Published in final edited form as:
Epidemiology. 2012 January ; 23(1): 95–106. doi:10.1097/EDE.0b013e31823b504c.

Fecal indicators in sand, sand contact, and risk of enteric illness among beachgoers

Christopher D. Heaney[1,2], **Elizabeth Sams**[2], **Alfred P. Dufour**[3], **Kristen P. Brenner**[3], **Richard A. Haugland**[3], **Eunice Chern**[3], **Steve Wing**[1], **Stephen Marshall**[1], **David C. Love**[4], **Marc Serre**[5], **Rachel Noble**[5], and **Timothy J. Wade**[2]

[1]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7435, USA

[2]Epidemiology Branch, Environmental Public Health Division, National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, 27711 North Carolina, USA

[3]National Exposure Research Laboratory, United States Environmental Protection Agency, Cincinnati, Ohio, USA

[4]Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, Maryland, 21205 USA

[5]Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7431, USA

Abstract

Background—Beach sand can harbor fecal indicator organisms and pathogens, but enteric illness risk associated with sand contact remains unclear.

Methods—In 2007, visitors at two recreational marine beaches were asked on the day of their visit about sand contact. Ten to 12 days later, participants answered questions about health symptoms since the visit. F^+ coliphage, *Enterococcus*, *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp. in wet sand were measured using culture and molecular methods.

Results—We analyzed 144 wet sand samples and completed 4,999 interviews. Adjusted odds ratios (aORs) were computed, comparing those in the highest tertile of fecal indicator exposure with those who reported no sand contact. Among those digging in sand compared with those not digging in sand, a molecular measure of *Enterococcus* spp. (calibrator cell equivalents/g) in sand was positively associated with gastrointestinal (GI) illness (aOR = 2.0 [95% confidence interval (CI) = 1.2–3.2]) and diarrhea (2.4 [1.4–4.2]). Among those buried in sand, point estimates were greater for GI illness (3.3 [1.3–7.9]) and diarrhea (4.9 [1.8–13]). Positive associations were also observed for culture-based *Enterococcus* (colony-forming units/g) with GI illness (aOR digging = 1.7 [1.1–2.7]) and diarrhea (2.1 [1.3–3.4]). Associations were not found among non-swimmers with sand exposure.

Conclusions—We observed a positive relationship between sand contact activities and enteric illness as a function of concentrations of fecal microbial pollution in beach sand.

High densities of *E. coli*, *Enterococcus*, and other microbial indicators of fecal pollution are present in beach sand, sometimes in higher concentrations than in nearby bathing waters.[1–6]

Corresponding author: Christopher D. Heaney, Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD, 21205, USA, Phone: (443) 287-4989, Fax: (410) 955-0617, cheaney@jhsph.edu.

Halliday and Gast[7] reviewed studies that measured densities of *E. coli* and *Enterococcus* — fecal indicator bacteria used to indicate the degree of fecal contamination at recreational beaches across the United States — and found that fecal indicator bacteria density in sand was two to 38 times higher than in water. Fecal indicator bacteria such as *E. coli* and *Enterococcus* indicate the potential presence of pathogenic microorganisms. Although the density of *E. coli* and *Enterococcus* in water has been shown to predict gastrointestinal (GI) illness among swimmers,[8] the illness risks associated with fecal pollution in beach sand remains unclear. Whitman et al.[9] demonstrated potential for hand-to-mouth transfer and exposure to *E. coli* and the fecal indicator virus F^+ coliphage (MS2) among persons in contact with beach sand at a Chicago, IL beach. In addition to fecal indicator organisms, enteric pathogens have been detected in beach sand.[4,10–14] Factors influencing the level of fecal contamination of beach sands are numerous; these may include publicly owned treatment works discharges in close proximity to beaches,[15,16] non-point sources such as agricultural and urban runoff, warm-blooded domestic and wild animals, bathers,[3,4,17] and potential re-growth of autochthonous *E. coli* and *Enterococcus* in sand.[18,19]

The occurrence of fecal indicator organisms in sand has led to questions about whether beach sand can transmit pathogens associated with fecal contamination — a matter of increasing concern to beach managers, public health officials, and beachgoers. Despite knowledge of the presence of fecal indicator organisms and pathogens in beach sand, dose-response relationships between fecal indicator densities in sand and specific illnesses have not been well-characterized. Because the beach-going public may spend more time on the beach than in the water. and because young children typically spend time at the water's edge playing in sand, it is important to understand the relation of fecal indicator densities in sand with the risk of enteric illness.

Using data from beachgoers participating in the 2007 trials of the National Epidemiological and Environmental Assessment of Recreational water study,[20–22] we explored whether increased daily average estimate of fecal indicator organism (F^+ coliphage, *Enterococcus*, *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp.) in wet sand were associated with an increased risk of enteric illness among beachgoers engaged in sand contact activities.

METHODS

Study Design/Participant Sampling

The National Epidemiological and Environmental Assessment of Recreational water study evaluated microbial water quality and followed cohorts of visitors to freshwater and marine beaches in the US. The data collection methods have been described previously.[20–22] In brief, we interviewed beachgoers as they arrived and as they were leaving the beach regarding their contact with beach sand, swimming behaviors, and other beach activities. Ten to 12 days later, one adult in the household was interviewed by telephone about symptoms experienced by participating household members. Because of the acute nature and short duration of illnesses considered during this study, re-enrollment in the study was allowed 28 days after a previous enrollment.

Beach Descriptions

Two recreational marine beach sites with a nearby publicly owned treatment-works outfall were chosen for the 2007 studies. Fairhope Municipal Park Beach is located on Mobile Bay in Fairhope, Alabama, and Goddard Memorial State Park Beach is located on Greenwich Bay near Warwick, Rhode Island. These beaches had publicly owned treatment-works discharges within 1.5 miles (2.4 km) of the beach location.

