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Enterovirus detection by reverse transcriptase polymerase chain reaction from the coastal waters of southern California

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ABSTRACT

Assays for the detection of enteroviruses by reversetranscriptase-polymerase chain reaction (RT-PCR) were performed on 63 coastal seawater samples taken from the coastline of the Southern California Bight, with the majority of the samples taken from Santa Monica Bay, California. These samples were taken at sites either influenced by freshwater outlets or proximal to high-use sandy beaches that are popular recreational areas. The RT-PCR is a primer-based molecular biology technique that can be used to detect the genomes of specific groups or types of RNA viruses. Our results indicate that the concentration methods and RT-PCR protocol used in this study could be used to detect enteroviruses from 20l samples of coastal seawater. Of the 63 samples, 21 (33%) were positive for enteroviruses, 35 (56%) were negative, and 7 (11%) were inconclusive. No direct correlation was observed between RT-PCR results and measurable rainfall (>0.5 inch either immediately prior to or during sampling), but our analyses demonstrated that positive results for enteroviruses were significantly more likely during the winter "wet" season than during the summer "dry" season. Results of 60 and 54 samples did not demonstrate any significant logistical correlation to total and fecal coliforms, respectively ($p>0.05$). Correlation analysis of 14 samples showed a significant, but weak, logistical correlation to levels of enterococci ($r = 0.50$, $p < 0.05$) in samples from only Santa Monica Bay. Inconclusive results occurred for approximately 1/9 of the samples, where inhibition of PCR occurred due to substances in the seawater. Optimization of our concentration procedure has improved the RT-PCR method over time and has reduced the incidence of inconclusive results; e.g., during the last two years, only one analysis was inconclusive. Our results indicate that there is no strong relationship between the presence of enteroviruses and levels of indicator bacteria.

INTRODUCTION

Human pathogens in coastal waters are a major concern in densely populated coastal regions such as southern California. These disease-producing viruses are released into the urban ocean from sewage outfalls and storm drains and pose potential public health risks to humans using the ocean as a food and recreational source. The Santa Monica Bay (SMB) in Los Angeles, California, receives urban runoff year-round from a system of storm drains. At the same time, the beaches of SMB receive thousands of visitors on a daily basis for surfing, sunbathing, swimming, and diving.

For decades, bacterial indicators have been used to infer the sanitary quality of recreational waters. However, viruses have long been known to be important etiological agents of waterborne disease. Human pathogenic viruses can be found in coastal waters contaminated by sewage, as they are found in high concentrations in human feces and urine. Assays using bacteria as indicators of fecal contamination may not be adequate for predicting the presence of pathogenic viruses. Shortcomings in microbiological water quality standards have been revealed on several occasions, where pathogenic viruses were isolated from seawater that met current standards of bacterial indices (Melnick 1985). Previous studies indicate that several viruses can be

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contracted by swimming or diving in contaminated ocean waters (Cabelli *et al.* 1982, Haile *et al.* 1999, Seyfried *et al.* 1985a, Seyfried *et al.* 1985b). Other reports indicate that coliform bacteria standards are not adequate for predicting virological quality of seawater for bathing, and outbreaks of gastroenteritis have been caused by swimming in water with acceptable coliform counts (Cabelli *et al.* 1982); however, it is not known whether this is a general phenomenon in all waters.

Viral pathogens found in the coastal marine environment can include hepatitis A virus, rotavirus, Norwalk virus, adenovirus, and other enteroviruses. These viruses can cause illness and a range of diseases when ingested, including paralysis, meningitis, respiratory tract infections, vomiting, diarrhea, eye infections, and myocarditis. For example, rotaviruses, often found in human fecal material, can be released into recreational waters via sewage spills or urban runoff, and can be responsible for severe gastroenteritis in humans (Ansari *et al.* 1991, Kapikian 1993). Enteroviruses have recently gained attention for their use as indicators of wastewater contamination (Kopecka *et al.* 1993, Pina *et al.* 1998, Reynolds *et al.* 1998). The enterovirus family includes 66 human serotypes including poliovirus, Coxsackievirus, echovirus, and other enteroviruses (Muir *et al.* 1998). This family of viruses is responsible for 10 to 30 million infections in the United States annually, causing a variety of symptoms for illnesses ranging from mildly damaging to deadly. A member of the enterovirus family, poliovirus, is commonly found in human sewage. The vaccine strain of the virus, although of low virulence and no public health risk, is shed into the fecal matter of children who have been recently vaccinated.

