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Pathogenic Human Viruses in Coastal Waters

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INTRODUCTION

All of the known pathogenic viruses that pose a significant public health threat in the marine environment are transmitted via the fecal-oral route. This group, known collectively as enteric viruses, is a continually growing list. These viruses belong primarily to the families *Adenoviridae* (adenovirus strains 3, 7, 40, and 41), *Caliciviridae* (Norwalk virus, astroviruses, caliciviruses, Snow Mountain agent, and small round structured viruses), *Picornaviridae* (poliovirus, coxsackieviruses, echoviruses, enteroviruses, and hepatitis A virus [HAV]), and *Reoviridae* (reoviruses and rotaviruses). The enteric viruses are associated with a variety of diseases in humans (Table 1), from ocular and respiratory infections to gastroenteritis, hepatitis, myocarditis, and aseptic meningitis. The elderly, the very young, and the immunocompromised are the most susceptible and are more likely to develop severe infections (40). While diarrhea has been one of the primary manifestations of infection, it is now recognized that more serious chronic diseases are associated with viral infections and that the risks of such infections need to be better defined. The primary site of viral infection and replication is the intestinal tract. Virus particles are shed in feces for weeks at levels as high as 10^{10} g^{-1} (rotaviruses), with average levels between 10^6 g^{-1} (enteroviruses) and 10^8 g^{-1} (HAV) (32, 36, 166). While person-to-person and surface-to-person transmission can occur, viruses in wastewater that contaminate drinking water sources, recreational waters, and shellfish harvesting waters pose the greatest risk to the public (14, 42, 90, 99, 107, 122; C. P. Gerba and C. E. Enriquez, Proc. First Ann. Chem. Symp., 1997).

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The majority of the world's population is found along the coasts. Often, wastewater is disposed directly or indirectly into coastal waters. Approximately 9.3×10^7 people (37%) of the total U.S. population reside in coastal areas and discharge about 1.0×10^{10} gallons of treated wastewater day^{-1} (101). In 2000, there were over 11,000 beach closings or advisories (freshwater and marine beaches) in the United States, a number that had almost doubled from the previous year, and a majority of these closings were due to wastewater pollution (102). On a global scale, coastal development is twice that of inland sites, with ~90% of the generated wastewater being released untreated into marine waters (20, 60).

The discharge of viral pathogens in treated sewage is not regulated, and monitoring relies on bacterial indicator detection to predict virus contamination (54). Virus levels in wastewater, measured by cell culture assay, range from 1.82×10^2 to 9.2×10^4 $liter^{-1}$ in untreated sewage and from 1.0×10^{-3} to 1.0×10^2 $liter^{-1}$ in treated wastewater depending on the level of treatment (101, 131, 133). Some of the wastewater that is discharged into the marine environment is only partially treated and is not disinfected (examples of partially treated discharges where only the undissolved solids are removed before release of the sewage effluent from the plant can be found in Los Angeles, San Diego, and Hawaii). Combined sewer overflows, which are systems that receive rainwater and untreated wastewater and overflow during high precipitation events, have been sources of coastal pollution in areas like Puget Sound, Wash. (130). Finally, communities with high-density septic tanks (on-site, individual disposal systems) also contribute to poor water quality and increased viral pollution of coastal waters (52, 87).

Prior to 1950, little was known about human viral infections except for those associated with the more widespread diseases such as hepatitis. The first isolations of viruses from water were

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TABLE 1. Examples of viral waterborne pathogens of concern

Pathogen	Diseases	Yr first described (reference)	Methods of detection (references)
Coxsackievirus (members of the enterovirus group belonging to *picornaviridae*)	Aseptic meningitis, herpangina, paralysis, exanthema, hand foot and mouth disease, common cold, hepatitis, infantile diarrhea, acute hemorrhagic conjunctivitis	1947[a] (22)	Tissue culture methods, molecular methods (65, 96)
Hepatitis viruses	Fever, nausea, abdominal pain, anorexia and malaise, associated with mild diarrhea, arthralgias, scleral icterus; cytologic damage, necrosis and inflammation of the liver (HAV)	1950[b] (12)	Tissue culture methods, molecular methods, Immunofluorescence (70, 117, 150, 152)
Norwalk-like viruses (belonging to *Caliciviridae*)	Diarrhea, vomiting, abdominal pain, cramping, low fever, headache, nausea, tiredness (malaise), muscle pain (myalgia)	1968,[a] 1968[b] (40)	Transmission EM, ELISA, molecular methods (69, 143)
Rotavirus (belonging to *Reoviridae*)	Vomiting, abdominal distress, diarrhea, dehydration, fever	1973[a] (9)	Tissue culture methods, molecular methods (49)

[a] Human pathogen.
[b] Waterborne transmission.

reported in the late 1930s and early 1940s and focused on poliovirus and other enteroviruses in feces and wastewater (22, 114). The method employed a gauze pad to adsorb the virus as the water passed through it and the subsequent use of cell culture (monkey kidney cells) to cultivate and identify the virus. Improvements in the filtration methods to recover viruses from water and the emergence of newly discovered pathogenic human enteric viruses associated with drinking water and shellfish outbreaks (the discovery of rotavirus and Norwalk virus) occurred in the 1970s and 1980s (42, 90).

The occurrence of pathogenic human enteric viruses in marine waters is not well characterized, and our understanding has been hampered by the limited number of scientific studies, lack of available and accurate detection assays, and erroneous assumptions in regard to virus viability and infectivity. The contamination of marine waters with viruses has been, and will continue to be, an important public health issue. The spread of viral diseases through recreational water exposure and ingestion of contaminated shellfish is a primary public health concern. The key to understanding and controlling viral contamination of our coastal environments is the application of new tools for monitoring and studying these microorganisms.

PUBLIC HEALTH RISK

Health Effects

The enteric viruses cause a wide range of diseases and symptoms. Gastroenteritis, otitis, and respiratory diseases are most often reported in studies of exposure to contaminated marine waters. Viral etiology is rarely identified, even though viruses are believed to cause a majority of waterborne marine illnesses.

Adenoviruses, which were first described in 1953 (134), have been identified as a significant cause of acute upper respiratory tract infections. While most infections caused by these viruses are asymptomatic, respiratory, gastrointestinal, and ocular infections are commonly reported. These viruses can cause significant problems for immunosuppressed or immunocompromised persons (146, 154). There are over 48 serotypes of adenoviruses, that comprise six subgroups (A to F), which have been identified as causative agents of human disease. Adenoviruses can cause a wide range of disease types, including respiratory, ocular, and gastrointestinal infections (35). The enteric adenoviruses are types 40 and 41 and are responsible for the majority of adenovirus-mediated cases of gastroenteritis (19, 28). Other types of adenoviruses which can cause gastroenteritis are types 3 and 7 in systemic infections. Most individuals become infected with adenoviruses before they reach 20 years of age. The adenoviruses are commonly found in wastewater-impacted marine environments (68, 79, 118, 120, 160).