Beach Sand Collection and Analysis

Wet beach sand samples were collected at 8:00 AM along with water samples each day of the study. Sand samples were collected using a 2.25-inch diameter stainless steel soil auger at a distance of 1 meter perpendicular to the lowest point of the water level (when the waves receded to their lowest point on the shoreline) at the same three sampling points where water samples were collected on each beach. No dry sand samples were collected. Sampling points were located at least 60 m apart to encompass the length of the beach. Beach sand samples were tested for fecal indicator bacteria *Enterococcus*, *Bacteroidales*, fecal *Bacteroides* (which targets the most common human *Bacteroides* spp.), and *Clostridium* spp. using a quantitative polymerase chain reaction (qPCR) calibrator cell equivalent method.[23–26] Culture-based tests of *Enterococcus* were performed by local laboratories within 6 hours of collection following EPA Method 1600[27] with some modifications (eAppendix 1, http://links.lww.com). Samples for the F^+ coliphage analysis were sent on ice at 4oC by overnight express and processed by a modification[28] of EPA Method 1601[29] to accommodate the analysis of sand instead of water samples (eAppendix 1, http://links.lww.com). Fecal indicator-organism concentrations are reported as qPCR calibrator cell equivalents per gram of dry weight sand for *Enterococcus*, *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp. Calibrator cell equivalents were calculated according to the comparative delta-delta cycle threshold method reported by Wade et al.[20] *Enterococcus* measured by EPA Method 1600[27] are reported in colony-forming units per gram of dry weight sand, and F^+ coliphage as a most probable number per gram of dry weight sand following a modification[28] of Method 1601.[29] Results below detection and potential inhibition for qPCR-based data were handled as described previously.[20]

Definition of Sand Contact

Upon leaving the beach, participants reported whether they had dug in the sand or built sand castles (digging in the sand), or had their body buried in the sand during their beach visit. Participants also reported whether they got sand in their mouth, ate or drank after playing in the sand, and washed their hands before eating or drinking after playing in sand. Participants were asked to report whether they had contact with wet or dry sand. In addition to sand exposures on the day of enrollment, participants reported other beach activities such as any water contact (swimming), or eating raw meat, runny eggs, or shellfish in the 3 days prior to enrollment.

Health Assessment

Ten to twelve days following the beach visit, participants were contacted by phone and asked if they had experienced any physical symptoms of enteric illness since the day of enrollment. The GI illness definition followed previously published research on waterborne illness[20,30] and was considered as any of the following: diarrhea (three or more loose stools in a 24-hour period); vomiting; nausea and stomach ache; and nausea or stomach ache plus interference with regular activities (missed time from work or school, or missed regular activities as a result of the illness). Diarrhea was also considered as a separate outcome. In addition, participants were asked about activities since the enrollment-day interview, including: number of times they went to the same beach, went swimming at another beach, went swimming in a pool, or ate raw or under-cooked foods (e.g., red meat, fish, shellfish, eggs).

Statistical Analysis

Participants with complete data for the exposure (sand contact), outcome (enteric illness), and potential confounding covariates (age, sex, race/ethnicity, swimming status, and beach) were included in analyses. Participants with prevalent enteric illness at enrollment were excluded from follow-up analyses. Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for enteric illness (GI illness; diarrhea) and associations with beach sand contact activities and densities of fecal indicators in beach sand.

Measurements of fecal indicator organism densities in beach sand were $\log_{10}$ –transformed. Fecal indicator organism samples below the lower detection limit were assigned a value of one-half the lower detection limit and subsequently $\log_{10}$ – transformed. We analyzed the daily average of the three 8:00 AM sand samples collected on each beach as a reflection of sand quality each day of the study.

Because a large proportion of F^+ coliphage data were below the detection limit, we considered only categorical classifications involving simple presence/absence and above versus below the median concentration (most probable number/g). For fecal indicator bacteria, we evaluated categorical classifications of above versus below the median, as well as tertiles (colony-forming units/g; calibrator cell equivalents/g). Categorical models involved comparisons of those in contact with sand on days when a fecal indicator was present at a specific average density (e.g., highest tertile) with those who were not in contact with sand (did not dig; were not buried in sand). For these models, the aOR can be interpreted as the risk (odds) of enteric illness (GI illness; diarrhea) among those who dug in the sand on days when average sand quality was at a specific level (e.g., highest tertile) divided by the risk of enteric illness among those who did not have contact with sand. Tests of linear trend were completed by the ranks of each categorical fecal indicator exposure variable in a logistic regression model as a linear term.

To evaluate the impact of multiple fecal indicators in sand, we created an index variable defined as the number of fecal indicators present in sand at each of the three sample locations. The potential range of this fecal indicator index variable was from 0 to 18 and was examined in tertiles. This variable was created as an alternative method to describe the burden of fecal contamination in the sand.

Robust variance estimates were used to account for the non-independence of observations within household.[31,32] We considered covariates strongly associated with beach sand contact and illness, or those regarded by investigators to be potential confounding factors, for inclusion in regression models. Information on other covariates was collected for the larger study (e.g., contact with animals, contact with other persons with diarrhea, number of other visits to the beach, asthma, chronic GI illnesses, eating any food or drink while at the beach, eating raw or undercooked meats, shellfish, and fish). If there was a substantial difference between fully adjusted and reduced model results, we used a backwards elimination approach. For each analysis, the set of covariates was reduced through a change-in-estimate procedure.[33] Adjusted odds ratio (full [aOR_{full}] and reduced [$aOR_{reduced}$]) values were compared by using the formula: $\ln|aOR_{full}/aOR_{reduced}| \times 100$. A criterion of 5% change was used; results from fully adjusted models are presented if the reduced model resulted in a change in estimate of greater than 5%. At a minimum, age, sex, race/ethnicity, swimming (defined as any contact with water which includes wading, body immersion, head immersion, and swallowing water), and beach were included in all models. Data management was completed using SAS version 9 (SAS Institute Inc., Cary, NC, USA), and statistical analyses were completed using Stata version 11 (StataCorp LP, College Station, TX, USA).

RESULTS

A total of 7,041 beachgoers were offered enrollment. Of these, 1,280 (18%) declined to participate. Of those who agreed to participate, 4,999 (87%) completed the telephone interview 10 to 12 days after visiting the beach and were eligible for analysis. Of these interviews, 4,948 (99%) respondents provided complete information on age, sex, race/ethnicity, contact with beach sand, and any swimming.

Respondents at the two beaches differed by age, race/ethnicity (defined as white/non-white), miles traveled to the beach, and proportion of persons who reported contact with sand (Table 1). Overall, respondents were 64% white and 57% female, with a median age of 30 years. Factors associated with sand contact have been described previously.[34] Similar to previous observations,[34] those who dug in the sand were younger than those who did not (median age 10 years and 36 years, respectively) but at baseline similar proportions of both groups reported vomiting and other GI symptoms (Table 1). Digging in the sand was strongly associated with swimming, as 81% of swimmers reported digging in the sand compared with only 19% of non-swimmers. This large difference in the proportion who swam was also seen for those buried and not buried in the sand (Table 1). Participants at Fairhope Beach were more likely to have reported digging in the sand (38%; 766/2015) and being buried in the sand (5%) (Table 1).

The frequency of detection and densities of fecal indicators in sand at Fairhope Beach and Goddard Beach are summarized in eAppendix 2 (http://links.lww.com). The detection of fecal indicators in sand ranged from 100% for qPCR-based *Clostridium* spp. to 87% for qPCR-based *Enterococcus*, 72% for culture-based *Enterococcus*, 68% for qPCR-based fecal *Bacteroides* spp., 53% for qPCR-based *Bacteroidales*, and 17% for culture-based F^+ coliphage (eAppendix 2). We did not observe spatial variability in fecal indicator measures in sand.

