
98. **Kroneman A, Verhoef L, Harris J, Vennema H, Duizer E, van Duynhoven Y, Gray J, Itturriza M, Böttiger B, Falkenhorst G, Johnsen C, von Bonsdorff C-H, Maunula L, Kuusi M, Pothier P, Gallay A, Schreier E, Höhne M, Koch J, Szücs G, Reuter G, Krisztalovics K, Lynch M, McKeown P, Foley B, Coughlan S, Ruggeri FM, Di Bartolo I, Vainio K, Isakbaeva E, Poljsak-Prijateli M, Hocenar Grom A, Zimsek Mijovski J, Bosch A, Buesa J, Sanchez Fauquier A, Hernandéz-Pezzi G, Hedlund K-O, Koopmans M.** 2008. Analysis of integrated virological and epidemiological reports of norovirus outbreaks collected within the foodborne viruses in Europe network from 1 July to 30 June 2006. J. Clin. Microbiol. **46:**2959–2965. http://dx.doi.org/10.1128/JCM.00499-08.
99. **Han T-H, Kim C-H, Chung J-Y, Park S-H, Hwang E-S.** 2011. Emergence of norovirus GII-4/2008 variant and recombinant strains in Seoul, Korea. Arch. Virol. **156:**323–329. http://dx.doi.org/10.1007/s00705-010-0844-4.
100. **Kim HS, Hyun J, Kim H-S, Kim J-S, Song W, Lee KM.** 2013. Emer-

gence of GII.4 norovirus in South Korea during the winter of 2012-2013. J. Microbiol. Biotechnol. **23:**1641–1643. http://dx.doi.org/10.4014/jmb.1308.08053.

101. **Bitler EJ, Matthews JE, Dickey BW, Eisenberg JNS, Leon JS.** 2013. Norovirus outbreaks: a systematic review of commonly implicated transmission routes and vehicles. Epidemiol. Infect. **141:**1563–1571. http://dx.doi.org/10.1017/S095026881300006X.
102. **Le Guyader FS, Haugarreau L, Miossec L, Dubois E, Pommepuy M.** 2000. Three-year study to assess human enteric viruses in shellfish. Appl. Environ. Microbiol. **66:**3241–3248. http://dx.doi.org/10.1128/AEM.66.8.3241-3248.2000.
103. **Miossec L, Le Guyader F, Haugarreau L, Pommepuy M.** 2000. Importance de la pluviométrie sur la contamination virale du milieu littoral lors de phénomènes épidémiques dans la population. Rev. Epidemiol. Sante Publique **48:**2S62–2S71.
104. **Lowther JA, Gustar NE, Powell AL, Hartnell RE, Lees DN.** 2012. Two-year systematic study to assess norovirus contamination in oysters from commercial harvesting areas in the United Kingdom. Appl. Environ. Microbiol. **78:**5812–5817. http://dx.doi.org/10.1128/AEM.01046-12.
105. **Rajko-Nenow P, Keaveney S, Flannery J, O'Flaherty V, Doré W.** 2012. Characterisation of norovirus contamination in an Irish shellfishery using real-time RT-qPCR and sequencing analysis. Int. J. Food Microbiol. **160:**105–112. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.10.001.
106. **Rippey SR.** 1994. Infectious diseases associated with molluscan shellfish consumption. Clin. Microbiol. Rev. **7:**419–425.
107. **Formiga-Cruz M, Tofiño-Quesada G, Bofill-Mas S, Lees DN, Henshilwood K, Allard AK, Conden-Hansson A-C, Hernroth BE, Vantarakis A, Tsibouxi A, Papapetropoulou M, Furones MD, Girones R.** 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. http://dx.doi.org/10.1128/AEM.68.12.5990-5998.2002.
108. **Seo DJ, Lee MH, Son NR, Seo S, Lee KB, Wang X, Choi C.** 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. http://dx.doi.org/10.1016/j.foodcont.2014.01.020.
109. **Moresco V, Viancelli A, Nascimento MA, Souza DSM, Ramos APD, Garcia LAT, Simões CMO, Barardi CRM.** 2012. Microbiological and physicochemical analysis of the coastal waters of southern Brazil. Mar. Pollut. Bull. **64:**40–48. http://dx.doi.org/10.1016/j.marpolbul.2011.10.026.