Reverse transcriptase-polymerase chain reaction (RT-PCR) has been used for the detection of enteroviruses in many different types of environmental samples (Tsai *et al.* 1993). This method is more rapid than traditional cell-culture methods, which require days to weeks to assess the results. Another limitation of cell-culture methods is that no single cell line can detect all enterovirus types. In this study, we used streamlined ultraconcentration and RT-PCR protocols for the direct detection of enterovirus genomes in coastal seawater to determine whether standard bacteriological indicator results were correlated to the presence of waterborne enteroviruses in seawater.

METHODS

Description of Sites

Samples were taken along the coastline of the Southern California Bight (SCB) with most of the samples taken within SMB. The majority of samples were taken from two locations: Santa Monica Pier (34.00 N, 118.49 W) and Playa del Rey Jetty (33.96 N, 118.46 W). Other sites that were sampled multiple times were Malibu Surfrider State Beach (34.04 N, 118.69 W) and Ballona Creek (33.96 N, 118.46 W). All of the sites sampled are either popular recreational beach areas receiving thousands of visitors each year, or sites where the coastal waters (like Playa del Rey Jetty and Malibu Surfrider State Beach) are influenced heavily by freshwater outlets that flow year-round (like Ballona Creek and Malibu Creek, respectively). The coastal waters near Santa Monica Pier are influenced by two storm drains, Ashland and Pico-Kenter. Collectively, these freshwater outlets are responsible for more than 95% of the total non-point source inputs to the SCB each year and have been shown in the past to adversely affect microbiological water quality in SMB (e.g., Haile *et al.* 1999). The selection of sites for sample collection was not random, but was biased toward areas that were more likely to be contaminated.

Sample Preparation

Methods were modified from the basic approach of Tsai *et al.* (1993). Methods of ultraconcentration were modified to enable the concentration of larger volume seawater samples, and involved prefiltration to remove material larger than viruses. For detection of enteroviruses by RT-PCR, large volumes of seawater (20 to 40 L) were retrieved from the sample site using an acid-rinsed (5% HC-l acid), and triple sample-rinsed bucket. The samples were placed on ice and transported immediately to the laboratory at the University of Southern California (USC). The concentration of the samples was initiated within 1 h of the sampling time. Samples were dispensed into a 40 L stainless steel pressure vessel and serially pressure filtered (at <18 kPa) through two 142-mm-diameter stainless steel filtration units. The first unit housed a glass fiber filter (Whatman, nominal pore size 1.2 μm), and the second unit housed a 0.22 μm Millipore Durapore filter. The filtrate was retained in a carboy, and while on ice was concentrated to a final volume of 40 to 150 mL with the use of a spiral cartridge filtration system (Amicon, Inc., 30 kDa molecular weight cutoff, SY130). The filtrate was further concentrated with the use of Centriprep-30 and Centricon-30 concentration units, to a final volume of approximately 100 μL. The previous use of the spiral cartridge concentration method for the concentration of viruses has demonstrated a recovery in excess of 80%, determined on the basis of the concentration of countable virus particles in the sample (Suttle *et al.* 1991).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

The detection of enteroviruses by RT-PCR used modified pan-enterovirus "universal" primers originally designed by DeLeon *et al.* (1990), which were designated as EV-L and EV-R. The upstream primer sequence was 5'-CCTCCGGCCCCTGAATG-3', and the downstream primer sequence was 5'-ACCGGATGGCCAATCCAA-3'. This primer set can amplify at least 25 enteroviral types, and the relatively short length of the PCR amplicon ensures efficient amplification. Perkin Elmer's RNA PCR core kits were used for the RT-PCR. A 2 µL subsample of the concentrated seawater sample was added to 16 µL of RT reaction mixture. Each sample was heated at 99° C for 5 min and cooled at 4° C before the addition of 1 µL of RT (50 U/uL) and 1 µL of Rnase inhibitor (20 U/uL). The RT phase was carried out in the following stages: 25° C for 10 min, 42° C for 30 min, 99° C for 5 min, and 4° C for 10 min. The total volume of the finished RT mixture (20 µL) was mixed with the PCR reaction mixture 80 µL, as suggested by the manufacturer, for a total PCR reaction volume of 100 µL. The target templates produced amplified DNA fragments of 196 bp after 40 cycles. Visualization of the amplified DNA was achieved by staining a 1.5% agarose gel with ethidium bromide and illuminating the sample with UV light. One hundred base pair ladders were used as lane markers for size comparison. Sensitivity assays were performed by spiking ocean samples with known amounts of poliovirus and conducting subsequent amplification with pan-enterovirus primers. This procedure allowed us to determine the sensitivity of each assay, which our preliminary results suggested varied depending upon the presence of interfering substances in seawater.