Relationships Among Sand Contact, Measures of Fecal Indicators in Sand, and Enteric Illness

The incidence of GI illness and diarrhea was 6.3% and 4.2%, respectively, during the 10–12-day follow-up period. GI illness and diarrhea incidence was highest among children younger than 5 years (9.5% and 5.2%, respectively) and lowest among those aged 55 and older (5.5% and 4.3%, respectively).

Because there were few days when F^+ coliphage was present in sand (17% of samples positive), we examined only presence versus absence and above versus below the median value of samples with results above the detection limit (Table 2). There was no increase in risk of GI illness or diarrhea among those digging in sand when F^+ coliphage was present (Table 2). Among those buried in sand, 14% (7/50) had diarrhea when F^+ coliphage was present compared with 4% (130/3,709) among those who were not buried in sand, and there was some evidence of an increasing trend of aORs across exposure categories (Table 2). However, due to small numbers of cases, overall results were not suggestive of a consistent association of F^+ coliphage with the sand exposures considered and GI illness and diarrhea (Table 2).

Enterococcus measured by Method 1600 (colony-forming units/g) showed positive associations with GI illness and diarrhea among those digging in the sand, and associations among those buried in the sand were stronger. Compared with people who did not dig in sand, the aORs of illness among people who reported digging in sand with *Enterococcus* levels in the highest tertile were 1.74 (95% CI = 1.11–2.72) for GI illness and 2.07 (95% CI = 1.27–3.38) for diarrhea (Table 3). Similar associations (but less precise) were observed for

GI illness and diarrhea among people buried in sand (Table 3). There was a trend of increasing risk for *Enterococcus* colony-forming units/g in sand (assessed as above vs. below the median or in tertiles) with enteric illnesses among both sand-exposure groups; however, the increase in aORs was not monotonic (Table 3).

Enterococcus measured in sand by a rapid molecular method (calibrator cell equivalents/g) was also associated with GI illness and diarrhea (Table 4). The aORs of illness among people who reported digging in sand with *Enterococcus* in the highest tertile were 1.98 (1.23–3.19) for GI illness and 2.44 (1.41 – 4.22) for diarrhea compared with people who did not dig in sand (Table 4). The aORs of GI illness and diarrhea among people buried in sand with the highest tertile of *Enterococcus* were 3.25 (1.33–7.92) and 4.90 (1.79 –13.4), respectively, compared with people who reported not being buried in sand (Table 4). The lower precision of aORs for being buried in the sand reflect smaller numbers of participants with that exposure. There was a trend of increasing *Enterococcus* calibrator cell equivalents/g in sand (above vs. below the median and tertiles) with both enteric illnesses among both sand exposure groups (Table 4).

Positive associations were observed between *Bacteroidales* with GI illness and diarrhea for each of the sand exposures (Table 5). Positive associations were observed between *Bacteroidales* and diarrhea for the above versus below the median for both digging in the sand (1.86 [1.14–3.05]) and being buried in sand (3.44 [1.31–9.04]). Positive associations of *Bacteroidales* tertiles with diarrhea were also observed for both sand contact groups, although numbers of events in some *Bacteroidales* tertiles were small (Table 5). There was a trend of increasing *Bacteroidales* calibrator cell equivalents/g in sand (above vs. below the median and tertiles) with diarrhea, among both sand-exposure groups (Table 5).

We observed less consistent relationships of fecal *Bacteroides* with illness among both sand-contact groups (Table 6). With one exception, all comparisons of fecal *Bacteroides* in sand at densities above the median value showed smaller aORs than for densities below the median value (Table 6). Adjusted ORs for fecal *Bacteroides* tended to be higher in the middle than in the highest tertiles (Table 6).

We also observed inconsistent relationships of *Clostridium* spp. estimates (calibrator cell equivalents/g) in sand with GI illness and diarrhea among both sand-contact groups (Table 7). Overall, categorical comparisons of *Clostridium* spp. density (median and tertiles) showed smaller point estimates at the higher *Clostridium* spp. density categories compared with lower density categories (Table 7).

A composite index variable suggested that a larger number of fecal indicators in sand was positively associated with GI illness and diarrhea. There was some evidence of an increasing trend of aORs across fecal-index tertiles, particularly for digging in sand and diarrhea (Table 8).

The fecal indicator-enteric illness associations were also present among the subset of people who reported getting sand in their mouth. Among this group, 16% (14/90) had GI illness symptoms when *Enterococcus* (measured as calibrator cell equivalents/g) were in the highest tertile compared with 6% (242/4074) among those who did not get sand in their mouth (data not shown); the aOR was 2.53 (95% CI = 1.27–5.04).

DISCUSSION

Estimates of fecal contamination in wet sand measured by molecular methods (*Enterococcus* and *Bacteroidales*) and culture-based methods (*Enterococcus*) were positively associated with enteric illness among those digging in sand and being buried in sand at two recreational beach sites. This association was observed for a definition of enteric illness based on composite symptoms (GI illness) and a more narrow definition (diarrhea alone). Although there was some evidence of positive associations between sand-contact activities and enteric illness for culture-based F^+ coliphage and molecular fecal *Bacteroides* and *Clostridium* spp. estimates, there was inconsistency across the exposure classifications considered (above vs. below the median and tertiles). Our ability to make conclusions for the F^+ coliphage measure was limited because of its low frequency of detection in beach sand samples (17%).

This is one of the first studies to show an association between beach sand contact and enteric illness as a function of microbial sand quality. One previous study observed a relationship between sand contact and GI illness, but exposure was assessed as a function of time spent in contact with wet sand, not as a function of an objective measure of microbial sand quality.[35] Two other studies of beach sand exposure and health effects (which included objective measures of beach sand fecal indicator densities) did not show consistent relationships between fecal contamination in beach sand, sand-contact activities, and illness (including GI illness).[36,37] Another study,[34] which lacked objective measures of fecal contamination of sand, observed an increased risk of enteric illness associated with beach sand contact activities (digging in sand and being buried in sand).

