Water quality indicators and the risk of illness in non-point source impacted recreational waters

John M. Colford, Jr.[1], Timothy J. Wade[2],
Kenneth C. Schiff, Catherine C. Wright[1],
John G. Griffith, Sukhminder K. Sandhu[1],
Susan Burns[1], Jackie Hayes[1], Mark Sobsey[3],
Greg Lovelace[3] and Stephen B. Weisberg

ABSTRACT

Numerous studies have demonstrated relationships between indicator bacteria and human illness at marine beaches impacted by point sources of pollution with known human fecal contributions, but extrapolating current water quality thresholds built upon these relationships at locations where nonhuman sources of fecal pollution is uncertain. A good example is Mission Bay, CA where tremendous resources have been expended eliminating human sources of fecal pollution, yet 20% of ongoing microbiological monitoring samples during dry weather exceed water quality objectives. This study answered two questions: 1) did water contact increase the risk of illness in the two weeks following exposure to water in Mission Bay? and 2) did the risk of illness increase with increasing levels of microbial indicators of water quality? Baseline health at the time of exposure and again two weeks later were measured in a cohort of 8,797 beachgoers during the summer of 2003. Nearly 2,000 water samples were analyzed for bacterial indicators (enterococcus, fecal coliforms, and total coliforms) using both traditional and non-traditional methods (chromogenic substrate or quantitative polymerase chain reaction), novel bacterial indicator (*Bacteroides*), and viruses (somatic and male-specific phage, adenovirus, Norwalk-like virus) and associations between water exposure and water quality indicators with health outcomes were assessed. While the incidence of diarrhea and skin rash were elevated in swimmers compared to non-swimmers, there was no statistically increased risk in 12 other symptoms measured, including highly credible gastrointestinal illness (HCGI). The incidence of illness was not associated with indicators traditionally used to monitor beaches nor with the non-traditional water quality indicators. These results contrast with most other recreational bathing studies, most likely because of the lack of human sources of fecal pollution.

INTRODUCTION

Fecal indicator bacteria are monitored at marine recreational bathing beaches to assess the risk of contracting swimming-related illnesses. In southern California, more than 85,000 samples are collected and over $3 million are spent annually to assess public health risk using bacterial tests as indicators of fecal contamination (Schiff *et al.* 2002). The focus on bacteria as a public health monitoring tool is based on the relationship between the density of fecal indicator bacteria and the occurrence of illnesses among those with water exposure.

Numerous studies have demonstrated the relationship between fecal indicator bacteria at marine beaches and swimming-related illnesses (Pruss 1998, Wade *et al.* 2003). Prominent among these studies were those conducted by Cabelli (1983, Cabelli *et al.* 1979) which reported a relationship between enterococcus and illness at several beaches. Haile *et al.* reported an association between swimming-related illnesses and enterococcus, fecal coliforms, and total coliforms in Santa Monica Bay, California (1999). The Cabelli and Haile studies were the focal point for the establishment of water quality thresholds at marine beaches using fecal indicator bacteria in the United States and the State of California, respectively.

While previous studies successfully demonstrated the value of fecal indicator bacteria, virtually all were conducted at locations where human sewage was the predominant contamination source. Haile *et*

---

[1] *University of California Berkeley, School of Public Health, Division of Epidemiology, Berkeley, CA*
[2] *United States Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC*
[3] *University of North Carolina, Chapel Hill, NC*

al. was the only study to have focused on urban runoff as a source, but even this non-point source was known to contain human sources of fecal contamination (1999). Most beach water quality problems in California are attributable to non-point source runoff (Noble *et al.* 2003, Schiff *et al.* 2003), and it is not certain that human health relationships for waterborne bacterial indicators would remain the same when non-human sources predominate (Calderon *et al.* 1991). Because animals can shed bacterial indicators without accompanying human pathogens (NRC 2004), there is uncertainty about the present practice of extrapolating water quality thresholds that are based on the risk of swimming-associated illnesses from human point source to non-point sources dominated by animal-associated fecal contamination. A poor correlation between bacterial indicators and virus concentrations has been found in urban runoff (Jiang *et al.* 2001, Noble and Fuhrman 2001), in contrast to the significant relationships that have been found when examining water bodies influenced by human sources (e.g., septic tanks; Lipp *et al.* 2001).

A cohort study conducted in Mission Bay, California, found state water quality standards to be exceeded more than 20% of the time (Schiff and Kinney 2001). Several million dollars have been expended to remove human contamination by inspecting and repairing the sanitary sewerage system surrounding the bay and diverting larger storm drains away from the bay. Recent source tracking studies suggested that human fecal material constitutes a minor proportion (<10%) of fecal inputs to the Bay (City of San Diego and MEC/Weston 2004). However, California water quality standard exceedences continue (Hanley 2002).

To address the pressing need for faster, more specific water quality measurements to protect swimmers' health, microbiologists are developing new test methods. Chromogenic substrate assays have become increasingly popular because they are faster and easier than traditional methods, while producing comparable results (Griffith *et al.* 2006). Genetic-based techniques are not yet commercially available for fecal indicator bacteria, but researchers are capable of obtaining results in a matter of hours (Noble and Weisberg 2005). Finally, genetic-based techniques are exploring new microbial indicators, such as *Bacteroides*, a group of obligate anaerobes that are abundant intestinal flora (Cabelli *et al.* 1982). These techniques also provide new tools for measuring pathogens directly, including human specific virus (Tsai *et al.* 1993, Noble *et al.* 2003). Regardless of rapidity, specificity, or cost, the efficacy of any new public health monitoring tool can only be evaluated through an epidemiological study that documents relationships to the incidence of swimming-related illness.

The goal of this study was to examine health effects experienced by swimmers and the relationship of these effects to water quality indicators in this system where non-human fecal sources dominate. The study was designed to answer two questions. First, did water contact increase the risk of illness in the two weeks following exposure to water in Mission Bay during the summer of 2003? Second, did the risk of illness increase with increasing levels of traditional microbial indicators of water quality? As a corollary question, the increased risk of illness with increasing levels of new, non-traditional microbial methods or indicators of water quality was also examined.