Negative and positive controls were performed for each RT-PCR run. For the negative controls, 2 µL of deionized, RNase-free H_2O was added to the PCR reactions rather than concentrated seawater. The positive controls were concentrated seawater samples spiked with 2 µL of Poliovirus Type 1 LsC at 10^5 to 10^{-2} PFU/100 µL of high titer stock. Using the positive controls, our detection limit was estimated at 1 PFU (data not shown). Positive controls from the Perkin Elmer GeneAmp RNA PCR core kit were run with each set of reactions to ensure the activity of all components in the PCR Kit.

Bacterial Indicators

To determine the relationship between the incidence of enteroviruses and levels of standard bacteriological indicators, our results were compared to standard bacterial indicator assay results from samples taken at the same location and at the same time (APHA 1992). Quantification of indicator bacteria by standardized methods was performed as described by Standard Methods (APHA 1992). Total and fecal coliforms and enterococci were analyzed at each site. However, data for enterococci at some sites are missing as sampling was conducted only on a weekly basis. In a few cases, seawater samples were analyzed in the University of Southern California laboratory for the presence of total and fecal coliforms, using the Colilert® method (Idexx, Inc.; APHA 1992). These analyses were not performed by a State-certified laboratory, but still followed quality control recommendations and protocols (APHA 1992). Test samples were run in the laboratory at USC, compared to results from the Environmental Monitoring Division at the Hyperion Treatment Plant, and revealed no significant difference between the total and fecal coliform assay results (data not shown).

RESULTS

A total of 63 environmental samples were analyzed for the presence of enteroviruses by RT-PCR. Results of the RT-PCR assay were identified as positive, negative, or inconclusive. Inconclusive results were basically false negatives; i.e, results from samples that were negative when first analyzed, but, when spiked with vaccine-strain poliovirus (positive control), demonstrated a negative result. Inconclusive results demonstrated the presence of inhibitory substances in the PCR reactions, leading to these false negatives. Diluting the sample before performing the RT-PCR sometimes resulted in a barely detectable positive result, further support for the presence of inhibitory substances. Of the 63 samples, 7 (11%) were inconclusive, 21 (33%) were positive for human enteric viruses, and 35 (56%) were negative (Table 1). The incidence of detection of enteroviruses was very similar between Santa Monica Pier and Playa del Rey Jetty, where detection occurred 35% of the time. No apparent correlation was found between RT-PCR results and measurable rainfall (>0.5 inch either immediately prior to or during sampling, rainfall data not shown). However, a significant relationship was found between the presence of enteroviruses and season, with enteroviruses less likely to be detected during the "dry" season (from May 1 to October 31), and more likely to be detected during the "wet" season (from November 1 to April 30; $p < 0.05$).

The results of the detection of enteroviruses via RT-PCR were compared to bacterial indicator densities in the same samples. No overall significant logistical correlation was found between enteroviruses and either total or fecal coliforms ($p > 0.05$). Bacterial indicator levels were typically higher during the "wet" season and high levels of bacteria sometimes coincided with the presence of enteroviruses;