Coxsackieviruses have emerged as important waterborne enteric pathogens. In a study of viruses in raw sewage and treated effluent in Puerto Rico, it was demonstrated that 95% of the enteroviruses detected were coxsackie B5 virus (21). Others have reported that approximately 30% of the isolates from untreated wastewater (Athens, Greece) were coxsackie B2, B4, and B5 virus, with an estimated overall coxsackievirus concentration of 35.8 to 172.8 cytopathic units per liter (79, 80). Coxsackieviruses have been associated with myocarditis, paralytic disease, aseptic meningitis, insulin-dependent diabetes, and cold-like illnesses (76, 77, 162).

Norwalk-like viruses (also called small round structured viruses) have been identified in drinking water (72, 84, 85), recreational water (73), and marine shellfish (81) tissue in a number of studies investigating their role in human disease (25, 29, 50, 55, 90). This diverse group of RNA viruses often causes gastroenteritis with diarrhea and/or vomiting, fever, and respiratory symptoms lasting approximately 2 days. These viruses were first identified by electron microscopy and include Norwalk virus, Snow Mountain agent, astroviruses and caliciviruses. These viruses are a major cause of shellfish-associated disease and may be the most significant cause of adult viral gastroenteritis (4, 5, 25, 29, 55, 63, 84, 86, 90, 91, 148).

Shellfish preferentially accumulate microorganisms during periods of low water temperatures (between 11.5 and 21.5°C), which results in a higher incidence of human viral gastroenteritis acquired through shellfish consumption during these periods (13). This preferential accumulation of microorganisms by shellfish, coupled with the enhanced survival of viruses at lower temperatures, may explain the seasonality of shellfish-borne viral disease. This group of viruses is heat stable and more resistant to chlorine disinfection than are bacteria. It has been suggested that caliciviruses are much more prevalent than

were previously thought and have been reported in wastewater at 10^7 RNA-containing particles liter^{-1} (91).

Indigenous marine caliciviruses excreted into the water column by mammals may cause disease in swine (vesicular exanthema) and humans (149). These authors noted that infected whales may excrete an estimated 10^6 caliciviruses g of feces^{-1} as enumerated by electron microscopy (149).

Rotavirus was first identified as a causative agent of gastroenteritis in 1973 and is thought to be responsible for the deaths of 4×10^6 to 5×10^6 individuals annually worldwide (9). It is one of the major causes of infantile viral gastroenteritis worldwide, but adults are also susceptible. Several outbreaks of waterborne disease have been attributed to this organism. It is estimated that over one million cases of severe diarrhea in the 1- to 4-year-old age group are caused by rotaviruses annually in the United States, with up to 150 deaths (62). All of the rotavirus outbreaks have been associated with direct fecal contamination of an untreated or compromised water supply or suboptimal treatment of drinking water. Rotaviruses have been detected in surface waters worldwide, with average concentrations ranging from 0.66 to 29 liter^{-1} (43). The highest concentrations have been reported in surface waters receiving untreated wastewater effluent.

Other viruses may be of concern for non-industrialized countries. A review of hepatitis E virus (HEV) reported this virus to be a significant cause of hepatitis in tropical and subtropical environments, resulting in a high mortality (20%) of pregnant women (150). It has been suggested that swine feces may serve as a source of HEV contamination of recreational and shellfish-harvesting coastal waters (150).

Recreational Risks

In the United Kingdom, studies of marine recreational waters began in the 1970s. The European Economic Directive "Quality of Bathing Water" (76/160/EEC) required that cultivatable viruses be absent in 10 liters of water (<1 PFU/10 liters) At that time in Europe (unlike the United States), no mandate for wastewater treatment existed and untreated wastewater was discharged directly to the marine environment through outfalls. The approach to protect public health was to monitor bathing beaches in order to identify impact. At beaches associated with sewage outfalls, viruses were detected at levels between 1 and 520 PFU/10 liters (147). Illness was observed in adults and children using beaches where human viruses were detected (8).

Documented outbreaks in marine waters are rare; however, numerous epidemiological studies have found swimmers to be at increased risk of disease after swimming at polluted beaches (25, 34, 60, 105, 148). The etiology of illnesses in these studies was not identified, and the pollution was documented using bacterial indicator systems. Major epidemiological studies have been undertaken in the United Kingdom, United States, and China. In Hong Kong it was estimated that beach swimmers were five times more likely to develop gastroenteritis than nonswimmers, with no less than 400,000 illnesses attributed to exposure to polluted beach water in 1990 (16). Results from interviewing 25,000 beach users in Hong Kong in 1992 concluded that swimmers were 2 to 20 times more likely to exhibit eye, skin, and respiratory symptoms than were nonswimmers (81). In an effort to protect public health, Hong Kong developed a beach rating system with *Escherichia coli* as the water quality indicator for 41 of its recreational beaches (three samples per month with a geometric mean limit of 180 CFU/100 ml in March through October).

Research has correlated enterococcus (*Enterococcus* spp., formerly group D streptococci) levels with both gastrointestinal and respiratory disease (34, 75). Bathers swimming in waters containing ≥60 CFU of streptococci/100 ml of water were at risk of developing some form of illness compared to nonbathers (34). Significant gastrointestinal health effects were noted when enterococcus levels exceeded 32 CFU/100 ml measured in bathing water at chest depth (75). At a beach in Ramsgate, United Kingdom, 24% of 1883 individuals reported at least one symptom of illness after wading, swimming, or surfing at the beach and the relative risk was significant for bathers (relative risk = 1.31) versus nonbathers (8).

Based on epidemiological studies, the U.S. Environmental Protection Agency (UEPA) recommends the use of enterococci for monitoring the safety of marine bathing waters (26). Although epidemiological recreational water studies support the enterococcal standard, many states continue to rely on the coliform standard (119). An epidemiological study in Santa Monica Bay, Calif., found that 373 individuals of every 10,000 who swam near active storm drains were at risk of developing some symptom of disease (56). This led to a significant change in monitoring and to approaches to protect public health, including the closure of Huntington Beach in 1999. The 4-month closure, which was due to microbial standard violations, resulted in the loss of millions of dollars in tourism income to the business community and almost two million dollars in beach closure investigation fees (164). One of the investigation recommendations was that virus monitoring be implimented.

Despite knowledge of nearshore water pollution in Key West, Fla., a competitive race, Swim around Key West, was held in 1999. Surveys obtained from 160 swimmers who participated in the event reported that 31% had a least one symptom of disease following the event (105), with gastroenteritis being the most commonly reported symptom. It is difficult to relate outbreaks to epidemiological data after marine recreational exposure, and it is more feasible to monitor for pathogens of concern, including the viruses.