METHODS

Overview

The study was designed as a prospective cohort (Wade *et al.* 2003, NRC2004). Participants were recruited each sampling day and their current health and degree of exposure to the water were recorded. Water quality was synoptically measured at multiple sites and over multiple time periods. Ten to 14 days later, the participants were contacted by phone and interviewed about symptoms of illness that occurred after their beach visit. Regression models were used to evaluate the association between exposure to indicators of water quality and illness and to compare illnesses between swimmers and non-swimmers.

Sampling sites

Study sites were selected to maximize the number of potential study participants. Beach-goers were recruited at six Mission Bay beaches on weekends and holidays, beginning Memorial Day weekend and continuing through Labor Day 2003. Water quality samples were collected at the same six beaches. Eighteen sampling sites were targeted, with the number of sites per beach ranging from two to five, depending upon beach length and anticipated swimming activity. Data were collected on 29 days.

Water quality data collection and analysis (indicator organisms)

Twelve measures of water quality were collected during the study. Three traditional indicators (enterococcus, total coliforms, fecal coliforms) were measured by traditional methods (membrane filtration; MF) and each was also measured using the chromogenic substrate (CS) method. Enterococcus was also determined by a new genetic method, quantitative polymerase chain reaction (QPCR). The final five measures were new indicators (*Bacteroides*, somatic coliphage, male-specific coliphage, adenovirus, and Norwalk-like virus).

Water samples were collected with varying frequency, depending on the specific indicator. The indicators, sampling frequency, and laboratory analysis methods are shown in Table 1. Additional details regarding laboratory procedures are available in the project technical report (Colford *et al.* 2005).

Human health data collection

All study instruments and protocols were approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley.

Beach recruitment

Interviewers canvassed the study beaches. Eligibility criteria included: 1) no previous participation in the study; 2) at least one family member of the household at the beach was 18 years old or older; 3) home address in the United States, Canada, or Mexico (interviews were conducted in either English or Spanish); and 4) had not swam (face or head under water) in the ocean or in a lake in the previous seven days. If an individual or household was eligible and agreed to participate, the interviewers obtained signed consent from the individual or all participating adult members of the household. Adults gave signed consent for children less than 18 years of age. Interviewers marked the screening form to identify the water sampling site that was closest to the location of the individual or family on the beach. Participants were given an incentive and asked to complete a questionnaire prior to their departure that day. The questionnaire assessed possible exposures at the beach and exposures or illnesses experienced during the 2 - 3 days prior to the beach visit. Participants who failed to complete the survey at the beach were contacted within three days by telephone.

Follow-up interview

Approximately 14 days following their beach visit, participants were telephoned and asked to complete a 10- to 15-minute interview. This interview consisted of the following types of questions: 1) demographic information; 2) swimming and other exposures since the beach day; 3) pre-existing health problems (e.g., chronic diarrhea); and 4) acute

Table 1. Methods, analytical laboratories, and sampling intensity used for water quality measurements during the Mission Bay Epidemiology Study.

Parameter	Method	Analytical Laboratory	Sampling intensity
Enterococcus	EPA 1600	City of San Diego	Beach composite once per day
Enterococcus	96 well Quantitray	City of San Diego	Hourly sample at every site
Enterococcus	Quantitative PCR	EMSL	Two samples per day at each site
Fecal Coliforms	APHA Method 9222D	City of San Diego	Hourly sample at every site
Fecal Coliforms (*E. coli)*	96 well Quantitray	City of San Diego	Beach composite once per day
Total Coliforms	APHA Method 9222B	City of San Diego	Hourly sample at every site
Total Coliforms	96 well Quantitray	City of San Diego	Beach composite once per day
Bacteroides	Quantitative PCR	EMSL	Two samples per day at each site
Somatic Phage	Modified EPA 1601	University of North Carolina	Beach composite once per day
Male-specific Phage	Modified EPA 1601	University of North Carolina	Beach composite once per day
Adenovirus 40 and 41	Quantitative PCR	University of North Carolina	Beach composite once per day
Norwalk-like Virus	Quantitative PCR	University of North Carolina	Beach composite once per day

health conditions experienced since the visit to the beach. As with the previous interviews, the head of household answered questions for children less than 18 years of age.

Health outcomes measured

The health outcomes ascertained through the interview included gastrointestinal illness, respiratory symptoms, dermatologic symptoms, and other non-specific symptoms. Gastrointestinal symptoms included nausea, vomiting, diarrhea, and stomach cramps. In addition, two categories of highly credible gastrointestinal illness (HCGI) were measured. HCGI-1 was defined as either: 1) vomiting; or 2) diarrhea and fever; or 3) cramps and fever. This is consistent with the way GI illness was defined by Haile *et al.* (1999). Respiratory outcomes included cough, cough with phlegm, nasal congestion or runny nose, sore throat, and significant respiratory disease (SRD). Significant respiratory disease was defined as: 1) fever plus nasal congestion; or 2) fever plus sore throat; or 3) cough with phlegm. This definition is also consistent with Haile *et al.* (1999) Dermatologic outcomes included skin rashes and infected cuts or scrapes. Non-specific symptoms included fever, redness or eye irritation, earache, and ear discharge.

Data Analysis

Two principal groups of analyses were conducted. The first was a set of models to evaluate any differences in illness rates between swimmers and non-swimmers. The analyses were repeated for two definitions of swimming. In the first set of analyses, “swimming” was defined as participants answering “yes” when asked if they had any water contact during their day at the beach. The analyses were also repeated using a definition of “swimming” as those who answered “yes” when asked if they had swallowed any water. Non-swimmers were those who answered “no” when asked if they had contact with water during their day at the beach.

The second group of analyses consisted of regression models designed to evaluate the association between the risk of illness in swimmers and water quality (as measured by the indicators). In these models, the main outcome was a binary indicator of illness and a continuous measure of exposure, modeled as the geometric mean for the indicator on the beach and day of the swimmer’s exposure. As a secondary analysis, enterococcus was treated as a dichotomous variable using California state water quality thresholds as cutpoints (>35 vs. <35 and >104 vs. <104). In all models involving water quality indicators, a value of zero was used for water quality exposure values below the detection limit of the test.