Investigators have observed many-fold higher concentrations of fecal microbial indicators (including *E. coli* and *Enterococcus*) in beach sand compared with nearby bathing water.[36,37] Halliday and Gast[7] recently completed a comprehensive review of studies of fecal microbial pollution of beach sand and observed that densities of fecal indicator bacteria in wet sand were up to thirty-eight times higher than in nearby bathing waters. It has been hypothesized that sand could serve as a source of fecal contamination for bathing water, especially the surf zone along the shoreline.[3,4,38] Others have demonstrated that sand can serve as both a source and a sink of fecal microbial contamination.[14] Debate continues about the applicability of fecal indicator bacteria (e.g., *E. coli* and *Enterococcus*) as a measure of beach water quality and fecal contamination of beach sand.[4,39,40] Several studies have examined potential for re-growth of *E. coli* and *Enterococcus* in sand.[18,19] Beach sand may serve as a source of autochthonous fecal indicator bacteria (*E. coli; Enterococcus*) to nearby bathing water in the absence of inputs from point sources of fecal contamination (and associated pathogens). Beach sand has been implicated as contributing unnecessarily to beach advisories based on results of water-quality fecal indicator bacteria tests.[4,14] EPA guidelines for monitoring fresh and marine recreational waters are based on *E. coli* and *Enterococcus*,[41,42] but no guidelines exist for fecal contamination of sand.

Our results suggest that *Enterococcus* density in sand increases the rate of GI illness and diarrhea among beachgoers who have contact with sand. The positive association of a qPCR-based measure of *Enterococcus* in sand with enteric illness was more consistent than that observed for a traditional culture-based measure of *Enterococcus* and several alternative measures of sand fecal pollution (culture-based F^+ coliphage, and qPCR-based *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp.). These alternative measures have been considered by some to be more specific indicators of human sewage sources of fecal contamination.[43] Previous research has demonstrated a consistent positive association between *Enterococcus* calibrator cell equivalents and swimming-associated illness among both adults and children.[21,22] Culturable fecal indicator bacteria cells (e.g., total and fecal coliforms, *E. coli*, and *Enterococcus*) are considered a better measure of viable bacteria associated with fecal pollution than qPCR-based measures, which reflect the genetic material of bacterial cells and may have differential environmental fates.[44–48]

The two beaches were located near publicly owned treatment-works sewage outfalls. It is possible that fecal contamination from municipal sewage reached recreational beaches through tidal flow, wave action, on-shore wind direction, or currents. However, some evidence suggests that diffuse sources (including coastal birds, other animal populations, bather density, and run-off) may contribute most of the fecal contamination to beach sand.[49,50] It is unclear if the observed relationships of *Enterococcus* with enteric illness can be generalized to beaches not influenced by municipal sewage outfalls (i.e., non-point source pollution beaches), to freshwater beaches, or to tropical beaches where the population dynamics of fecal indicators in sand may be different.

Swimming was strongly associated with sand contact; 81% of swimmers vs. 19% of non-swimmers reported digging in sand and 89% of swimmers vs. 11% of non-swimmers reported being buried in sand. As a result, our study has little power to evaluate associations between sand contact and illness among non-swimmers because there were very few sand-exposed cases among non-swimmers. For example, among non-swimmers who dug in sand with *Enterococcus* in the highest tertile, there were no exposed diarrhea cases and one exposed GI illness case. Therefore, although we adjusted for swimming in all analyses of sand contact, it is possible that associations between sand contact and illness reflect exposure to contaminated water as well as sand. In our previous study,[51] which included more beaches but lacked measures of fecal contamination of sand, digging in sand was associated with GI illness (adjusted incidence proportion ratio = 1.26 [95% CI = 1.03–1.54]) and diarrhea (1.26 [0.98–1.62]) among non-swimmers, suggesting that sand may be an additional route of exposure to pathogens.

Beachgoers’ contact with sand was assessed by questionnaire and included a question asking if they had contact with wet or dry sand. However, only wet sand was collected and analyzed for microbial measurements. We used microbial quality in wet sand as a proxy for quality of all sand. To examine the potential influence of misclassification of exposure by wet versus dry sand contact, we examined associations restricted to participants with wet sand contact only. The results were similar to those among all participants. For example, among tertiles of the qPCR-based *Enterococcus* measure, the aORs of associations with GI illness among those digging in wet sand only were 1.02 (95% CI = 0.58–1.80), 1.31 (0.71–2.43), and 2.48 (1.46–4.21), respectively.

Overall, associations were stronger among those buried in sand (which represents a more intense form of sand exposure) than among those digging in sand. Defining sand exposure as any sand contact, including either digging in the sand or buried in the sand, did not alter our conclusions (data not shown).

Research on health effects among beachgoers has focused largely on swimming-associated illness and microbial water quality. We show a relation of sand contact activities with GI illness and diarrhea as a function of objective measures of fecal pollution of 5beach sand. Limitations of the study include a small sample size for investigation of associations among subgroups (children, non-swimmers), use of wet sand as a proxy for exposure to dry sand contact activities, lack of tests of specific enteric pathogens in beach sand, lack of analyses taking account of water quality, and lack of objective measures of enteric illness self-reports by microbial or immunologic tests of biospecimens. Laboratory confirmation of infection with pathogens via tests of saliva, blood, or stool could improve the classification of incident symptoms.

Further investigation of sand exposure and its association with enteric and non-enteric illness appears warranted based on these results. It is unknown whether the relation of *Enterococcus* in sand with GI illness and diarrhea can be extended to non-enteric illnesses (e.g., skin rash, upper respiratory illness, eye irritation, earache, infected cuts/wounds). Further studies at a broader geographic range of beach sites, including non-point source runoff, freshwater, and tropical beaches, may advance understanding of sand exposures associated with illness risk and the association of densities of fecal-indicator organisms in beach sand with illness risk among beachgoers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: US Environmental Protection Agency Cooperative Training in Environmental Sciences Research EPA CR83323601; National Institute of Environmental Health Sciences Environmental Epidemiology Training Grant (T32 ES007018), UNC Department of Biostatistics; Kellogg Health Scholars Program–Community Track, UNC Center for Health Promotion and Disease Prevention. This document has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Environmental Protection Agency.

We thank the many individuals and organizations who helped with data collection on the NEEAR water study project, including Rebecca Calderon, Ann Williams, Joel Hansel, Holly Wirick, Ed Hudgens, Mike Ray, Danelle Lobdelle, Scott Rhoney, Beth Leamond, Lucas Neas, Stephanie Friedman, Westat Corp., and the beach interviewers. We thank Mark Sobsey for the use of his laboratory to perform coliphage analyses. We thank Jyotsna Jagai at EPA for reviewing the manuscript.