Risk assessment models can be used to evaluate health risks in the absence of epidemiological data by using pathogen-monitoring information. In a comprehensive study in Hawaii, an area that most would view as a pristine aquatic environment, enteroviruses were found in 6% of samples screened (18). Risk assessment models used to estimate the maximum risks from exposure to this level of viruses predicted 1.3 infections per 100 swimmers (18).

Viruses attached to particulate matter (inorganic soil particles or organic detritus) are more likely to cause disease than are free-floating viruses. Researchers used a model to predict the risk of infection from both unattached and particle-associated viruses in the Oder Lagoon (Southern Baltic). Unattached viruses were only a risk in a river and at the river mouth during the summer bathing period. Viruses attached to particles extended the range of risk from the river mouths out into associated regions of the lagoon (137). Risk modeling has also incorporated epidemiological data such as the number of in-

TABLE 2. Occurrence of human viruses in marine environments

Virus(es)	Location	Maximum % of samples positive via cell culture or PCR/RT-PCR	Virus concn/10 liters assayed where data available	Reference
Enteroviruses	Galveston Bay, Tex.	40.0 (cell culture)	Data not included	38
Enteroviruses	Texas Gulf coast	59.0 (cell culture)	0.1–4.4	45
Enteroviruses	Galveston Bay, Tex.	72.0, 14.0 for sediment and water (cell culture)	0.1–10.5	121
Enteroviruses	Waikiki and neighboring beaches, Hawaii	8.0 for beach samples, 50.0 for sewage outfall samples (cell culture)	<0.1–130,000	125
Adenoviruses, enteroviruses, and HAV	Nearshore waters of Barcelona, Spain	78.0, 44.0, and 33.0, respectively (PCR/RT-PCR)	Presence/absence only	118
Enteroviruses and adenoviruses	Beaches of Patras, Greece	83.4.0 and 90.0, respectively, (RT-PCR/PCR)	1.2–6.0 (via cell culture, enteroviruses only)	160
Enteroviruses, HAV, and Norwalk virus	Canals and nearshore waters of the Florida Keys	79.0, 63.0, and 11.0 respectively, (RT-PCR)	Presence/absence only	52
Reoviruses	Beaches along the Catania coastline of Sicily	27.0 (cell culture)	Presence/absence only	7
Enteroviruses and reoviruses	Beaches of Pesaro, Italy	32.6 and 49.3, respectively (cell culture)	Presence/absence only	116
Reoviruses	Nearshore and canal waters around the mouth of the Tiber River, Italy	3.0 (cell culture)	2.0–5.0	6
Enteroviruses	Nearshore waters, Florida Keys	93.0 (RT-PCR)	0.1 via cell culture for one water column sample; remaining presence/absence only via RT-PCR	89
Enteroviruses	Sarasota estuary, Fla.	25.0 for samples (cell culture) 91.0 for stations positive (RT-PCR)	17.0–77.0 (via cell culture)	87
Enteroviruses	Charlotte Harbor estuary, Fla.	21.9 (cell culture), 75.0 during the 1997–1998 El Niño	100.0–200.0	88
Adenoviruses	Beaches from Malibu, Calif., to the Mexican border	33.3 (PCR)	8,800.0–75,000.0 (via MPN-nested PCR)	68
Reoviruses and enteroviruses	Fano Beach and Albani Channel Harbour, Italy	30.0 and 0.0, respectively (RT-PCR)	Presence/absence only	100
Enteroviruses	Santa Monica Bay, Calif.	32.0 (RT-PCR)	Presence/absence only	103

dividuals with symptoms, bacterial indicator data, pathogen concentrations, and dose-response functions to calculate the recreational risk in specific aquatic environments (27).

VIRUS OCCURRENCE AND FATE IN MARINE WATERS

Occurrence Studies

Studies performed during the last 5 years on the occurrence of viruses in coastal waters are from geographically diverse areas. Groups of researchers have conducted investigations in Greece, Italy, California, Florida, and Hawaii, using cell culture and/or molecular techniques (Table 2).

In Italy, Pianetti et al. investigated nearshore waters along the beaches of Pesaro on the Adriatic Sea and found that 32% of 144 samples were positive for viable enteroviruses by cell culture (116). They concluded the viral pollution was originating from waste disposal systems (Foglia River discharge and regional public and resort wastewater disposal systems) being used in the region which periodically impact regional beach water quality. Previous studies by another group of researchers in the same area (estuarine waters of the Foglia River and Pesaro beach water samples) found poliovirus type 3 by reverse transcriptase polymerase chain reaction (RT-PCR) in cell culture-positive samples (98). Muscillo et al. surveyed wasters off of Fano's beaches and in Albani Channel Harbour (Adriatic coastline of Italy) and found that 30% of 72 samples were positive for the presence of reoviruses by RT-PCR (100). No enteroviruses were detected.

Aulicino et al. surveyed waters of the Catania coastline on Sicily (Ionian Sea) and nearshore sites around the mouth of the Tiber River (Tyrrhenian Sea) in Italy (6, 7). Reoviruses were detected in 27% (12 of 44) and 3% (12 of 58) of the samples in these two studies, respectively. No enteroviruses were detected in either of these studies. In the case of the Tyrrhenian Sea study, the authors attributed advanced wastewater treatment and disinfection as reasons for good water quality at a majority of the monitored sites. In Southwest Greece along the beaches of Patras (beaches on both the Gulf of Patras and the Corinthian Gulf), enteroviruses and adenoviruses were detected in 17% (21 of 120) and 28% (34 of 120)

of the samples by nested PCR, respectively (160). When these samples were screened via cell culture, the enterovirus concentration was 290 PFU/100 liters. Only 9% (11 of 120) of the samples were positive for enteroviruses by cell culture, in contrast to the 17% positive sample rate via nested PCR.

A Hawaiian water quality study found that 8% of beach samples were positive for enteroviruses by cell culture (125). Concentrations were on average 3.7 PFU/100 liters, with the highest level detected at 10 PFU/100 liters. This study of Mamala Bay, Oahu, that included Waikiki and other beaches, suggested the viral pollution was originating from a wastewater outfall that received only primary treatment. This work led to recommendations that better sewage treatment be implemented.

Extensive studies in Florida have shown that enteroviruses and HAV can be routinely detected in nearshore marine environments. In Charlotte Harbor (important recreational and shellfish waters) on the Gulf Coast of Florida, enteroviruses were detected by cell culture assay in 75% of the sites screened (88). This study also found that combined surface water temperature, rainfall data, and regional river discharge rates could predict virus presence 98% of the time. In Sarasota, Fla., just north of Charlotte Harbor, enteroviruses were detected in 25% of the samples and at 81% of the sites by cell culture, with concentrations ranging from 0.17 and 0.59 PFU/100 liters (87). Using RT-PCR, 91% of the sites were shown to be positive for the presence of enteroviruses. Enteroviruses identified in these samples included echovirus types 20 and 24 and poliovirus. Their presence was attributed to the high density of septic tanks in this coastal community (87).