Multivariate models included potential confounding factors, including age, gender, ethnicity, income, allergies, swimming after the beach interview, collecting shells at the beach, digging in the sand, playing with seaweed or algae, chronic or pre-existing illnesses, contact with other sick people, use of insect repellant at the beach, use of sunblock, showering immediately after swimming, consumption of raw or undercooked eggs or meat, and consumption of food at the beach. All variables, except age, were categorized as 1 or 0. Race was collapsed into two categories, white and non-white.

All analyses were conducted using a nested interaction model that effectively assigned non-swimmers a zero exposure value, while including an indicator of swimming. The model permits comparisons among swimmers with different levels of indicator exposure, as well as comparisons among swimmers versus non-swimmers independent of indicator level and is parameterized as follows:

$$1\mathrm{n}\,(p/(1-p)) = a + \beta_1 x_1 + \beta_2(x_1 * x_2) + \beta_3 x_3 + \beta_4(x_3 * x_2)$$

where p = probability of illness, x_1= 1 if any contact with water, 0 otherwise; x_2 is a water quality indicator value (continuous); and x_3 is a 1/0 indicator of other specific water exposure (body contact, head under water, etc.)

In the multivariate analyses, a backwards deletion procedure was used to identify factors that most affected the water quality/illness relationship (Rothman and Greenland 1982).

The risk of illness output from the models was expressed as an odds ratio. For models comparing swimmers and non-swimmers, the odds ratio can be interpreted as the odds of a specific illness in swimmers divided by the odds of illness in non-swimmers. For models assessing the association between water quality indicators among swimmers, the odds ratios can be interpreted as the increase in the odds of illness per defined unit of increase in the water quality measure among swimmers. Odds ratios were calculated by exponentiating the regression coefficient provided by the model output.

Models adjusted for relevant covariates (see above) were used to estimate the percentages of swimmers and non-swimmers ill for any health outcomes with a statistically significant elevated adjusted odds ratio (OR). The adjusted attributable risk estimates were determined by estimating adjusted probabilities of swimmers and non-swimmers from a multivariate logistic model, weighting the covariates as the mean value for each covariate. The adjusted attributable risk was then calculated as the difference between the probability of illness among swimmers with mean levels of covariates and non-swimmers with mean levels of covariates. These results are expressed as the number of excess cases of illness predicted among 1,000 swimmers, along with a 95% confidence interval (CI) of this estimate.

RESULTS

Water quality

A total of 1,897 water samples were collected. All but five of these samples were analyzed successfully in the laboratory. The majority of samples had quantifiable levels of indicator bacteria (Table 2). About 16% of the samples exceeded state water quality thresholds for traditional fecal indicator bacteria, with enterococcus accounting for most of the exceedances and total coliforms the least .

Table 2 also shows the range of concentrations for virus measurements. Pathogenic virus was detected in only one sample. The majority of samples had quantifiable levels of somatic phage, but not for male-specific phage, which is thought to be more strongly associated with fecal material.

Health outcomes

A total of 12,469 individuals and 5,062 households were enrolled in the study. Of these, 8,797 (71%) of the enrolled participants and 3,501 (69%) of the households completed the follow-up telephone interview. Fifty-seven percent (n = 4,971) of those that completed the follow-up interview were swimmers, compared to 3,742 non-swimmers. Table 3 shows the individual and household sociodemographic characteristics of the study group.

Health outcomes for swimmers versus non-swimmers

A significant increase in diarrhea (OR 1.36, 95% CI 1.04 - 1.78) and skin rash (OR 2.25, 95% CI 1.60 - 3.16) was observed among swimmers when swimming was defined as having any water contact (Table 4). When swimming was defined as having swallowed water, there was a significant association between exposure and diarrhea (OR 1.89, 95% CI 1.34 - 2.66), cramps (OR 1.53, 95% CI 1.08 - 2.15), skin rash (OR 2.11, 95% CI 1.37 - 3.24), and eye irritation (OR 1.69, 95% CI 1.23 - 2.3; Table 4).
No significant elevations were found in any of the other health outcomes measured, regardless of the

Table 2. Range of concentrations for traditional and non-traditional water quality indicators (number/100 ml) and frequency of exceedance (N = 1,897) of the State of California's water quality threshold.

	Total No. of Samples	No. of Samples Below Detection	Geomean (No. per 100ml)	Max. (No. per 100ml)	No. of Samples Exceeding State Water Quality Threshold	State Water Quality Threshold
Enterococcus-CS	1,897	585	29	57,940	265	>104
Fecal Coliform	1,897	304	25	48,000	99	>400
Total Coliform	1,897	808	102	45,000	5	>10,000
Total:Fecal Ratio	1,897	N/A	N/A	N/A	75	Total:Fecal < 10 When Total > 1,000
Enterococcus-QPCR*	790	46	65	141,053	351	>104
Bacteroides	790	294	102	3,718,815	N/A	N/A
Adenovirus	151	150	-	0.01	N/A	N/A
Norwalk-like Virus	151	151	-	-	N/A	N/A
Male-specific Phage	141	125	0.2	0.8	N/A	N/A
Somatic Phage	141	45	0.6	36.6	N/A	N/A

*Quantitative polymerase chain reaction

Table 3. Individual sociodemographic characteristics collected from study participants at all beaches from Mission Bay.

Characteristic	Surveyed Participants							
	All (N = 8,797)		Swimmers (N = 4,971)		Non-swimmers (N = 3,742)		Missing (N = 84)	
	n	%	n	%	n	%	n	%
Age								
0 - 5	1,214	13.8	870	17.5	326	8.7	18	21.4
5.1 - 12	1,808	20.6	1,461	29.4	332	8.9	15	17.9
12.1 - 30	2,366	26.9	1,215	24.4	1,127	30.1	24	28.6
30.1 - 55	2,928	33.3	1,251	25.2	1,654	44.2	23	27.4
>55	332	3.8	76	1.5	253	6.8	3	3.6
Missing	149	1.7	98	2.0	50	1.3	1	1.2
Gender								
Male	4,761	54.1	2,624	52.8	2,100	56.1	37	44.0
Female	3,948	44.9	2,292	46.1	1,609	43.0	47	56.0
Missing	88	1.0	55	1.1	33	0.9	0	0.0
Race								
White	2,495	28.4	1,181	23.8	1,307	34.9	7	8.3
African American	369	4.2	165	3.3	194	5.2	10	11.9
American Indian/ Alaskan Native	62	0.7	35	0.7	27	0.7	0	0.0
Asian/Pacific Islander	463	5.3	177	3.6	281	7.5	5	6.0
Hispanic/Latino	4,723	53.7	3,052	61.4	1,616	43.2	55	65.5
Mixed Race	407	4.6	241	4.8	163	4.4	3	3.6
Other	227	2.6	96	1.9	128	3.4	3	3.6
Missing	51	0.6	24	0.5	26	0.7	1	1.2

definition of swimming.