References

1. Kinzelman J, McLellan SL, Daniels AD, Cashin S, Singh A, Gradus S, Bagley R. Non-point source pollution: determination of replication versus persistence of Escherichia coli in surface water and sediments with correlation of levels to readily measurable environmental parameters. J Water Health. 2004; 2(2):103–14. [PubMed: 15387134]
2. Skalbeck JD, Kinzelman JL, Mayer GC. Fecal indicator organism density in beach sands: Impact of sediment grain size, uniformity, and hydrologic factors on surface water loading. Journal of Great Lakes Research. 2010; 36(4)
3. Wheeler Alm E, Burke J, Spain A. Fecal indicator bacteria are abundant in wet sand at freshwater beaches. Water Res. 2003; 37(16):3978–3982. [PubMed: 12909116]
4. Whitman RL, Nevers MB. Foreshore sand as a source of Escherichia coli in nearshore water of a Lake Michigan beach. Appl Environ Microbiol. 2003; 69(9):5555–62. [PubMed: 12957945]
5. Bonilla TD, Nowosielski K, Cuvelier M, Hartz A, Green M, Esiobu N, McCorquodale DS, Fleisher JM, Rogerson A. Prevalence and distribution of fecal indicator organisms in South Florida beach sand and preliminary assessment of health effects associated with beach sand exposure. Mar Pollut Bull. 2007; 54(9):1472–82. [PubMed: 17610908]
6. Obiri-Danso K, Jones K. Distribution and seasonality of microbial indicators and thermophilic campylobacters in two freshwater bathing sites on the River Lune in northwest England. J Appl Microbiol. 1999; 87(6):822–832. [PubMed: 10664907]
7. Halliday E, Gast RJ. Bacteria in Beach Sands: An Emerging Challenge in Protecting Coastal Water Quality and Bather Health. Environ Sci Technol. 2010
8. Wade TJ, Pai N, Eisenberg JN, Colford JM Jr. Do U.S. Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. Environ Health Perspect. 2003; 111(8):1102–9. [PubMed: 12826481]
9. Whitman RL, Przybyla-Kelly K, Shively DA, Nevers MB, Byappanahalli MN. Hand-mouth transfer and potential for exposure to E. coli and F(+) coliphage in beach sand, Chicago, Illinois. J Water Health. 2009; 7(4):623–9. [PubMed: 19590129]
10. Davies CM, Long JA, Donald M, Ashbolt NJ. Survival of fecal microorganisms in marine and freshwater sediments. Appl Environ Microbiol. 1995; 61(5):1888–1896. [PubMed: 7646026]
11. Beversdorf LJ, Bornstein-Forst SM, McLellan SL. The potential for beach sand to serve as a reservoir for Escherichia coli and the physical influences on cell die-off. Journal of Applied Microbiology. 2006; 0(0)

12. Byappanahalli MN, Whitman RL, Shively DA, Sadowsky MJ, Ishii S. Population structure, persistence, and seasonality of autochthonous Escherichia coli in temperate, coastal forest soil from a Great Lakes watershed. Environ Microbiol. 2006; 8(3):504–13. [PubMed: 16478456]
13. Desmarais TR, Solo-Gabriele HM, Palmer CJ. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Appl Environ Microbiol. 2002; 68(3):1165–72. [PubMed: 11872464]
14. Ishii S, Hansen DL, Hicks RE, Sadowsky MJ. Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in Lake Superior. Environ. Sci Technology. 2007; 41(7):2203–2209.
15. Elmanama AA, Fahd MI, Afifi S, Abdallah S, Bahr S. Microbiological beach sand quality in Gaza Strip in comparison to seawater quality. Environ Res. 2005; 99(1):1–10. [PubMed: 16053922]
16. Ghinsberg RC, Bar Dov L, Rogol M, Sheinberg Y, Nitzan Y. Monitoring of selected bacteria and fungi in sand and sea water along the Tel Aviv coast. Microbios. 1994; 77(310):29–40. [PubMed: 8159125]
17. Whitman RL, Nevers MB, Byappanahalli MN. Examination of the Watershed-Wide Distribution of Escherichia coli along Southern Lake Michigan: an Integrated Approach. Appl Environ Microbiol. 2006; 72(11):7301–10. [PubMed: 16980417]
18. Feng F, Goto D, Yan T. Effects of autochthonous microbial community on the die-off of fecal indicators in tropical beach sand. FEMS Microbiol Ecol. 2010; 74(1):214–25. [PubMed: 20629750]
19. Yamahara KM, Walters SP, Boehm AB. Growth of enterococci in unaltered, unseeded beach sands subjected to tidal wetting. Appl Environ Microbiol. 2009; 75(6):1517–1524. [PubMed: 19151188]
20. Wade TJ, Sams E, Brenner KP, Haugland R, Chern E, Beach M, Wymer L, Rankin CC, Love D, Li Q, Noble R, Dufour AP. Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: A prospective cohort study. Environ Health. 2010; 9(1):66. [PubMed: 21040526]
21. Wade TJ, Calderon RL, Brenner KP, Sams E, Beach M, Haugland R, Wymer L, Dufour AP. High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. Epidemiology. 2008; 19(3):375–383. [PubMed: 18379427]
22. Wade TJ, Calderon RL, Sams E, Beach M, Brenner KP, Williams AH, Dufour AP. Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness. Environ Health Perspect. 2006; 114(1):24–8. [PubMed: 16393653]
23. Haugland RA, Siefring SC, Wymer LJ, Brenner KP, Dufour AP. Comparison of Enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Res. 2005; 39(4):559–568. [PubMed: 15707628]
24. Siefring S, Varma M, Atikovic E, Wymer L, Haugland RA. Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. J Water Health. 2008; 6(2):225–237. [PubMed: 18209285]
25. Converse RR, Blackwood AD, Kirs M, Griffith JF, Noble RT. Rapid QPCR-based assay for fecal Bacteroides spp. as a tool for assessing fecal contamination in recreational waters. Water Res. 2009; 43(19):4828–37. [PubMed: 19631958]
26. Chern EC, Brenner KP, Wymer L, Haugland RA. Comparision of fecal indicator bacteria densities in marine recreational waters by QPCR. Water Quality, Exposure and Health. 2009; 1:203–214.
27. EPA. Method 1600: Enterococci in water by membrane filtration using membrane-enterococcus indoxyl-beta-D-glucoside agar (mEI). In: Water, Oo, editor. Publication EPA 821-R-02-022. United States Environmental Protection Agency; Washington, DC: 2002.
28. Love DC, Sobsey MD. Simple and rapid F+ coliphage culture, latex agglutination, and typing assay to detect and source track fecal contamination. Appl Environ Microbiol. 2007; 73(13):4110–4118. [PubMed: 17483282]
29. Heaney CD, Sams E, Dufour AP, Brenner KP, Haugland R, Wymer L, Chern E, Love DC, Wing S, Serre M, Seed JR, Noble R, Wade T. Weather and environmental factors associated with F+ coliphages and fecal indicator bacteria in beach sand at two recreational marine beaches. Appl Environ Microbiol. Submitted, under review.