Over the last 20 years, extensive residential development in the Florida Keys, without the development of municipal wastewater treatment and disposal systems, has led to the majority of canal front homes using septic tanks for waste disposal. In these canals and in nearshore water sites of the Florida Keys, 79% of sample sites were positive for enteroviruses, 63% were positive for HAV, and 11% were positive for Norwalk viruses when samples were screened by RT-PCR (52). A total of 95% of sites were positive for at least one of the viral groups overall. Studies conducted in nearshore water of the Florida Keys showed that 93% of coral mucus samples were positive for the presence of enterovirus nucleic acid sequences as confirmed by PCR plus dot blot probing (89). The research in Florida has been able to demonstrate that: (i) the bacterial indicator levels used as recreational standards were not predictive of viral pollution; (ii) bacterial indicator levels were often under the standard limit, despite the presence of human viruses; and (iii) all sites were under the direct influence of either septic tank drain fields or compromised sewage treatment systems.

In Southern California, 33% (4 of 12) of marine samples were positive for adenoviruses. Most-probable-number (MPN) concentration estimates indicated that there were 880 to 7,500 adenoviruses per liter of water (68). These marine sites were located outside of river discharge points, and the authors noted that as in the Florida studies, bacteria indicators did not correlate with the presence of viruses. The authors did note that F^+-specific RNA coliphages (RNA bacteriophages which require pilus production by strains of pilus-producing *E. coli* for attachment, i.e., F^+ *E. coli*) did correlate with the presence of adenovirus (68). A study conducted in Santa Monica Bay, Calif., found enterovirus RNA in 32% of 50 samples by RT-PCR (103). These authors noted that while no correlation could be established between the presence of individual microbial water quality indicators and enterovirus RNA, there was a significant correlation between a recently adopted combined microbial indicator standard (total coliforms, fecal coliforms, and enterococci) and enterovirus RNA (103). In a 1979 study, 40% of the samples which passed bacterial indicator water quality standards contained viable enteroviruses (38). Other studies have shown that enterovirus contamination cannot be assessed by using bacterial indicators (46, 160).

Survival and Transport

A number of studies have investigated the ability of viruses to survive in marine systems and be transported from their sources to marine beaches or shellfish waters. The use of viral tracers was particularly successful in the study of septic tanks and package plants utilizing 90-ft injection wells in the Florida Keys. In Key Largo, Fla., bacteriophage were flushed down a toilet in a home with a septic tank and drain field adjacent to Port Largo Canal. The bacteriophage appeared in the canal in 11.2 h and in nearshore waters in 23 h. Estimated migration rates were as high as 24.2 m h^{-1}, a rate 500-fold greater than that seen using subsurface flow meters (113). Bacteriophage flushed down two package plant injection wells located in Key Largo and Long Key appeared in groundwater monitoring wells in 8 h and in surface waters in 10 and 53 h, respectively. Migration rates from the Key Largo canal ranged from 2.5 to 3.5 m h^{-1}, and the rates in Long Key were 0.1 to 2.0 m h^{-1}. The higher rates in Key Largo were due to different influences of tidal pumping from site to site (111). In a similar study, viral tracer was observed in an adjacent canal in 3.25 h in a septiotank study conducted in Marathon Key (Boot Key Harbor, the middle Keys). Another injection well study on Saddlebunch Key (lower Keys) found migration rates as high as 141.0 m h^{-1}. In both studies, movement was toward the reef tract and Atlantic Ocean, with rapid movement being facilitated via tidal channels (112). All of the bacteriophage tracer studies demonstrated that septic tanks and wastewater treatment plants utilizing shallow-water injection wells do contribute to the deterioration of regional coastal surface water quality. This is of particular concern when waters such as those off the Florida Keys are heavily utilized for recreational activity in addition to being ecologically sensitive to poor water quality, a common trait of all coral reef ecosystems. Several physical factors such as tidal pumping and the porous nature of limestone contribute to the rapid groundwater migration rates observed in many of Florida's coastal communities (145).

Along the Catania coastline of Italy, reoviruses were detected only during the autumn and winter months, indicating that surface water temperature may play a key role in the survival of enteric viruses in marine environments (7). Another study noted that reoviruses were found in 95% of samples during the winter and in only 15% of the samples during the summer (59). In Charlotte Harbor, Fla., enteroviruses were detected by cell culture only in December and February, when lower surface water temperatures coincided with an increase in the number of water quality indicators relative to warmer months (88). This study also noted that an increase in rainfall and river discharge due to the 1997 to 1998 El Niño season

decreased estuarine salinity and increased the probability of detecting viable enteroviruses (88). A 3-year study of French coastal waters indicated that the presence of viruses (enteroviruses, HAV, Norwalk-like virus, astrovirus and rotavirus) in shellfish usually coincided with incidence of human disease and episodes of rain. These authors hypothesized that short, heavy winter rains caused an overload of sewage treatment facilities, resulting in contamination of shellfish beds (92). Whether the source of feces in a given region is due to indigenous wildlife or human populations, precipitation events have been shown to significantly influence microbial water quality by increasing the bioload (33, 53, 88, 109, 129, 161, 165).

In a marine virus survival study, 90% inactivation of poliovirus and parvovirus was observed in 1 to 3 days at the highest temperature compared to 10 days at the lowest temperatures. The temperatures used in this study ranged from 6 to 28°C (163). In the Florida Keys, where 79% of the samples were positive for enterovirus presence by RT-PCR in the summer (the average water temperature ranged from 29 to 33°C), none of the samples were positive by cell culture (52). In a follow-up study to determine if water temperature influenced the viability of viruses being released into nearshore waters of the Florida Keys, six of the original samples were screened for the presence of viable viruses when surface water temperatures averaged ~25°C. Two of the six sites were positive for enteroviruses by cell culture (J. L. Jarrell, E. K. Lipp, D. Griffin, J. Lukasik, D. Wait, M. Sobsey, and J. B. Rose, submitted for publication). This study further noted in a seeded marine study that viruses survived longer at moderate (22°C) than at higher (30°C) temperatures while viral nucleic acid persisted for a longer period (≥60 days) than did infectious viruses (≤51 days). These data emphasize the need to couple PCR with a viability assay (if one exists) to account for the detection of inactivated versus infectious human viruses.

A study at an offshore sewage sludge dumping site noted that viable enteric viruses could be detected in the sediments 17 months after dumping was halted (46). Enteroviruses associated with marine solids were shown to remain infectious for 19 days compared to 9 days for unassociated enteroviruses in the water column (121). The increase in the survival of enteroviruses associated with particulate matter has been observed by others (17, 82, 83). A 3-log-unit reduction in viable HAV and poliovirus type 1 at 5 days occurred in the water column (using a cell culture assay for a seeded estuarine study) (108). Marine mammal caliciviruses remained viable for over 14 days when the water temperature was 15°C (149). In a cell culture-based assay of feline caliciviruses, infectious viruses were detected for 30 days at or below 10°C (71).