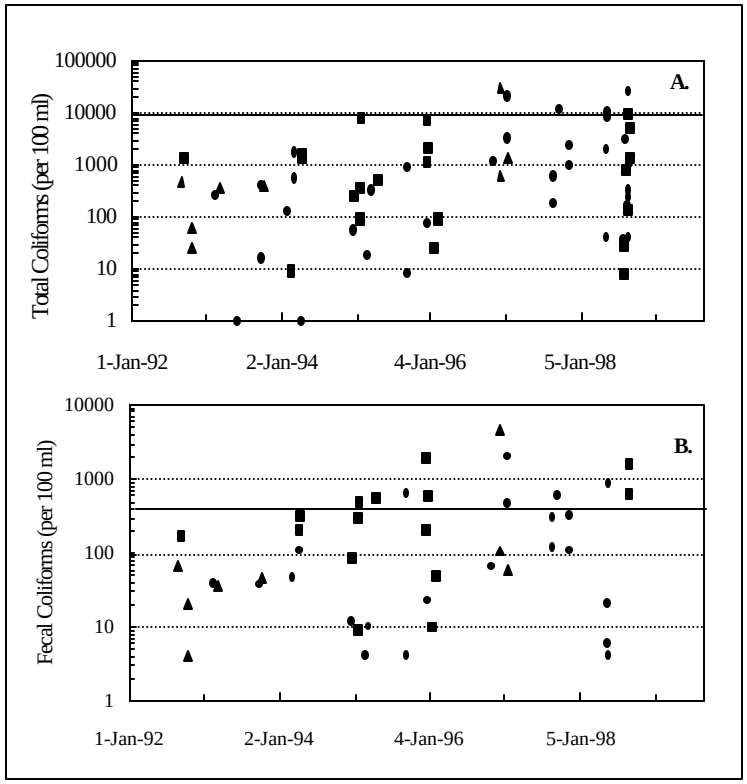
TABLE 1. Bacterial and viral indicator results at all sample sites. N/A = Data not available. Bacterial indicator results in bold indicate threshold exceedence of at least one indicator at that site based upon thresholds used in the State of California. * indicates samples that were taken during or immediately after a heavy rain (> 0.5 inch).

Sample Date	Location	Total Coliform	Fecal Coliform	Enterococci	Enterovirus Detection
30-Aug-92	Santa Monica Pier	N/A	N/A	N/A	Inconclusive
7-Sep-92	Santa Monica Pier	N/A	N/A	N/A	Positive
20-Oct-92	Santa Monica Pier	N/A	N/A	N/A	Inconclusive
15-Feb-93	Santa Monica Pier	250	40	N/A	Negative*
5-Mar-93	Santa Monica Pier	375	35	N/A	Inconclusive
4-Jun-93	Playa del Rey	1	N/A	1	Negative
28-Sep-93	Playa del Rey	16	N/A	3	Negative
29-Sep-93	Santa Monica Pier	410	38	N/A	Negative
8-Oct-93	Santa Monica Pier	410	45	N/A	Inconclusive
2-Feb-94	Playa del Rey	130	N/A	18	Negative
14-Feb-94	Playa del Rey	9	N/A	13	Positive*
7-Mar-94	Playa del Rey	1700	N/A	10	Negative
7-Mar-94	Santa Monica Pier	560	45	N/A	Negative
8-Apr-94	**Santa Monica Pier**	**1540**	**210**	**N/A**	**Positive**
13-Apr-94	Playa del Rey	1	N/A	1	Negative
13-Apr-94	**Santa Monica Pier**	**1400**	**320**	**N/A**	**Positive**
14-Apr-94	Santa Monica Pier	1300	110	N/A	Negative
18-Dec-94	Playa del Rey	56	12	N/A	Negative*
18-Dec-94	Santa Monica Pier	250	86	N/A	Positive*
19-Jan-95	Playa del Rey	92	9	N/A	Positive*
19-Jan-95	Santa Monica Pier	380	300	N/A	Positive*
24-Jan-95	Playa del Rey	8200	490	N/A	Positive
1-Mar-95	Playa del Rey	19	4	N/A	Negative
14-Mar-95	Playa del Rey	320	10	N/A	Negative
15-Apr-95	**Santa Monica Pier**	**500**	**560**	**N/A**	**Positive**
11-Sep-95	Playa del Rey	8	4	N/A	Negative
11-Sep-95	Santa Monica Pier	880	620	39	Negative
13-Dec-95	**Playa del Rey**	**7200**	**1900**	**N/A**	**Positive***
13-Dec-95	**Santa Monica Pier**	**1200**	**210**	**N/A**	**Positive***
21-Dec-95	**Santa Monica Pier**	**2200**	**600**	**N/A**	**Positive***
21-Dec-95	Playa del Rey	76	23	N/A	Negative*
13-Jan-96	Playa del Rey	25	10	N/A	Positive*
31-Jan-96	Playa del Rey	90	48	N/A	Positive*
31-Oct-96	Playa del Rey	1200	64	48	Negative*
10-Dec-96	**Playa del Rey**	**640**	**110**	**5400**	**Inconclusive***
10-Dec-96	**Santa Monica Pier**	**29000**	**4600**	**N/A**	**Inconclusive***
15-Jan-97	Playa del Rey	1400	58	N/A	Inconclusive*
15-Jan-97	**Santa Monica Pier**	**20000**	**2000**	**N/A**	**Negative***
15-Jan-97	**Paradise Cove**	**3200**	**460**	**N/A**	**Negative***
22-Aug-97	Santa Monica Pier	600	300	N/A	Negative
24-Aug-97	Santa Monica Pier	190	120	N/A	Negative
17-Sep-97	**Santa Monica Pier**	**12000**	**600**	**37**	**Negative**
10-Nov-97	Santa Monica Pier	920	320	N/A	Negative*
10-Nov-97	Malibu Surfrider	2400	1100	N/A	Negative*
13-May-98	Malibu Surfrider	40	21	42	Negative*
13-May-98	Malibu Surfrider	2000	6	N/A	Negative*
20-May-98	**Malibu Surfrider**	**8000**	**850**	**190**	**Negative**
20-May-98	**Malibu Surfrider**	**10000**	**4**	**N/A**	**Negative**
3-Aug-98	Tijuana River	30	8	10	Positive
3-Aug-98	Los Penosquitos Lag.	8	4	2	Positive
3-Aug-98	San Diego River	36	38	54	Negative
10-Aug-98	San Luis Rey River	800	80	24	Positive
10-Aug-98	Moonlight Beach	3000	230	nm	Negative
17-Aug-98	Aliso Creek	140	20	64	Positive
17-Aug-98	San Juan Creek	160	70	20	Negative
18-Aug-98	**Los Angeles River**	**9000**	**1700**	**2**	**Positive**
24-Aug-98	Goleta Creek	314	314	20	Negative
24-Aug-98	Mission Creek	240	85	10	Negative
24-Aug-98	**Arroyo Burro**	**24,192**	**589**	**99**	**Negative**
24-Aug-98	Carpinteria Creek	41	20	10	Negative
31-Aug-98	**Calleguas Creek**	**1100**	**170**	**140**	**Negative**
31-Aug-98	**Malibu Surfrider**	**1353**	**616**	**175**	**Positive**
31-Aug-98	**Ballona Creek**	**5000**	**1600**	**170**	**Positive**