30. Colford JM Jr, Wade TJ, Schiff KC, Wright CC, Griffith JF, Sandhu SK, Burns S, Sobsey M, Lovelace G, Weisberg SB. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. Epidemiology. 2007; 18(1):27–35. [PubMed: 17149140]
31. Rogers WH. sg17: Regression standard errors in clustered samples. Stata Technical Bulletin. 1993; 13:5.
32. Royall RM. Model robust confidence intervals using maximum likelihood estimators. International Statistical Review. 1986; 54:6.
33. Rothman, K.; Greenland, S.; Lash, T. Modern Epidemiology. 3. Philadelphia: Lippincott Williams & Wilkins; 2008.
34. Heaney CD, Sams E, Wing S, Marshall S, Brenner K, Dufour AP, Wade TJ. Contact with beach sand among beachgoers and risk of illness. Am J Epidemiol. 2009; 170(2):164–72. [PubMed: 19541858]
35. Bonilla TD, Nowosielski K, Cuvelier M, Hartz A, Green M, Esiobu N, McCorquodale DS, Fleisher JM, Rogerson A. Prevalence and distribution of fecal indicator organisms in South Florida beach sand and preliminary assessment of health effects associated with beach sand exposure. Mar Pollut Bull. 2007
36. Marino FJ, Morinigo MA, Martinez-Manzanares E, Borrego JJ. Microbiological-epidemiological study of selected marine beaches in Malaga (Spain). Wat Sci Tech. 1995; 31(5–6):5.
37. Seyfried PL, Tobin RS, Brown NE, Ness PF. A prospective study of swimming-related illness. II. Morbidity and the microbiological quality of water. Am J Public Health. 1985; 75(9):1071–5. [PubMed: 4025657]
38. Solo-Gabriele HM, Wolfert MA, Desmarais TR, Palmer CJ. Sources of *Escherichia coli* in a coastal subtropical environment. Appl Environ Microbiol. 2000; 66(1):230–237. [PubMed: 10618229]
39. Oshiro R, Fujioka R. Sand, soil, and pigeon droppings: Sources of indicator bacteria in the waters of Hanauma Bay, Oahu, Hawaii. Wat Sci Tech. 1995; 31:5–6.
40. Yamahara KM, Layton BA, Santoro AE, Boehm AB. Beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. Environ Sci Technol. 2007; 41(13):4515–4521. [PubMed: 17695890]
41. EPA. Agency USEP. Ambient water quality criteria for bacteria. Washington DC: Office of Water; 1986.
42. EPA. Agency USEP. 40 CFR Part 131. Vol. RIN 2040-AE63. Washington DC: 2004. Water quality standards for coastal and Great Lakes recreation waters.
43. Whitman RL, Przybyla-Kelly K, Shively DA, Nevers MB, Byappanahalli MN. Hand-mouth transfer and potential for exposure to E. coli and F+ coliphage in beach sand, Chicago, Illinois. J Water Health. 2009; 7(4):623–9. [PubMed: 19590129]
44. Berg G, Dahling DR, Brown GA, Berman D. Validity of fecal coliforms, total coliforms, and fecal streptococci as indicators of viruses in chlorinated primary sewage effluents. Appl Environ Microbiol. 1978; 36(6):880–4. [PubMed: 104657]
45. Blatchley ER 3rd, Gong WL, Alleman JE, Rose JB, Huffman DE, Otaki M, Lisle JT. Effects of wastewater disinfection on waterborne bacteria and viruses. Water Environ Res. 2007; 79(1):81–92. [PubMed: 17290975]
46. Bonadonna L, Briancesco R, Cataldo C, Divizia M, Donia D, Pana A. Fate of bacterial indicators, viruses and protozoan parasites in a wastewater multi-component treatment system. New Microbiol. 2002; 25(4):413–20. [PubMed: 12437220]
47. Lucena F, Duran AE, Moron A, Calderon E, Campos C, Gantzer C, Skraber S, Jofre J. Reduction of bacterial indicators and bacteriophages infecting faecal bacteria in primary and secondary wastewater treatments. J Appl Microbiol. 2004; 97(5):1069–76. [PubMed: 15479424]
48. Payment P, Plante R, Cejka P. Removal of indicator bacteria, human enteric viruses, Giardia cysts, and Cryptosporidium oocysts at a large wastewater primary treatment facility. Can J Microbiol. 2001; 47(3):188–93. [PubMed: 11315109]
49. Noble MA, Xu JP, Robertson GL, Rosenfeld LK. Distribution and sources of surfzone bacteria at Huntington Beach before and after disinfection on an ocean outfall-- a frequency-domain analysis. Mar Environ Res. 2006; 61(5):494–510. [PubMed: 16644005]

50. Boehm AB, Weisberg SB. Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies. Environ Sci Technol. 2005; 39(15):5575–83. [PubMed: 16124289]

TABLE 1

Characteristics of Those Who Did or Did Not Dig in the Sand, and Those Who Were or Were Not Buried in the Sand.

	Digging in the sand		Body buried in the sand	
Characteristic	**No (n = 3586) No. (%)**	**Yes (n = 1362) No. (%)**	**No (n = 4730) No. (%)**	**Yes (n = 218) No. (%)**
Age (yrs)				
0–4	183 (5)	287 (21)	429 (9)	50 (23)
5–10	219 (6)	423 (31)	550 (12)	92 (42)
11–19	356 (10)	172 (13)	507 (11)	21 (10)
20–54	2186 (61)	435 (32)	2568 (54)	53 (24)
55+	642 (18)	36 (3)	676 (14)	2 (<1)
Sex				
Male	1505 (42)	603 (44)	2009 (42)	99 (45)
Female	2081 (58)	759 (56)	2721 (58)	119 (55)
Race/Ethnicity				
White	2315 (65)	870 (64)	3061 (65)	124 (57)
Black	468 (13)	222 (16)	654 (14)	36 (17)
Asian	60 (2)	24 (2)	82 (2)	2 (1)
American Indian	7 (<1)	5 (<1)	9 (<1)	3 (1)
Hispanic/Latino	636 (18)	186 (14)	778 (16)	44 (20)
Multiethnic/other	100 (3)	55 (4)	146 (4)	9 (4)
Conditions in the 3 d prior to the beach visit				
Vomiting	50 (1)	24 (2)	69 (1)	5 (2)
Diarrhea or lose bowels	102 (3)	33 (2)	131 (3)	4 (2)
History of chronic GI illness	124 (3)	21 (2)	145 (3)	0 (0)
Water contact status				
No	2154 (60)	257 (19)	2387 (50)	24 (11)
Yes	1432 (40)	1105 (81)	2343 (50)	194 (89)
Contact with animals 48 h prior to beach visit, or between beach visit and phone interview	2192 (61)	970 (71)	3011 (64)	151 (69)
Consumption of raw meat 48 h prior to beach visit or between beach visit and phone interview	508 (14)	113 (8)	607 (13)	14 (6)
Beach				
Fairhope Beach	1249 (35)	766 (56)	1905 (40)	110 (50)

Characteristic	Digging in the sand		Body buried in the sand	
	No (n = 3586) No. (%)	Yes (n = 1362) No. (%)	No (n = 4730) No. (%)	Yes (n = 218) No. (%)
Goddard Beach	2337 (65)	596 (44)	2825 (60)	108 (49)

TABLE 2

Relation of F^+ Coliphage in Sand with GI Illness and Diarrhea by Status of Sand Contact and by Categorical Classification.