This work has shown that temperature, rainfall, type of disposal system, and coastal processes such as tides and currents are responsible for the survival and transport of viruses in marine systems. The rates of survival and transport may be geographically dependent. Further research on comparative studies on coastal processes and particularly on the role of sediments is needed.

EVOLUTION OF DETECTION ASSAYS

In 1970, the Committee on Environmental Quality Management of the Sanitary Engineering Division of the American Society of Civil Engineers Committee Report stated that the methods for detecting, identifying, and enumerating viruses in water samples were inadequate (61). In 1971, when reference 61 was written by Hill et al., cell culture was the only method of viral detection for environmental samples. Many techniques such as radioimmunoassay, immunofluorescence (IF), complement fixation, and enzyme-linked immunosorbent assay (ELISA) were routinely used to identify viruses in clinical specimens but were either cost prohibitive or not sensitive enough for use with environmental samples. Today the standard method of virus detection continues to be cell culture.

Modern viral detection assays hold the greatest promise for current and future studies. These include molecular based techniques based on PCR and other amplification technologies. Regardless of the detection assay employed, research has demonstrated the need for efficient concentration from large volumes of water when attempting to detect viruses in all but the most polluted waters. Historically, the use of filter cartridges and the manipulation of water chemistry have been employed to accomplish this.

Sample Collection

Viral collection protocols evolved from the early use of gauze pads for capturing viruses. In the mid-1970s, the concentration of enteroviruses from 400 liters of marine water to volumes as low as 10 ml, with a recovery rate of 60 to 80% using cartridge filters, was demonstrated (30). It was shown that elution solutions at pH 9.0 were efficient at recovering viruses adsorbed to membrane filter surfaces (31). These methods used a pump to move marine water through a membrane filter located in a filter housing. After filtration, the viruses which were attached to the filter surface were released by using an eluate (1 to 3 liters) at a basic pH. Viruses were then concentrated in the eluate to 10 to 70 ml by using a series of chemical and pH manipulations and centrifugation. Concentrated viruses were then stored under refrigeration until an aliquot was used for cell culture assay. Fiberglass filters were utilized in a study in which rotaviruses were detected in large volumes of seawater (47). Another study investigated a microporous filtration method with recovery rates of 41% from 400 liters of water (155). This basic membrane filtration approach to collecting viruses from large volumes of water, although tedious, is still used today in many environmental virus studies (3). Variations of the original method exist for efficient recovery of viruses from freshwaters or marine waters by using a variety of filter types. A recently developed protocol which utilizes negatively charged membranes for filtration of 2 liters of marine water followed by real-time PCR for detection demonstrated recovery rates greater than 61% for poliovirus in seeded samples (74). This protocol is rapid and efficient and was used successfully to detect Norwalk virus and enteroviruses in Tokyo Bay (74).

An alternate method to membrane filtration, which has been successfully used to detect viruses in marine water, is vortex flow filtration (VFF). This technology utilizes a flow pattern (toroidal vortices) that keeps cell-sized particles from contacting the filter surface as water is removed from the sample. Typically 20-liter samples are concentrated to ~50 ml over 2.5 to 3 h. The benefit to this technology is that it is automated and

requires minimal supervision. The drawbacks are the cost of the equipment, the limitation on the volume of sample that can be concentrated relative to the existing membrane filtration protocol, and the time required for filtration. Samples seeded with T2 phage reported a recovery rate of 72% for T2 bacteriophage (110). Pathogenic human viruses have been detected in a number of marine water quality studies using this concentration method (52, 157). A similar ultrafiltration protocol known as tangential-flow filtration (TFF) is widely used in the biotechnology sector and has been successfully used to detect viruses in marine water (68, 159). While TFF has shown promise, some investigators have noted problems in regard to quantitative work with marine waters and a lower recovery rate in comparison to VFF (68, 128).

In an assay that was developed using silica particles and centrifugation, enteroviruses were detected in seeded and unseeded samples from an original marine water volume of 10 liters. This assay, like the outlined membrane filtration protocol, utilized pH manipulation to alternately capture and release viruses from a matrix (106).

Cell Culture

Until the 1990s, cell culture assay was the most widely used protocol for the detection of viruses in water (30, 37, 38, 46, 47, 121, 141). It had long been recognized that cell culture for the detection of viruses in waters and sediments has numerous shortcomings, such as long turnaround time, low sensitivity, and high cost. In addition, cell culture can be labor-intensive and host cells for many viruses such as the Norwalk-like viruses have not been identified (44, 151). Although the limitations of cell culture are clear, there is no alternative protocol to determine if viruses present in a given volume of water are viable and thus capable of causing infection. In a typical cell culture protocol, an aliquot of sample is exposed to host cells and incubated over time. If the viable viruses are present, destruction of host cells occurs, a process known as the cytopathic effect (115). Incubation periods vary depending on the protocol used, but the typical range is 2 to 4 weeks and can be double that depending on confirmation steps. Given the length of time required to complete the assay, it is clearly not a proactive monitoring protocol. To date, cell culture is used in parallel or in conjunction with emerging detection assays to address the issue of viability and to compare the sensitivity of assays (2, 48, 78, 87, 139; M. Abbaszadegan, P. Stewart, M. LeChevallier, M. Yates, and C. Gerba, Proc. Water Qual. Technol. Conf., 1995). The only alternatives to the cell culture assay prior to the mid- to late 1980s were the use of electron microscopy (EM) and hybridization or antibody-based assays to enumerate or detect viruses (11, 47, 58, 64).

Immunology

In the 1980s, several researchers tried some of the immunological methods used in clinical laboratories on environmental samples. Steinmann (151) used enzyme-linked immunosorbent assay (ELISA) and EM to detect rotavirus in concentrated sewage. Dowell et al. (25) used a nitrocellulose enzyme immunosorbent assay technique (NC-EIA) to monitor viruses in environmental waters. Both NC-EIA and ELISA are fast and easy and can be adapted for instrumentation, large-scale studies, and quantitative analysis. However, these methods can detect only high concentrations of viral particles. Ten million virus particles per ml of sample must be present to be detected by EM, and viral concentrations in coastal environments would probably be several orders of magnitude lower than this. Additionally, immunological techniques such as ELISA, radioimmunoassay, and IF can identify only viruses for which a specific antibody has been produced and cannot distinguish between infectious and non-infectious particles.

However, if IF is used in conjunction with cell culture, it can increase sensitivity, provide information on infectivity of the virus, and yield results in 24 to 48 h. One of the first uses of IF with cell culture for the detection of rotaviruses was described by Gerba et al. (39). However, again, only viruses for which appropriate cell lines and specific antibodies have been produced can be detected by this method.