We explored the relationship between participant age and health outcomes after water exposure (Table 5). Among participants with any water contact, the strongest and only significant association with diarrhea was among children ages 5 to 12 years (OR 2.80, 95% CI 1.07 - 7.27). The OR increased with increased exposure in the 5 - 12 year old age group (OR 5.30, 95% CI 1.96 - 14.33). Skin rash was significantly associated with several age groups among participants either with any water contact or who reported swallowing water. Significant associations were also found among those who swallowed water and who reported skin rash (ages 0 - 5 years and 5 - 12 years) and eye irritation (ages 5 - 12 years).

Table 6 shows the attributable risk calculations for diarrhea, stratified by age group. The estimated excess of cases among swimmers vs. non-swimmers was greatest in participants age 5 - 12 years with any water contact (27.4 excess cases per 1000 swimmers) and

Table 3. (Continued)

Characteristic	Surveyed Participants (N = 3,501 Households)	
Household size (# of persons)	**n**	**%**
1	1,269	36.2
2	649	18.5
3	532	15.2
4	511	14.6
5	290	8.3
6	140	4.0
7	68	1.9
≥8	42	1.2
Missing	0	0.0
Country of Residence (HH)		
United States	3,170	90.5
Mexico	66	1.9
Canada	2	0.1
Missing	263	7.5
Average Annual Income (HH)		
≤ 10,000	284	8.1
10,001 to 20,000	639	18.3
20,001 to 30,000	444	12.7
30,001 to 40,000	360	10.3
40,001 to 50,000	294	8.4
50,001 to 60,000	231	6.6
60,001 to 70,000	181	5.2
70,001 to 80,000	210	6.0
80,001 to 100,000	229	6.5
>100,000	309	8.8
Missing	321	9.2

Table 4. Adjusted odds ratios for health outcomes relative to various types of water exposure. Bolded numbers indicate statistical significance.

Health Outcome	Adjusted OR (95% CI)		
	Any Water Contact	Water on Face	Swallow Water
Gastrointestinal			
Diarrhea	**1.36 (1.04 - 1.78)**	**1.54 (1.16 - 2.06)**	**1.89 (1.34 - 2.66)**
HCGI-1	0.96 (0.68 - 1.37)	1.03 (0.71 - 1.50)	1.01 (0.62 - 1.66)
HCGI-2	0.93 (0.49 - 1.75)	1.10 (0.57 - 2.13)	1.12 (0.51 - 2.45)
Nausea	0.88 (0.64 - 1.23)	1.11 (0.77 - 1.61)	1.41 (0.91 - 2.17)
Cramps	1.07 (0.81 - 1.42)	1.14 (0.86 - 1.51)	**1.53 (1.08 - 2.15)**
Vomiting	0.85 (0.58 - 1.26)	0.92 (0.61 - 1.37)	0.86 (0.49 - 1.52)
Skin Rash	**2.25 (1.60 - 3.16)**	**2.39 (1.72 - 3.31)**	**2.11 (1.37 - 3.24)**
Eye Irritation	1.19 (0.93 - 1.52)	1.29 (0.99 - 1.68)	**1.69 (1.23 - 2.30)**
Ear			
Earache	0.96 (0.65 - 1.44)	1.00 (0.64 - 1.56)	1.10 (0.63 - 1.93)
Ear Discharge	0.40 (0.16 - 1.01)	0.47 (0.19 - 1.13)	0.82 (0.22 - 3.00)
Fever	0.96 (0.70 - 1.32)	1.04 (0.74 - 1.47)	1.15 (0.76 - 1.75)
Respiratory			
SRD	1.08 (0.80 - 1.45)	1.03 (0.75 - 1.43)	0.99 (0.62 - 1.57)
Sore Throat	0.89 (0.69 - 1.16)	0.96 (0.71 - 1.32)	0.87 (0.56 - 1.34)
Cough	0.74 (0.54 - 1.02)	0.77 (0.54 - 1.11)	0.82 (0.47 - 1.41)

among those who had swallowed water (59.0 excess cases per 1000 swimmers).

Relationship between health outcomes and water quality among swimmers

No correlation was observed between the risk of illness and increased levels of traditional water quality indicators for enterococcus, fecal coliform or total coliform. Using diarrhea as an example, odds ratios were not statistically elevated due to increases in enterococcus (Table 7). The lack of relationship resulted despite numerous approaches to assigning water quality exposure (i.e., combining or separating sites at a beach) or calculation of indicator metrics (i.e., daily geomean, daily maxima, or various cutpoints). Of particular note, exposure to indicator measures above the two different California state water quality thresholds did not correlate with a significant increased risk of illness (Table 8).

We found no correlation between increased risk of illness and levels of *Bacteroides*, enterococcus using rapid methods (QPCR), human pathogenic virus (adenovirus and Norwalk-like virus), or somatic phage (data not shown, results available in Colford *et al.* 2005). The relationship with viruses could not be adequately evaluated because no Norwalk-like virus was found and adenovirus was only found in one sample, though the low counts were consistent with the absence of increased health risk for the other health outcomes evaluated.

Significant associations between the levels of male-specific coliphage and HCGI-1, HCGI-2, nausea, cough, and fever were observed (Table 9). However, a low number of participants were exposed to the water at times when male-specific coliphage was detected (Table 10). Therefore, the relationships between male-specific coliphage and various health outcomes should be interpreted cautiously.