but enteroviruses could also be detected when levels of total and fecal coliforms were extremely low (Figures 1a and 1b). Although a trend of increasing total and fecal coliform numbers is apparent in Figures 1a and 1b, this could be a result of non-random sampling (e.g., we sampled more frequently at Playa del Rey Jetty in later years). Total and fecal coliforms demonstrated a strong correlation to one another over the entire study period ($r = 0.76$), which is expected because fecal coliforms are a subset of the total coliform group. A significant correlation, although weak within Santa Monica Bay, was found between enterococci levels and enteroviruses ($r = 0.50$, $p < 0.05$). However, this relationship was based upon a small sample size because the enterococci sampling was conducted only on a weekly basis ($n = 14$). No significant correlation was found between enterococci levels and enteroviruses when considering the entire SCB ($p > 0.05$)

Recent laws in the State of California require that a beach must be posted or closed to recreational use if the water exceeds the following bacterial indicator thresholds: total coliforms, 10,000 cfu or MPN/100 mL; fecal coliforms, 400 cfu or MPN/100 mL; and enterococci, 104 cfu or MPN/100mL. Also, a total/fecal ratio standard applies when the total coliform level is over 1,000 and the total-coliform-to-fecal-coliform ratio is less than 10. Based upon these criteria, we attempted to identify whether a threshold-based logistical correlation existed between the detection of enteroviruses and the exceedence of the bacterial indicator thresholds. No significant relationship was found between the exceedence of the numeric threshold of a single bacterial indicator and the presence of enteroviruses ($p > 0.05$). However, within SMB, a significant relationship was found between the exceedence of any one of the four bacterial indicator thresholds and the presence of enteroviruses ($r = 0.71$, $p < 0.05$). This relationship was not evident when analyzing data from samples taken along the entire SCB shoreline.