PCR Amplification

A few of the most recent studies utilizing PCR and RT-PCR, nested PCR, and integrated cell culture PCR for marine samples are described in Table 3. Of the 10 studies, 5 focused on virus detection in shellfish and the other 5 examined viral contamination of marine waters. This compilation of work demonstrates that a diverse group of enteric viruses can be detected, including Norwalk and Norwalk-like viruses, HAV, small round structured viruses, enteroviruses, rotavirus, and adenoviruses. Some studies reported the sensitivity in regard to PFU as measured in cell culture. As few as 0.1 PFU could be detected, but other studies reported the sensitivity of the assay based on viral particles (PCR units), and only Jiang et al. (68) reported the sensitivity per volume of water concentrated. A variety of methods and primers and probes were used, and thus far no standardization of methods has been forthcoming. The American Academy of Microbiology has strongly recommended to the EPA, European Union, World Health Organization, and other organizations that they financially support the round-robin testing of PCR methods for detection of viruses as well as other microorganisms. This is needed so that a consensus method and standardization for the application of PCR for virus detection in all types of water can be developed (132).

Since the late 1980s, PCR has been used for the detection and quantitation of viral pathogens in the environment. PCR was first developed and employed in laboratory-based studies (95, 135). Some of the earliest studies employing RT-PCR for the detection of enteroviruses included laboratory and field studies (15; R. DeLeon, C. Shieh, S. Baric, and M. D. Sobsey, Proc. Water. Qual. Technol. Conf., 1990). Standard RT-PCR has since been used in a number of marine-based water quality monitoring studies (52, 87–89, 118, 122).

A enterovirus primer set first used in environmental monitoring in the late 1980s is currently one of the most widely used primer-probe combinations employed in environmental monitoring due to good sensitivity, specificity, and reliability (DeLeon et al., Proc. Water Qual. Technol. Conf., 1990). An evaluation of this primer set against four other existing or new sets demonstrated that the original was superior or equivalent in sensitivity and specificity (unpublished data). This primer-

TABLE 3. Compilation of select viral studies in marine systems by PCR[a]

Author (yr)	Amt and sample type	Concn method	Viral extraction or elution	Nucleic acid extraction	Method	Virus isolated	Primers (5′ to 3′)	Probe	Amplicon, target	Sensitivity and range detected
Atmar (1995)	1.5 g of oyster (*Crassostrea virginica*) and hard-shell clam (*Mercenaria mercenaria*) tissue (stomach and diverticula)	Homogenization, centrifugation	PEG 6000 centrifugation	Proteinase K, phenol-chloroform extraction, ethanol precipitation	RT-PCR confirmation by Southern hybridization	HAV	GGAAATGTCT CAGGTACTTT CTTTG GTTTTGCTCCT CTTTATCAT GCTATG	CTCCAGAATCA TCTCC	VP1, 248 bp	100 PFU
						NV	CTTGTTGGTTT GAGGCCA TAT ATAAAAGTTG GCATGAACA	GGCCTGCCATC TGGATTGCC	Polymerase gene region, 470 bp	Oysters (5–200 PCR units), clams (10–100 PCR units) (1 PCR unit is ca. 42 viral genomes)
Green (1998)	50 g of shellfish flesh	homogenization, sonification centrifugation	Freon TF centrifugation	Reaction mix of glass powder matrix and guanidine isothiocyanate	Nested RT-PCR	SRSV group I	TCNGAAATGG ATGTTGG CGATTTCATC ATCACCATA Internal: GAATT CCATCGCCC ACTGGCT ATCTCATCATC ACCATA	NA	4679–4871, 192 bp	100- to 10,000-fold greater than single round RT-PCR
						SRSV group II	AGCCNTNGAA ATNATGGT CGATTTCATC ATCACCATA Internal: GAATT CCATCGCCC ACTGGCT	NA	4338–4607, 269 bp	
Griffin (1999)	110 liters of marine water	Filterite DFN 0.45-10UN	1.5% beef extract–0.05 M glycine, concentrated using organic flocculation	RNEasy minikit (Qiagen, Santa Clarita, Calif.)	RT-PCR confirmation by dot blotting	EV	CCTCCGGCCC CTGAATG ACCGGATGGC CAATC	TACTTTGGGTG TCCGTGTTTC	5′-untranslated region, 197 bp	10^3 PFU
						HAV	CAGCACATCA GAAAGGT GAG CTCCAGAATC ATCTCCAAC	TGCTCCTCTTTA TCATGCTATG	VP1 and VP2 capsid protein protein interphase, 192 bp	0.1 PFU
						NV	CAAATTATGA CAGAATCC TTC GAGAAATATG ACATGGAT TGC	ATGTCATCAGG GTCAAAG AGG	Viral polymerase, 260 bp	~10^6 virions
						SRSV (Ando)	TGTCACGATC TCATCATC ACC TGGAATTCCA TCGCCCAC TGG	ATGTCAGGGG ACAGGTTTGT ATGTCGGGGCC TAGTCCTGT ACATCGGGTGA TAGGCCTGT	RNA polymerase region, 123 bp	ND
Green (1999)	≥50 g of shellfish tissue (stomach and digestive gland)	Homogenization in 1:7 (wt/vol) 10% TPB–0.05 M glycine (pH9), centrifugation	Chloroform extraction, PEG 6000 precipitation	Proteinase K digestion, phenol-chloroform extraction, ethanol precipitation	RT-PCR and cell culture	EV	TCCGGCCCCT GAATGCG CACCGGATGG CCAATCC	NA	5′ nontranslated region, 196 bp	ND
					RT-PCR	RV	AAACGAAGTC TTCAACA TGG TGAAACTCAT TTCCATT CAT	NA	5′ terminus of gene 6, 154 bp	ND

					RT-PCR	HAV	CTTGGTTTCATGAATCTCT	NA	5′ nontranslated region, 314 bp	ND
							AGGCCCCAAAGAACAGTCCA			
Le Guyader (2000)	<1.5 g oyster (*Crassostrea gigas*) and mussel (*Mytilus galloprovincialis*) (stomach and diverticula)	Homogenization and centrifugation	PEG 6000 centrifugation	Proteinase K, phenol-chloroform extraction, ethanol precipitation	RT-PCR and hybridization	AV	GCTTCTGATTAAATCAATTTT	ACCATTTAAAATTGATTTAATC	6727–6797, 89 bp	ND
							CGAGTAGGATCGAGGGTA			
						EV	TCCGGCCCCTGAATGCGG	ACACGGACACCCAAAGTAGTCGGTTGG	446–642, 196 bp	
							CACCGGATGGCCAATCCAAT		162–596, 434 bp	
							CAAGCACTTCTGTTTCCCCGG			
							ATTGTCACCATAAGCAGCCA	TCAACAACAGTTTCTACAGA	2389–2192, 247 bp	
						HAV	GGAAATGTCTCAGGTACTTTCTTTG			
							GTTTTGCTCCTCTTTATCATGCTATG			
						NLV	GTAAAACGACGGCCAGACDATYC	CTCTGTGCACTTTCTGAAGT	See reference	
							CAGGAAACAGCTATGACATAAAAG	ACCTTGTGTGCCATGTCTGA		
							GGCCTGCCATCTGGATTGCC	CTATGTGCACTGTCAGGAGT		
							GAATTCCATCGCCCACTGGCT	ATGTCAGGGGACAGGTTTGT		
							GTGAACAGCATAAATCACTGG	ATGTCGGGGCCTAGTCCTGT		
							GTGAACAGTATAAACCACTGG			
							GTGAACAGTATAAACCATTGG			
						RV	GGCTTTAAAAGAGAGAATTTCCGTCTGG	GTATGGTATTGAATATACCAC	1–392, 392 bp	
							GATCCTGTTGGCCATCC	GTCACCATCATTGATTGAGTACTT		
								TTCATCATCTGAAATCTCATTTTTA		
								TGAATTATCATTTATTTCTGTTGCT		
								TTCGTGTCACTAATTTGAGTTGGA		