DISCUSSION

Swimmers experienced more diarrhea and skin rash than non-swimmers in Mission Bay. The prevalence of these symptoms increased with higher exposure (i.e., reported swallowing water), further suggesting that these symptoms were mediated by water contact. However, increased risk was not observed for more severe symptoms such as fever, vomiting, or multi-symptom categories such as HCGI. These more severe symptoms are the foundation for Federal and State water quality thresholds (Cabelli

Table 5. Health outcomes by age group and water exposure type. Bolded numbers indicate statistical significance.

Health Outcome	Any Water Contact			
	Age Group (years)			
	0 - 5	>5 - 12	>12 - 30	>30
Gastrointestinal				
Diarrhea	0.75 (0.40 - 1.40)	**2.80 (1.07 - 7.27)**	1.71 (0.96 - 3.05)	1.28 (0.85 - 1.93)
HCGI-1	0.86 (0.45 - 1.59)	1.33 (0.56 - 3.14)	0.73 (0.36 - 1.44)	1.37 (0.60 - 3.15)
HCGI-2	0.74 (0.31 - 1.75)	2.26 (0.28 - 18.45)	0.64 (0.15 - 2.74)	2.10 (0.30 - 14.73)
Nausea	1.90 (0.62 - 5.83)	1.40 (0.52 - 3.79)	0.46 (0.26 - 0.83)	1.11 (0.63 - 1.95)
Cramps	1.20 (0.53 - 2.70)	1.61 (0.77 - 3.37)	0.57 (0.34 - 0.94)	1.51 (0.93 - 2.43)
Vomiting	0.58 (0.31 - 1.10)	1.59 (0.54 - 4.68)	0.68 (0.31 - 1.47)	1.45 (0.64 - 3.30)
Skin Rash	**5.86 (1.81 - 19.0)**	**3.26 (1.30 - 8.15)**	1.60 (0.89 - 2.86)	**1.84 (1.04 - 3.25)**
Eye Irritation	0.53 (0.27 - 1.04)	1.84 (0.94 - 3.61)	1.21 (0.81 - 1.82)	1.23 (0.80 - 1.88)
Ear				
Earache	0.86 (0.31 - 2.39)	1.14 (0.37 - 3.49)	0.62 (0.30 - 1.25)	1.47 (0.73 - 2.96)
Ear Discharge	0.12 (0.01 - 1.66)	0.22 (0.03 - 1.59)	0.58 (0.14 - 2.37)	0.63 (0.10 - 3.86)
Fever	0.68 (0.39 - 1.16)	1.67 (0.67 - 4.15)	0.83 (0.44 - 1.56)	1.44 (0.73 - 2.84)
Respiratory				
SRD	0.63 (0.32 - 1.22)	1.23 (0.57 - 2.66)	1.01 (0.58 - 1.75)	1.43 (0.86 - 2.35)
Sore Throat	0.74 (0.33 - 1.69)	1.21 (0.57 - 2.57)	0.82 (0.51 - 1.30)	0.90 (0.61 - 1.35)
Cough	0.52 (0.27 - 1.02)	0.84 (0.38 - 1.87)	0.78 (0.41 - 1.49)	0.84 (0.49 - 1.45)

Table 5. (Continued)

Health Outcome	Swallow Water			
	Age Group (years)			
	0 - 5	>5 - 12	>12 - 30	>30
Gastrointestinal				
Diarrhea	0.97 (0.47 - 2.01)	**5.30 (1.96 - 14.33)**	1.76 (0.79 - 3.91)	1.78 (0.86 - 3.66)
HCGI-1	0.61 (0.25 - 1.49)	1.72 (0.65 - 4.59)	1.34 (0.49 - 3.67)	0.70 (0.08 - 6.29)
HCGI-2	0.74 (0.23 - 2.36)	2.83 (0.32 - 24.83)	0.92 (0.13 - 6.46)	3.15 (0.18 - 54.04)
Nausea	2.27 (0.76 - 6.81)	2.29 (0.84 - 6.23)	0.56 (0.21 - 1.46)	2.08 (0.81 - 5.33)
Cramps	2.05 (0.88 - 4.78)	**2.51 (1.18 - 5.33)**	0.52 (0.23 - 1.18)	1.83 (0.85 - 3.92)
Vomiting	0.41 (0.14 - 1.18)	2.15 (0.57 - 8.13)	1.27 (0.43 - 3.77)	1.13 (0.12 - 10.12)
Skin Rash	**10.42 (2.34 - 46.40)**	**4.10 (1.39 - 12.09)**	1.15 (0.46 - 2.86)	1.32 (0.39 - 4.46)
Eye Irritation	0.89 (0.41 - 1.92)	**2.87 (1.44 - 5.71)**	1.48 (0.82 - 2.67)	1.53 (0.72 - 3.25)
Ear				
Earache	0.25 (0.03 - 2.18)	2.09 (0.65 - 6.78)	0.78 (0.26 - 2.28)	0.89 (0.18 - 4.30)
Ear Discharge	tf*	tf	tf	tf
Fever	0.73 (0.36 - 1.45)	2.36 (0.88 - 6.28)	1.51 (0.64 - 3.55)	1.04 (0.22 - 4.91)
Respiratory				
SRD	0.62 (0.24 - 1.57)	1.18 (0.46 - 3.03)	1.03 (0.41 - 2.55)	0.71 (0.15 - 3.25)
Sore Throat	0.81 (0.26 - 2.50)	1.03 (0.42 - 2.52)	0.90 (0.38 - 2.10)	0.69 (0.25 - 1.86)
Cough	0.44 (0.15 - 1.32)	1.34 (0.48 - 3.70)	1.50 (0.53 - 4.27)	tf

*tf = too few individuals for analysis

Table 6. Frequency (percent ill) reporting diarrhea and calculated attributable risk.