FIGURE 1: Total coliform (A.) and fecal coliform (B.) results by sampling date. Solid line indicates beach water quality threshold for that type of indicator bacterial. Symbols represent enteric virus results for each sample: (■) positive, (●) negative, or (▲) inconclusive. Sampling over the five-year period was not random in location. Therefore, trends in total and fecal coliform numbers over time are probably related to the increased level of sampling at Playa del Rey Jetty and Malibu beaches, both within 100 yards of two of the largest freshwater outlets in southern California, Ballona Creek and Malibu Creek.



DISCUSSION

The detection of enteroviruses in coastal seawater samples can be successfully performed using RT-PCR, which is rapid and relatively reliable. In the United States, human fecal contamination is inferred from the presence of indicator bacteria, usually total and fecal coliforms, and enterococci. Epidemiological studies of waterborne illnesses, however, showed that the most common etiological agents were more likely to be viruses and protozoa, and these reports indicated that human pathogenic viruses, bacteria, and protozoa can all potentially infect those swimming or diving in contaminated waters (Cabelli 1983, Cabelli *et al.* 1982, Haile *et al.* 1999, Kay *et al.* 1994, Moore *et al.* 1994, Seyfried *et al.* 1985a, Seyfried *et al.* 1985b). Human pathogenic viruses such as enteroviruses can be detected in marine waters, and our results indicate that there is no apparent strong relation between the presence of enteroviruses and routinely monitored bacterial indicators in the coastal waters of SMB (Figures 1a and 1b). Furthermore, we detected the presence of enteroviruses when indicator bacteria levels were low or undetectable.

The relation, or lack thereof, between bacterial indicators and the presence of viral pathogens has been previously discussed and has revealed many complex issues. Because of the previous difficulty in detecting specific types of viral pathogens in recreational waters, bacterial indicators were always used as surrogates for testing of all microbio-

logical water quality. With the advent of molecular techniques, direct testing for virus particles might be warranted, especially as we consider the reasons that viruses may not behave like bacteria in wastewater and seawater. A lack of relation between the two may be derived in part to the biological and chemical differences between viruses and bacteria, the "patchiness" of pathogen dispersal in sewage, and problems in sampling (Fleisher 1990). Environmental factors, such as sedimentation, turbulence, sunlight intensity, temperature, nutrient status of water, and predation influence the persistence of indicator viruses and bacteria in different ways (Chamberlain and Mitchell 1978, Gerba and McLeod 1976, Ghoul *et al.* 1986, McNeill 1992, Perez-Rosas and Hazen 1988). Viruses are fundamentally different from bacteria, because they are dependent upon their hosts for replication. Some bacteria, specifically coliforms and enterococci, not only persist in the marine environment but grow if the temperature and conditions are right (McNeill 1992). Other studies demonstrated, however, that fecal coliform bacteria have lost their ability to be cultured by about 2 to 3 d; these studies also showed an indetectable decline in fecal coliform numbers in a non-culturable state during the same period (Garcia-Lara *et al.* 1991). For enteroviruses, no opportunity for replication occurs in the marine environment. The degradation of viruses occurs biochemically, but different viruses are likely to degrade at very different rates from one another (Finch and Fairbairn 1991; Noble 1998). Viruses not native to the marine environment tend to degrade faster in seawater than those that are native, but in circumstances where particulate matter is plentiful, such as sewage solids, human pathogenic viruses may be well protected from various causative agents of degradation such as ultraviolet irradiation (Noble and Fuhrman 1999). With all of these complex interactions in mind, specific bacterial or viral indicators may be more appropriate for use in estimation of potential public health risk at certain times (Fleisher *et al.* 1996).

Further complications block our ability to use a single microbiological indicator to predict the risk of recreational water contact to human health. Methods such as membrane filtration (MF) rely upon the growth of bacteria (into colonies) for enumeration. The interpretation of MF results is complicated by the occurrence of viable but non-culturable indicator bacteria that are not enumerated by standard methods, and result in an underestimation of indicator numbers (Byrd *et al.* 1991). As mentioned previously, cell culture methods used in the past to detect the presence of human pathogenic viruses in coastal waters can be useful in demonstrating the infectivity of the viruses detected. The RT-PCR method, on the other hand, is based solely upon the detection of viral genetic material and provides no information on the potential infectivity of the virus particle(s). The inhibitory substances found in environmental samples, especially in storm drains, make the detection of extremely low levels of virus genomes difficult by RT-PCR. This study demonstrates, however, that multiple indicators are useful for assessing the microbiological water quality of coastal waters. Current State of California regulations require testing for all three bacterial indicators, and the posting or closing of beaches based upon the exceedence of any one of the four single sample bacterial indicator thresholds or on 30-d geometric mean values. This study shows that the detection of enteroviruses was positively correlated with the exceedence of any one of the four bacterial indicator thresholds, supporting the use of multiple-indicator testing for beach water quality.