110. **Lowther JA, Henshilwood K, Lees DN.** 2008. Determination of norovirus contamination in oysters from two commercial harvesting areas over an extended period, using semiquantitative real-time reverse transcription PCR. J. Food Prot. **71:**1427–1433.
111. **Richards GP, Watson MA, Meade GK, Hovan GL, Kingsley DH.** 2012. Resilience of norovirus GII.4 to freezing and thawing: implications for virus infectivity. Food Environ. Virol. **4:**192–197. http://dx.doi.org/10.1007/s12560-012-9089-6.
112. **Kukkula M, Maunula L, Silvennoinen E, von Bonsdorff C-H.** 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. J. Infect. Dis. **180:**1771–1776. http://dx.doi.org/10.1086/315145.
113. **Bae J, Schwab KJ.** 2008. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. Appl. Environ. Microbiol. **74:**477–484. http://dx.doi.org/10.1128/AEM.02095-06.
114. **Bruggink LD, Marshall JA.** 2010. The incidence of norovirus-associated gastroenteritis outbreaks in Victoria, Australia (2002–2007) and their relationship with rainfall. Int. J. Environ. Res. Public Health **7:**2822–2827. http://dx.doi.org/10.3390/ijerph7072822.
115. **Skraber S, Ogorzaly L, Helmi K, Maul A, Hoffman L, Cauchie H-M, Gantzer C.** 2009. Occurrence and persistence of enteroviruses, noroviruses and F-specific RNA phages in natural wastewater biofilms. Water Res. **43:**4780–4789. http://dx.doi.org/10.1016/j.watres.2009.05.020.
116. **Wang D, Wu Q, Kou X, Yao L, Zhang J.** 2008. Distribution of norovirus in oyster tissues. J. Appl. Microbiol. **105:**1966–1972. http://dx.doi.org/10.1111/j.1365-2672.2008.03970.x.
117. **Asahina AY, Lu Y, Wu C, Fujioka RS, Loh PC.** 2009. Potential biosentinels of human waste in marine coastal waters: bioaccumulation of human noroviruses and enteroviruses from sewage-polluted waters by indigenous mollusks. J. Virol. Methods **158:**46–50. http://dx.doi.org/10.1016/j.jviromet.2009.01.013.
118. **Ueki Y, Shoji M, Suto A, Tanabe T, Okimura Y, Kikuchi Y, Saito N, Sano D, Omura T.** 2007. Persistence of caliciviruses in artificially contaminated oysters during depuration. Appl. Environ. Microbiol. **73:**5698–5701. http://dx.doi.org/10.1128/AEM.00290-07.
119. **Greening GE, Hewitt J, Hay BE, Grant CM.** 2003. Persistence of Norwalk-like viruses over time in Pacific oysters grown in the natural environment, p 367–378. *In* Villalba A, Reguera B, Romalde JL, Beiras R. (ed), Proceedings of the 4th International Conference on Molluscan Shellfish Safety, Santiago de Compostela, Spain, 4 to 8 June 2002.
120. **Vilariño ML, Le Guyader FS, Polo D, Schaeffer J, Kröl J, Romalde JL.** 2009. Assessment of human enteric viruses in cultured and wild bivalve molluscs. Int. Microbiol. **12:**145–151. http://dx.doi.org/10.2436/20.1501.01.92.
121. **Phillips G, Tam CC, Rodrigues LC, Lopman B.** 2011. Risk factors for symptomatic and asymptomatic norovirus infection in the community. Epidemiol. Infect. **139:**1676–1686. http://dx.doi.org/10.1017/S0950268810002839.
122. **Lowther JA, Gustar NE, Hartnell RE, Lees DN.** 2012. Comparison of Norovirus RNA levels in outbreak-related oysters with background environmental levels. J. Food Prot. **75:**389–393. http://dx.doi.org/10.4315/0362-028X.JFP-11-360.
123. **Le Guyader FS, Loisy F, Atmar RL, Hutson AM, Estes MK, Ruvoen-Clouet N, Pommepuy M, Le Pendu J.** 2006. Norwalk virus specific binding to oyster digestive tissues. Emerg. Infect. Dis. **12:**931–936. http://dx.doi.org/10.3201/eid1206.051519.
124. **Tan M, Jiang X.** 2007. Norovirus-host interaction: implications for disease control and prevention. Expert Rev. Mol. Med. **9:**1–22. http://dx.doi.org/10.1017/S1462399407000348.