	Adjusted Attributable Risk* (95% CI) per 1000	Percent ill - Adjusted	
	swimmers	Non-swimmers	Swimmers
Any Water Contact			
All Ages (years)	11.1 (0.0 - 22.1)	3.20	4.31
0 - 5	-14.1 (-47.7 - 18.5)	6.01	4.60
>5 - 12	27.4 (-8.9 - 63.7)	1.59	4.33
>12 - 30	14.3 (-7.0 - 35.6)	2.09	3.51
>30	10.0 (-8.6 - 28.6)	3.74	4.75
Swallow Water			
All Ages (years)	27.2 (8.9 - 45.6)	3.25	5.97
0 - 5	-1.6 (-42.5 - 39.4)	6.09	5.93
>5 - 12	59.0 (15.0 - 103.0)	1.48	7.38
>12 - 30	18.5 (-13.6 - 50.6)	2.55	4.40
>30	26.4 (-15.7 - 68.4)	3.63	6.28

*Expressed as excess cases per 1,000 swimmers

1983, Haile *et al.* 1999) and have been the focus of most previous epidemiology studies (Wade *et al.* 2003). Symptoms such as HCGI are considered more relevant because multi-symptom reactions that include fever are typically pathogen mediated, whereas symptoms like rash and diarrhea can result from saltwater irritation.

Unlike most previous marine recreational epidemiology studies, we found no relationship between illness rates and fecal indicator bacteria. Wade *et al.* reviewed 27 marine recreational water epidemiology studies and found increased relative risk with increasing fecal indicator concentrations in most of them (2003), particularly for enterococcus. However, in essentially all of these studies, water quality was impacted by known sources of human fecal contamination. There appears to be little human fecal contamination in Mission Bay as evidenced by a recent source tracking study that found the predominant source of fecal contamination was avian (Gruber *et al.* 2005). While animal sources can also harbor disease-causing agents, they are less likely to serve as vectors for human disease (NRC 2004).

The use of bacterial indicators as predictors of swimming-associated illnesses is based on the presumption that they have survival properties similar to the pathogens they are intended to mirror. This presumption is less likely to remain true when circulation is restricted and residence times increase, which can be days to weeks in Mission Bay (SIO 2003). Increased survival and perhaps even regrowth of fecal indicator bacteria, has been suggested in sediments and wrack lining beaches including Mission Bay (City of San Diego and MEC/Weston 2003, Weiskel *et al.* 1996). Regardless, the lack of relationship of non-human sources of fecal indicator bacteria to health risk suggests that water contact advisories posted at beaches in Mission Bay during the course of this study were not reflective of public health risk.

It is arguable whether viral measures in our study were better indicators of risk and could be used in place of bacterial indicators for health risk assessments in Mission Bay. We found that increasing density of male-specific phage was correlated with increased incidence of several health outcomes, including HCGI-1, HCGI-2, nausea, cough, and fever (Table 10). This is consistent with the success of this measure in freshwater application (Lee *et al.* 1997). However, we interpret these associations cautiously because male-specific coliphage was not detected often and few subjects were exposed to the water at those times (Table 10).

The human-specific viruses we measured in Mission Bay were rarely detected, consistent with our low rates of swimming-associated illnesses. We did not encounter high virus counts that would have allowed us to assess their effectiveness as predictors in the positive direction. Interpretation of viruses as negative predictors is compromised by technology limitations. We used the most advanced techniques available, but quantifying virus particles in seawater is difficult because DNA and RNA are lost due to complexation and interferences when concentrating and extracting nucleic acid material. Thus, we cannot be

Table 7. Adjusted odds ratio (95% CI) for the association of enterococcus, fecal coliform and total coliform levels (ln of daily geometric mean at the beach level) two weeks after exposure. The unit change in exposure in these models was set to represent a change of geometric mean of 30.

	Enterococcus		Fecal Coliform		Total Coliform	
Health Outcome	**Any Water Contact**	**Swallow Water**	**Any Water Contact**	**Swallow Water**	**Any Water Contact**	**Swallow Water**
Gastrointestinal						
Diarrhea	0.77 (0.33 —1.80)	0.31 (0.06 —1.59)	0.41 (0.18 —0.93)	0.33 (0.07 —1.48)	0.34 (0.15 —0.77)	0.47 (0.09 —2.50)
HCGI-1	0.76 (0.28 —2.04)	1.64 (0.23 —11.77)	0.65 (0.25 —1.67)	1.53 (0.22 —10.63)	0.58 (0.21 —1.59)	0.64 (0.08 —4.92)
HCGI-2	0.97 (0.18 —5.23)	1.65 (0.09 —31.24)	0.59 (0.11 —3.08)	3.49 (0.20 —61.77)	0.48 (0.09 —2.70)	0.43 (0.02 —8.79)
Nausea	0.72 (0.22 —2.38)	0.79 (0.10 —6.35)	0.56 (0.19 —1.68)	1.30 (0.17 —9.71)	0.39 (0.12 —1.25)	0.35 (0.04 —2.85)
Cramps	0.87 (0.38 —2.00)	0.67 (0.14 —3.09)	0.58 (0.26 —1.28)	0.62 (0.14 —2.72)	0.84 (0.34 —2.14)	0.56 (0.12 —2.59)
Vomiting	0.69 (0.22 —2.22)	1.98 (0.18 —21.55)	0.76 (0.23 —2.55)	2.63 (0.26 —26.55)	0.48 (0.14 —1.62)	0.69 (0.06 —8.20)
Skin Rash	0.84 (0.34 —2.04)	0.65 (0.11 —3.85)	0.86 (0.36 —2.06)	0.67 (0.12 —3.68)	1.35 (0.52 —3.51)	3.49 (0.59 —20.64)
Eye Irritation	0.74 (0.34 —1.58)	0.79 (0.17 —3.73)	0.69 (0.33 —1.41)	0.67 (0.15 —2.89)	0.64 (0.29 —1.40)	1.21 (0.24 —6.09)
Ear						
Earache	1.13 (0.34 —3.81)	0.45 (0.04 —5.15)	1.50 (0.51 —4.37)	0.38 (0.04 —3.37)	1.85 (0.53 —6.48)	0.46 (0.04 —4.99)
Ear Discharge	1.45 (0.05 —42.31)	1.09 (0.01 —189.13)	7.13 (0.31 —164.89)	0.79 (0.00 —129.89)	6.31 (0.27 —144.82)*	5.21 (0.02 —1139.85)
Fever	0.98 (0.39 —2.43)	1.09 (0.21 —5.82)	0.57 (0.24 —1.37)	0.72 (0.14 —3.76)	0.79 (0.29 —2.11)	0.40 (0.07 —2.20)
Respiratory						
SRD	1.15 (0.47 —2.79)	1.47 (0.23 —9.33)	0.58 (0.25 —1.33)	1.11 (0.16 —7.77)	0,68 (0.26 —1.75)	0.78 (0.10 —6.12)
Sore Throat	1.27 (0.52 —3.13)	0.34 (0.05 —2.13)	1.42 (0.61 —3.31)	0.25 (0.06 —2.03)	1.50 (0.59 —3.82)	0.40 (0.06 —2.61)
Cough	0.50 (0.14 —1.76)	0.06 (0.01 —0.70)	0.44 (0.14 —1.43)	0.16 (0.02 —1.58)	0.37 (0.10 —1.36)	0.14 (0.01 —1.45)