	GI Illness		Diarrhea	
	No. (%)[a]	aOR (95% CI)[b]	No. (%)[a]	aOR (95% CI)[b]
Digging In Sand	(n= 3835)		(n = 3864)	
Not digging [d]	142 (5)	1.00	86 (3)	1.00
Digging				
F^+ coliphage				
absent	57 (9)	1.57 (1.04–2.39)	38 (6)	1.81 (1.12–2.93)
present	26 (8)	1.19 (0.69–2.06)	19 (6)	1.43 (0.75–2.71)
Test for trend[e]		*P*=0.17		*P*=0.07
≤median (0.014 MPN/g[f])	72 (9)	1.51 (1.04–2.20)	48 (6)	1.72 (1.12–2.65)
> median	11 (8)	1.03 (0.43–2.50)	9 (7)	1.43 (0.55–3.73)
Test for trend[e]		*P*=0.17		*P*=0.05
Buried In Sand	(n = 3838)		(n = 3867)	
Not buried [d]	208 (6)	1.00	130 (4)	1.00
Buried				
F^+ coliphage				
absent	9 (8)	1.39 (0.63–3.09)	6 (6)	1.51 (0.56–4.05)
present	8 (16)	2.29 (0.74–7.07)	7 (14)	3.32 (0.94–11.7)
Test for trend[e]		0.11		0.05
≤median (0.014 MPN/g[f])	14 (10)	1.52 (0.76–3.05)	10 (6)	1.78 (0.74–4.29)
> median (0.014 MPN/g[f])	3 (30)	4.20 (0.34, 51.1)	3 (30)	6.99 (0.60–81.3)
Test for trend[e]		*P*=0.12		*P*=0.06

[a] The numbers are participants reporting new symptoms, among those without baseline symptoms.

[b] Robust variance estimates clustering on household.

[c] The numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

[d] Reference category.

[e] *P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

[f] MPN/g indicates most probable number per gram of dry weight sand.

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

TABLE 3

Relation of *Enterococcus* (colony forming units/g) in Sand with GI Illness and Diarrhea by Status of Sand Contact and by Categorical Classification.

	GI Illness		Diarrhea	
	No. (%)[a]	aOR (95% CI)[b]	No. (%)[a]	aOR (95% CI)[b]
Digging In Sand	(n= 4672)		(n = 4706)	
Not digging[d]	182 (5)	1.00	115 (3)	1.00
Digging				
Enterococcus				
< median (13 CFU/g[e])	47 (7)	1.21 (0.82–1.77)	30 (4)	1.26 (0.80–1.99)
≥median (13 CFU/g[e])	65 (11)	1.65 (1.10–2.47)	51 (8)	2.06 (1.33–3.18)
Test for trend[f]		*P*=0.02		*P*=0.001
tertile 1 (≥0 – 5 CFU/g[e])	28 (7)	1.30 (0.81–2.10)	16 (4)	1.22 (0.68–2.19)
tertile 2 (5 – 27 CFU/g[e])	41 (9)	1.26 (0.79–2.02)	33 (7)	1.64 (0.98–2.74)
tertile 3 (>27 CFU/g[e])	43 (11)	1.74 (1.11–2.72)	32 (8)	2.07 (1.27–3.38)
Test for trend[f]		*P*=0.01		*P*=0.002
Buried In Sand	(n= 4675)		(n = 4709)	
Not buried [d]	274 (6)	1.00	181 (4)	1.00
Buried				
Enterococcus				
< median of 13 CFU/g[e]	9 (7)	0.95 (0.47–1.90)	6 (4)	0.98 (0.42–2.29)
≥median of 13 CFU/g[e]	11 (15)	2.18 (0.85–5.60)	9 (13)	2.61 (0.87–7.89)
Test for trend[f]		*P*=0.18		*P*=0.14
tertile 1: ≥0 – 5 CFU/g[e]	6 (8)	1.32 (0.54–3.22)	4 (5)	1.40 (0.49–4.06)
tertile 2: >5 – 27 CFU/g[e]	6 (8)	1.04 (0.33–3.33)	5 (7)	1.31 (0.34–4.99)
tertile 3: >27 CFU/g[e]	8 (14)	1.87 (0.68–5.16)	6 (10)	2.06 (0.58–7.32)
Test for trend[f]		*P*=0.18		*P*=0.23

[a] The numbers are participants reporting new symptoms, among those without baseline symptoms.

[b] Robust variance estimates clustering on household.

[c] The numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

[d] Reference category

[e] CFU/g indicates colony forming units per gram of dry weight sand.

[f] *P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

TABLE 4

Relation of *Enterococcus* (calibrator cell equivalents/g) in Sand with GI Illness and Diarrhea by Status of Sand Contact and by Categorical Classification.

	GI Illness		**Diarrhea**	
	No. (%)^{a}	**aOR (95% CI)**^{b}	**No. (%)**^{a}	**aOR (95% CI)**^{b}
Digging in sand	(n= 4331)		(n = 4364)	
Not digging ^{d}	174 (5)	1.00	110 (3)	1.00
Digging				
Enterococcus				
< median 156 CCE/g^{e}	47 (7)	1.12 (0.75–1.68)	32 (5)	1.27 (0.78–2.06)
≥median 156 CCE/g^{e}	54 (11)	1.67 (1.10–2.55)	39 (8)	1.96 (1.23–3.12)
Test for trend^{f}		*P*=0.02		*P*=0.005
tertile 1 ≥0 – 107 CCE/g^{e}	36 (9)	1.15 (0.70–1.86)	27 (7)	1.39 (0.80–2.40)
tertile 2 >107 – 324 CCE/g^{e}	28 (7)	1.12 (0.67–1.89)	17 (4)	1.13 (0.61–2.09)
tertile 3 >324 CEE/g^{d}	37 (11)	1.98 (1.23–3.19)	27 (8)	2.44 (1.41–4.22)
Test for trend^{f}		*P*=0.02		*P*=0.006
Buried in sand	(n= 4334)		(n = 4367)	
Not buried ^{d}	256 (6)	1.00	167 (4)	1.00
Buried				
Enterococcus				
< median 156 CCE/g^{e}	7 (7)	0.91 (0.41–2.02)	3 (3)	0.61 (0.19–1.97)
≥median 156 CCE/g^{e}	12 (15)	2.32 (0.97–5.52)	11 (14)	3.43 (1.32–8.90)
Test for trend^{f}		*P*=0.11		*P*=0.05
tertile 1 ≥0 – 107 CCE/g^{e}	4 (7)	0.74 (0.27–2.04)	2 (3)	0.57 (0.14–2.31)
tertile 2 >107 – 324 CCE/g^{e}	4 (6)	0.95 (0.33–2.79)	2 (3)	0.77 (0.18–3.22)
tertile 3 >324 CEE/g^{d}	11 (18)	3.25 (1.33–7.92)	10 (16)	4.90 (1.79–13.4)
Test for trend^{f}		*P*=0.06		*P*=0.03

[a] The numbers are participants reporting new symptoms, among those without baseline symptoms.