Schwab (2001)	1.5 g of oyster (*Crassostrea virginica*) and hard-shell clam (*Mercenaria mercenaria*) tissue (stomach and diverticula)	Homogenization, centrifugation	PEG 6000 centrifugation	Proteinase K, phenol-chloroform extraction, ethanol precipitation	RT-PCR–DEIA and Southern hybridization	HAV	GGAAATGTCT CAGGTACTT TCTTTG GTTTTGCTCCT CTTTATCAT GCTATG	GTGATAGCTCC CACAGGTGC	VP1 region, 248 bp	Comparable to Southern blot
						NV	CTTGTTGGTTT GAGGCCA TAT ATAAAAGTTG GCATGAACA			
Lipp (2001)	100L estuarine surface water	Filterite DFN 0.45–10 UN	1.5% beef extract–0.05 M glycine (pH 9.5) concentration using organic flocculation	NA	Cell culture CPE enumerated by MPN	EV	NA	NA	NA	1.0–2.0 MPN 100 liters^{-1}
Jiang (2001)	20–40 liters of coastal marine water	TFF (30-kDa spiral filter cartridge)	Centriprep-30 ultracentrifugation unit	NA	Nested PCR	AV	GCCGCAGTGG TCTTACATG CACATC CAGCACGCCG CGGATGTCA AAGT	NA	143 bp	1 purified viral particle
		VFF (100-kDa filter cartridge)	NA				Internal: GCCAC CGAGACGTA CTTCAGC CTG TTGTACGAGT ACGCGGTAT CCTCGCG GTC			
Donaldson (2001)	100 µl	NA	NA	RNeasy minikit	Real-time RT-PCR	EV: CX A9, CX A16, PV1	GGCCCCTGAA TGCGGCT AAT	CGGACACCCAA AGTAGTCGG TTCCG	5′-untranslated region, 192 bp	9.3 viral particles/ml to 155 viral particles/g of sponge
	20 l	VFF (100-kDa filter cartridge)	NA				CACCGGATGG CCAATCCAA			
Reynolds (2001) marine water		Filterite electronegative filter	1.5% beef extract–0.05 M glycine (pH 9.5) concentration and flocculation	NA	ICC-PCR	EV	TGTCACCATA AGCAGCC TCCGGCCCCT GAATGCG GCT Internal: CCCAA AGTAGTCGG TTCCGC	NA	149 bp	EV ICC/PCR (0.1 PFU) HAV ICC/PCR (10 MPN/flask), PCR 12 (PFU/10 µl)
				Heat extraction for direct PCR	PCR, cell culture	HAV	CAGCACATCA GAAAGGT GAG CTCCAGAATC ATCTCAAC Internal: GCTTC CCATGTCAG AGTG		192 bp	Cell culture (0.002 MPN)

[a] Abbreviations: AV, adenoviruses; EV, enterovirus; CPE, cytopathic effect; CX, coxsackieviruses; DEIA, DNA enzyme immunoassay; HAV, Hepatitis A virus; ICC-PCR, integrated cell culture PCR; MPN, most probable number; NA, not applicable; ND, not done; NLV, Norwalk-like viruses; NV, Norwalk virus; PV, polioviruses; RT-PCR, reverse transcription-PCR; RV, rotaviruses; VP, viral capsid protein.

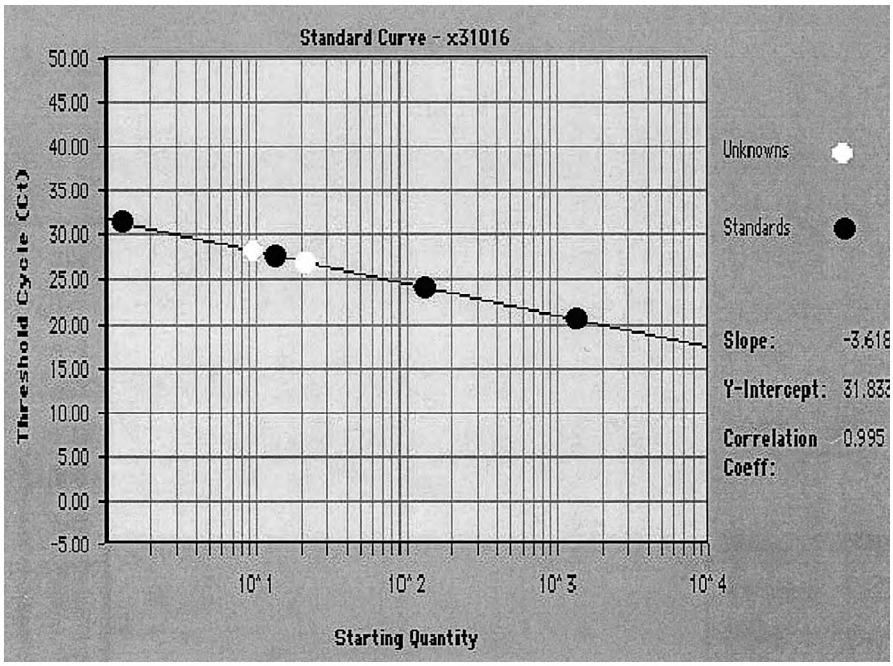

FIG. 1. Real-time PCR. A TaqMan poliovirus standard curve generated by plotting threshold cycle (C_T) against the log of the virus concentration. The C_T occurs where the sequence detection software begins to detect an increase in signal due to the exponential growth of PCR amplicons. Standards are shown here in black, two natural-water samples seeded with poliovirus are shown in white. A 2-ml volume of a poliovirus Sabin type 1 cell culture was lysed by vigorous shaking, and extracellular DNA was digested with pancreatic DNase I. Viral particles were purified using a glycerol step gradient (136), stained with SYBR Gold, and directly counted by epifluorescence microscopy (104). The stock dilution contained 9.1×10^3 viruses per μl. A 10-μl sample of the stock was purified and brought up in 70 μl of water. Four dilutions ranging from 1.3×10^0 to 1.3×10^3 were made using UV-treated 0.02-μm-pore-size-filtered deionized water to generate an RNA standard curve. RT-PCR on all standards was performed in triplicate. Two natural-water samples (white dots) were seeded with poliovirus and quantitated using this standard curve. The quantities of the two natural-water samples were 7.2×10^0 and 1.7×10^1 viruses per μl.

probe set was used by Griffin et al. (52) and the sequences are listed in Table 3.