Table 8. Adjusted odds ratios for health outcomes using two dichotomous measures of exposure to enterococcus. There were no statistically significant results.

Health Outcome	Adjusted OR (95% CI)	
	> 35 vs. ≤ 35	> 104 vs. ≤ 104
Gastrointestinal		
Diarrhea	1.01 (0.73-1.39)	1.22 (0.85-1.76)
HCGI-1	0.74 (0.51-1.06)	1.13 (0.73-1.75)
HCGI-2	0.69 (0.38-1.25)	0.80 (0.37-1.73)
Nausea	0.78 (0.51-1.20)	1.12 (0.65-1.92)
Cramps	0.91(0.66-1.24)	1.36 (0.95-1.95)
Vomiting	0.67 (0.43-1.03)	1.13 (0.67-1.92)
Skin Rash	0.83 (0.61-1.15)	1.00 (0.67 - 1.49)
Eye Irritation	0.97 (0.72-1.32)	0.77 (0.53-1.11)
Ear		
Earache	1.08 (0.68-1.70)	1.18 (0.70-2.00)
Ear Discharge	1.15 (0.30-4.36)	0.94 (0.19-4.62)
Fever	0.92 (0.65-1.31)	0.89 (0.58-1.37)
Respiratory		
SRD	0.96 (0.67-1.37)	1.13 (0.73-1.73)
Sore Throat	1.10 (0.80-1.50)	1.15 (0.77-1.72)
Cough	0.65 (0.40-1.05)	0.51 (0.25-1.03)

certain that the low levels we observed were due to their absence from the system or from difficulties in recovering viruses that were present. Our results do suggest that these non-bacterial measures may have the potential to be more effective than traditional bacterial indicators as predictors of illness when non-human sources are dominant.

Table 9. Adjusted odds ratios for various health outcomes after two weeks for any water contact (per unit increase) to male-specific coliphage. Statistically significant results are shown in bold face type.

Health Outcome	Adjusted OR (95% CI)
Gastrointestinal	
Diarrhea	1.14 (0.97 - 1.35)
HCGI-1	**1.26 (1.06 - 1.48)**
HCGI-2	**1.43 (1.13 - 1.82)**
Nausea	**1.34 (1.16 - 1.55)**
Cramps	1.04 (0.83 - 1.32)
Vomiting	1.21 (0.96 - 1.53)
Skin Rash	1.00 (0.77 - 1.31)
Eye Irritation	1.14 (0.95 - 1.36)
Ear	
Earache	Too few
Ear Discharge	Too few
Fever	**1.25 (1.09 - 1.44)**
Respiratory	
SRD	1.05 (0.85 - 1.31)
Sore Throat	1.04 (0.83 - 1.30)
Cough	**1.22 (1.02 - 1.48)**

While we found that traditional fecal indicators were ineffective predictors of health effects, Mission Bay may be an exception rather than the rule. Mission Bay has been subjected to extensive cleanup activities. Recent source tracking studies have identified that human fecal sources are now only a minor contributor to the overall bacterial load (City of San Diego and MEC/Weston 2004). Our study does suggest the need for further evaluation of traditional fecal indicator bacteria in circumstances where non-point, non-human contributions are the dominant fecal source. We found that non-traditional ways of quantifying fecal contamination, such as QPCR, were unassociated with health effects. This contrasts with a recent study which observed a relationship between enterococcus measured by QPCR and gastrointestinal illness at Great Lakes beaches (Wade *et al.* 2005). Unlike Mission Bay, however, these beaches were impacted by human fecal contamination.

Our findings are unique in this field and do not agree with earlier studies reporting associations between water quality indicators and illness. Like Mission Bay, many other enclosed marine beaches, particularly in California, suffer from impaired water quality due to non-point sources of bacteria and poor circulation. We do not recommend extrapolation to other enclosed beaches at this time, however, because we are uncertain if Mission Bay has unique site characteristics. Further studies to confirm the reduced risk of swimming related illnesses at beaches impacted by non-point sources of fecal pollution appear justified and will be an important element of developing revised water quality thresholds and/or improved indicators of water quality. Finally, our findings from Mission Bay were collected during periods of dry weather with no known sewage spills.

Table 10. Association of male-specific coliphage (defined as >0.10 cfu) with diarrhea.

	Diarrhea Reported	No Diarrhea Reported	Total
Male-specific Phage Present (>0.10 cfu)	8	153	161
Male-specific Phage Absent	195	3,878	4,073
Total	203	4,031	4,234

We would predict an increase in health risks, and likely an association with water quality indicators, if large sources of untreated human fecal material entered the Bay. Health risks to swimmers during wet weather remain unknown.

LITERATURE CITED

Cabelli, V. 1983. Health effects criteria for marine recreational waters. US Environmental Protection Agency. Cincinnati, OH.

Cabelli, V.J., A.P. Dufour, M.A. Levin, L.J. McCabe and P.W. Haberman. 1979. Relationship of microbial indicators to health effects at marine bathing beaches. *American Journal of Public Health* 69:690-6.

Cabelli, V.J., A.P. Dufour, M.A. Levin and L.J. McCabe. 1982. Swimming-associated gastroenteritis and water quality. *American Journal of Epidemiology* 115:606-16.