Even with the differences between bacterial and viral indicators and their behavior in water, a weak correlation was observed between the presence of enterococci and enteroviruses in SMB. This very weak relation precludes the use of these indicators as predictors of viral contamination. However, further studies using larger sample sizes could reveal a stronger relation. Another study by Wyer *et al.* (1995) analyzed a large number of samples and found statistically significant correlations between total coliforms, fecal coliforms, fecal streptococci, and enterovirus concentrations ($n= 690$, $p <0.001$). Similar to this study, however, the correlations reported by Wyer *et al.* (1995) were low, suggesting poor predictive relationships between indicators ($r <0.50$).

The RT-PCR method detects virus genomes within 24 h of sampling, although seawater concentrates can be held at -80° C for long periods of time for batch processing if necessary. Traditional cell culture methods, on the other hand, require at least 1 to 2 weeks for processing, and are not nearly as sensitive for the detection of all types of enteric viruses. However, RT-PCR provides only information on the presence of a specific type of viral RNA (in this case, enteroviral RNA), and does not provide an estimate of the level of infective viruses in the water sample. We suggest that the positive detection of enteroviral RNA indicates the likelihood of the presence of pathogenic viruses in the sample. Other studies have examined the relation between the presence of viral genomes and the respective infectivity of those viruses in environmental samples, and have found that viruses detected by RT-PCR are typically infective (Dubois *et al.* 1997). Research has shown that free RNA degrades relatively rapidly in seawater (Pichard and Paul 1991), making the amplification of free viral RNA an unlikely source of the RT-PCR products. The positive detection of viral genomes in coastal seawater is probably a reasonable indicator of the recent presence of intact viruses. The detection limit of our RT-PCR assay

was 1 PFU/100 µL (data not shown) and was comparable to the detection limits reported previously (Tsai *et al.* 1993, Rose *et al.* 1997). Because of their presence in high numbers in human fecal contamination, and their pathogenicity, enteroviruses are a perfect candidate for a viral index of human fecal contamination.

The positive detection of enteroviruses serves to confirm previous findings of human fecal contamination in southern California due to the presence of untreated runoff from a series of storm drains (Haile *et al.* 1999). Many other coastal areas face similar situations. Haile *et al.* (1999) demonstrated a relation between levels of indicator bacteria and symptoms of disease contracted from water contact near flowing storm drains. Although the bacteria tested are not direct indicators of human fecal contamination, they were positively linked to upper respiratory and gastrointestinal symptoms. Haile *et al.* (1999) also detected human enteric viruses in the same storm drains, Ashland and Pico-Kenter, that influence the waters around Santa Monica Pier. Haile (1999) found that the detection of viruses in these storm drains was also positively linked to higher public health risks for the swimmers. The same types of viruses detected in this study may be those pathogens that caused symptoms of skin rash, gastrointestinal discomfort, and upper respiratory tract infections to the subjects interviewed during the Santa Monica Bay Epidemiological Study (Haile *et al.* 1999).

This study suggests a need for the specific testing of freshwater outlets and storm drains and their adjacent coastal waters for viral pathogens. Routine monitoring for bacterial indicators may not always be useful for inferring viral contamination; i.e., specific virus assays may be needed at high-use sandy beaches, even when bacterial indicator levels appear to be low. The detection of human enteric viruses and natural distributions of viruses and bacteria may aid in the ability to predict local coastal health hazards and to monitor the effects of urban runoff on the coastal marine environment. Results from these studies have already proven to be useful to regulatory agencies and water quality monitoring program sponsors interested in preventing public health problems resulting from the exposure to pathogenic bacteria and viruses. Additional research is necessary to expand our understanding of microbial pollution in coastal watersheds, and especially to provide advancements in molecular methods for the rapid and accurate detection of human viral pathogens for routine use in water quality monitoring.

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