One of the central obstacles to the use of PCR in environmental monitoring is the susceptibility of the assay to inhibitors (divalent cations, humic acids, etc.) commonly found in aquatic samples. The traditional approach of separating viral nucleic acids from inhibitors was through the use of phenol-chloroform extraction protocols. The drawback to this protocol is time to completion and loss of viral genomes during the repetitive washing steps, which limited the ability to detect small numbers of viruses in water samples. A more rapid and sensitive assay was developed in the late 1980s which utilized heat to liberate viral nucleic acids (127). Several protocols involved using polyethylene glycol (PEG) and/or Pro-Cipitate (Bionostics Inc., Devens, Mass.) to concentrate and purify viral nucleic acids from water samples (67, 138, 156). Freon has been used to isolate Norwalk viruses from stool specimens followed by heat release of the Norwalk virus genomes from their capsids (140). One of the most widely used methods, due to outstanding reliability and simplicity, for purifying viral nucleic acids from environmental water samples was first described in a clinical setting (10). This assay uses silica beads to capture nucleic acids, which is the basis for many of the commercially available nucleic acid capture and purification kits. Other assays that used antibodies to capture viruses or genetic sequences include immunomagnetic or genomagnetic separation. Numerous studies have used antibody-coated vesicles or antibodies labeled with iron particles (immunomagnetic separation) to separate and purify viruses from particulate-laden samples (5, 51, 93, 139, 142).

Variants of the standard RT-PCR assay have been used to detect human viruses in laboratory and environmental studies. A nested RT-PCR assay which employs two primer sets (the second internal to the first used in successive reactions) was developed for sensitive (15 to 21 viruses) detection of Norwalk-like viruses in shellfish tissue (50). Nested multiplex RT-PCR was used for simultaneous detection of enteroviruses and herpesviruses in a clinical study (153). A seminested RT-PCR using the same primer on one end of the gene target and two more primers, one internal to the other, in a successive-PCR protocol was developed to detect HAV, enteroviruses, rotavirus, and Norwalk-like viruses in seafood samples (55). Booster nested RT-PCR, an assay which uses two primer sets, one internal to the other, in a single RT-PCR run was developed for sensitive detection of the Norwalk-like Sapporo virus. This assay proved more sensitive than standard RT-PCR followed by Southern hybridization (63). Triplex RT-PCR, which used three primer sets (each for a specific virus), was developed and used for simultaneous detection of poliovirus, HAV, and rotavirus in sewage and marine water samples (157).

Quantification of viral particles is difficult when using molecular detection assays. An MPN PCR which used multiple amplification reactions to estimate viral numbers was successful in enumerating adenoviruses in marine waters along the

coastline of Southern California (68). Real-time PCR was first developed for clinical quantitative PCR and has recently been adapted for environmental monitoring of enteroviruses in sewage and marine samples (24, 94, 158). Real-time PCR relies on the detection of a fluorescent signal produced during the amplification reaction that is proportional to the amount of amplicon being produced (Fig. 1). The benefit of this assay is that it is quantitative and the target amplicon is verified by using a fluorescently labeled probe which recognizes internal amplicon sequences. A detection limit of 13 viral particles in marine waters has been demonstrated (24).

In addition to the susceptibility of PCR to environmental inhibitors, the remaining limitation is the inability of the assay to determine virus viability. While some investigations which utilize docking proteins of host cells for capture of viruses particles, with the assumption being that if the virus can still recognize its host cell docking protein it must be viable, are under way, the only recognized viability assay is cell culture. For viruses such as the enteroviruses and HAV, for which cell culture assays exist, the integration of cell culture with PCR was a means of addressing this dilemma.

Integrated cell culture-PCR (ICC-PCR) is rapid and specific and yields information on the infectivity of a virus (123). ICC-PCR allowed the detection of infectious enteroviruses within 48 h with detection limits as low as 0.001 MPN/liter of sample (126). ICC-PCR sensitivity was later shown to be 1 PFU for enteroviruses at 20 h of incubation and 1 to 10 PFU for HAV at 72 to 96 h (124). This research also demonstrated that 11 samples in the sample set were positive via ICC-PCR in comparison to 3 samples via straight RT-PCR. A modification of this assay using seminested RT-PCR has been published (97). Drawbacks to this method include the difficulty in adapting it for large-scale studies and automation. As in standard cell culture, maintenance of cell lines can be cost prohibitive. However, this technique can be faster and more sensitive than traditional cell culture, and it is one of the few techniques that can determine viral infectivity.

CONCLUSION AND PERSPECTIVES

It has been recognized that the observed decrease in coastal marine water quality occurring in areas impacted by human waste and refuse is negatively affecting both human and ecosystem health (57, 60, 144). A majority of pathogens responsible for outbreaks of human illnesses acquired from marine recreational exposure have not been identified but are thought to be viruses. Contrary to popular belief, many cell culture-based assays have shown that viable pathogenic human viruses can readily be detected in marine water being impacted by human sewage. From the literature cited in this review, it is clear that this has been demonstrated repeatedly since the 1970s. A common trend among many of the cited occurrence studies is that bacterial indicator occurrence did not correlate with viral occurrence. In addition, in a majority of the studies that monitored marine waters for both bacterial indicators and pathogenic viruses, viruses were detected when indicator levels were below public health water quality threshold levels (54). This creates a significant dilemma for public health officials who are responsible for marine water quality monitoring. The data obtained from three decades of occurrence studies warrant investigating the use of human viruses as indicators of marine water quality and public health risk.

Although progress has been made in the search for an ideal viral detection method, problems remain. Although most molecular biology-based assays are sensitive, specific, rapid and cost efficient, there has been no development of a consensus method or standardization. The use of docking proteins, antibodies, and genetic sequences for viral capture holds promise in isolating a particular virus from inhibitory particulate matter found in water samples. Gene chips, with their theoretical ability to detect individual viral genomes and to simultaneously detect multiple viral types, should prove useful in monitoring assays when combined with target capture protocols, cell culture, and PCR.

In the not too distant future, it may be that climate and marine conditions will be used in models to predict the risk to human health in marine environments. As cited studies have shown, precipitation, salinity, and water temperature have been correlated with viral occurrence and infectivity. The current limitations to the accurate development of public health risk models is the lack of integrated occurrence studies which characterize factors such as soil type, sewage disposal system density, pathogen and indicator occurrence, and the influence of climate. Tools and techniques are now available to conduct these types of studies to make the global assessment of viral pollution in marine waters achievable.

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