Calderon, R., E. Mood and A. Dufour. Health effects of swimmers and nonpoint sources of contaminated water. *International Journal of Environmental Health Research* 1991;1:21-31.

City of San Diego and MEC Analytical Systems-Weston Solutions, Inc. (City of San Diego and MEC/Weston). 2004. Mission Bay clean beaches initiative bacterial source identification study. Prepared for the State Water Resources Control Board, Sacramento, CA. City of San Diego and MEC/Weston. San Diego, CA.

Colford, J.M., Jr., T.J. Wade, K.C. Schiff, C. Wright, J.B. Griffith, S.K. Sandhu and S.B. Weisberg. 2005. Recreational Water Contact and Illness in Mission Bay, California. Technical Report 449. Southern California Coastal Water Research Project. Westminster, CA.

Griffith, J.F., L.A. Aumand, I.M. Lee, C.D. McGee, L.L. Othman, K.J. Ritter, K.O. Walker and S.B. Weisberg. 2006. Comparison and verification of bacterial water quality indicator measurement methods using ambient coastal water samples. *Environmental Monitoring and Assessment* 116:335-344.

Gruber, S.J., L.M. Kay, R. Kolb and K. Henry . 2005. Mission Bay bacterial source identification study. *Stormwater* http://www.forester.net/sw_0505_mission.html.

Haile, R.W., J.S. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides and G. Wang. 1999. The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology* 1999 10:355-63.

Hanley, Y. 2002. 2002. Impact of rainfall on microbiological water quality of Mission Bay, California. Masters Thesis, California State University, San Diego. San Diego, CA.

Jiang, S., R. Noble and W. Chu. 2001. Human adenovirus and coliphages in urban runoff-impacted coastal waters of southern California. *Applied & Environmental Microbiology* 67:179-184.

Lee, J.V., S.R. Dawson, S. Ward, S.B. Surman and K.R. Neal. 1997. Bacteriophages are a better indicator of illness rates than bacteria amongst users of a white water course fed by a lowland river. *Water Science and Technology* 35:165-170.

Lipp, E.K., S.A. Farrah and J.B. Rose. 2001. Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. *Marine Pollution Bulletin* 42:286-293.

National Resource Council (NRC). 2004. Indicators for waterborne pathogens. National Academies Press. Washington, DC.

Noble, R.T. and J.A. Fuhrman. 2001. Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels. *Hydrobiologia* 460:175-184.

Noble, R.T., S.B. Weisberg, M.K. Leecaster, C.D. McGee, P. Dorsey, P. Vainik, M.V. Orozco-Borbón. 2003. Storm effects on regional beach water quality along the southern California shoreline. *Journal of Water and Health* 1:23-31.

Noble, R.T., S.M. Allen, A.D. Blackwood, W.-P. Chu, S.C. Jiang, G.L. Lovelace, M.D. Sobsey, J.R. Stewart and D.A. Wait. 2003. Use of viral pathogens and indicators to differentiate between human and non-human fecal contamination in a microbial source tracking comparison study. *Journal of Water and Health* 1:195-207.

Noble, R.T. and S.B. Weisberg. 2005. A review of technologies being developed for rapid detection of bacteria in recreational waters. *Journal of Water and Health* 3:381-392.

Pruss A. 1998. Review of epidemiological studies on health effects from exposure to recreational water. *International Journal of Epidemilogy* 27:1-9.

Rothman, K.J. and S. Greenland. 1998. Modern Epidemiology. Second ed. Lippincott-Ravens. Philadelphia, PA.

Schiff, K. and P. Kinney. 2001. Tracking sources of bacterial contamination in stormwater discharges from Mission Bay, California. *Water Environment Research* 73:534-542.

Schiff, K., J. Morton and S. Weisberg. 2003. Retrospective evaluation of shoreline water quality along Santa Monica Bay beaches. *Marine Environmental Research* 56:245-253.

Schiff, K., S. Weisberg and V. Raco-Rands. 2002. Inventory of ocean monitoring in the Southern California Bight. *Environmental Management* 29:871-876.

Scripps Institute of Oceanography (SIO). 2003. Mission Bay contaminant dispersion study. Final Report to the City of San Diego General Services Department (Agreement No. 228099) SIO, University of California San Diego. San Diego, CA.

Tsai, Y., M.D. Sobsey, L.R. Sangermano and C.J. Palmer. 1993. Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. *Applied Environmental Microbiology* 59:3488-3491.

Wade, T.J., R.L. Calderon, E. Sams, M.J. Beach, K.P. Brenner, A.H. Williams and A.P. Dufour. 2005. Rapidly measured indicators of recreational water quality are predictive of swimming associated gastrointestinal illness. *Environmental Health Perspectives* doi:10.1289/ehp.8273.

Wade, T.J., N. Pai, J.N. Eisenberg and J.M. Colford, Jr. 2003. Do US Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. *Environmental Health Perspectives* 111:1102-9.

Weiskel, P.K., B.L. Howes and G.R. Heufelder. 1996. Coliform contamination of a coastal embayment: Sources and transport pathways. *Environmental Science & Technology* 30:1872-1881.

ACKNOWLEDGEMENTS

We thank the many organizations that helped with data collection and sample processing on this project. Ric Amador and Laila Othman (City of San Diego) kindly coordinated fecal indicator bacterial analysis using traditional methodologies. Rich Haugland, Al Dufour and Charles LaCerra coordinated the analyses using quantitative polymerase chain reaction (EMSL Laboratories, Cincinnati, Ohio). Virus and coliphage measurements were coordinated by Mark Sobsey (at University North Carolina, Chapel Hill). The human health data collection was coordinated by Jackie Hayes (University California Berkeley). Data management and analytic support were provided in part by Srikesh Arunajadai and Shannon Carr (University California Berkeley). We also wish to thank Rebecca Calderon, Richard Gersberg, Mark Gold, Charles McGee, Alan Brookhart and John Witte for their advice in study design, data analysis and data interpretation. We thank our funding agency contract managers (from the City of San Diego and the San Diego Regional Water Quality Control Board), Christina Arias, Leslie Dobalian and Ruth Kolb.