[b] Robust variance estimates clustering on household.

[c] The numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

[d] Reference category.

[e] CCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.

[f] *P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

TABLE 5

Relation of *Bacteroidales* in Sand with Enteric Illness by Status of Sand Contact and by Categorical Classification.

	GI Illness		Diarrhea	
	No. (%)[a]	**aOR (95% CI)[b]**	**No. (%)[a]**	**aOR (95% CI)[b]**
Digging In Sand	(n= 4331)		(n = 4364)	
Not digging [d]	174 (5)	1.00	110 (3)	1.00
Digging				
Bacteroidales				
< median 165 CCE/g[e]	49 (8)	1.23 (0.83–1.82)	32 (5)	1.32 (0.84–2.06)
≥median 165 CCE/g[e]	52 (10)	1.51 (0.98–2.33)	39 (7)	1.86 (1.14–3.05)
Test for trend[f]		0.06		0.01
tertile 1 ≥0 – 54 CCE/g[e]	39 (10)	1.38 (0.88–2.16)	27 (7)	1.53 (0.92–2.54)
tertile 2 >54 – 390 CCE/g[e]	27 (7)	1.21 (0.72–2.02)	19 (5)	1.45 (0.78–2.68)
tertile 3 >390 CEE/g[e]	35 (10)	1.48 (0.88–2.47)	25 (7)	1.73 (0.97–3.07)
Test for trend[f]		*P*=0.12		*P*=0.04
Buried In Sand	(n= 4334)		(n = 4367)	
Not buried [d]	256 (6)	1.00	167 (4)	1.00
Buried				
Bacteroidales				
< median 165 CCE/g[e]	6 (6)	0.77 (0.33–1.79)	3 (3)	0.61 (0.19–1.96)
≥median 165 CCE/g[e]	13 (17)	2.55 (1.10–5.90)	11 (14)	3.44 (1.31–9.04)
Test for trend[f]		*P*=0.09		*P*=0.05
tertile 1 ≥0 – 54 CCE/g[e]	2 (3)	0.37 (0.09–1.44)	1 (2)	0.30 (0.04–2.12)
tertile 2 >54 – 390 CCE/g[e]	9 (16)	2.63 (1.05–6.57)	6 (10)	2.72 (0.85–8.70)
tertile 3 >390 CEE/g[e]	8 (13)	1.94 (0.71–5.29)	7 (11)	2.75 (0.88–8.63)
Test for trend[f]		*P*=0.07		*P*=0.05

[a] The numbers are participants reporting new symptoms, among those without baseline symptoms.

[b] Robust variance estimates clustering on household.

[c] The numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

[d] Reference category.

[e] CCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.

[f] *P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

TABLE 6

Relation of Fecal *Bacteroides* in Sand with Enteric Illness by Status of Sand Contact and by Categorical Classification.

	GI Illness		Diarrhea	
	No. (%)[a]	**aOR (95% CI)[b]**	**No. (%)[a]**	**aOR (95% CI)[b]**
Digging in sand	(n= 4331)		(n = 4364)	
Not digging [d]	174 (5)	1.00	110 (3)	1.00
Digging				
fecal *Bacteroides*				
< median 563 CCE/g[e]	61 (9)	1.40 (0.96–2.06)	43 (7)	1.62 (1.04–2.51)
≥median 563 CCE/g[e]	40 (8)	1.29 (0.81–2.04)	28 (6)	1.49 (0.89–2.51)
Test for trend[f]		*P*=0.15		*P*=0.05
tertile 1: ≥0 – 89 CCE/g[e]	37 (8)	1.07 (0.67–1.69)	26 (6)	1.23 (0.74–2.03)
tertile 2: >89 – 1,088 CCE/g[e]	39 (12)	2.11 (1.33–3.36)	26 (8)	2.27 (1.33–3.90)
tertile 3: >1,088 CEE/g[e]	25 (7)	1.08 (0.63–1.84)	19 (6)	1.40 (0.74–2.63)
Test for trend[f]		*P*=0.09		*P*=0.03
Buried in sand	(n= 4334)		(n = 4367)	
Not buried [d]	256 (6)	1.00	167 (4)	1.00
< median 563 CCE/g[e]	12 (11)	1.55 (0.72–3.33)	7 (6)	1.40 (0.46–4.29)
≥median 563 CCE/g[e]	7 (10)	1.38 (0.47–4.04)	7 (10)	2.29 (0.78–6.76)
Test for trend[f]		*P*=0.30		*P*=0.12
tertile 1: ≥0 – 89 CCE/g[e]	5 (7)	0.90 (0.35–2.36)	1 (1)	0.27 (0.04–1.94)
tertile 2: >89 – 1,088 CCE/g[e]	7 (13)	2.12 (0.77–5.85)	6 (11)	2.96 (0.89–9.83)
tertile 3: >1,088 CEE/g[e]	7 (12)	1.71 (0.58–5.11)	7 (12)	2.88 (0.96–8.65)
Test for trend[f]		*P*=0.16		*P*=0.04

[a] The numbers are participants reporting new symptoms, among those without baseline symptoms.

[b] Robust variance estimates clustering on household.

[c] The numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

[d] Reference category.

[e] CCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.

[f] *P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

CCE indicates calibrator cell equivalents and are reported as per dry weight of sand.

TABLE 7

Relation of *Clostridium* spp. in Sand with GI Illness by Status of Sand Contact and by Categorical Classification.

	GI Illness		Diarrhea	
	No. (%)[a]	**aOR (95% CI)[b]**	**No. (%)[a]**	**aOR (95% CI)[b]**
Digging in sand	(n= 4672)		(n = 4706)	
Not digging [d]	182 (5)	1.00	115 (3)	1.00
Digging				
Clostridium spp.				
< median 2468 CCE/g[e]	63 (9)	1.46 (1.00–2.13)	53 (8)	1.98 (1.31–2.99)
≥median 2468 CCE/g[e]	49 (8)	1.36 (0.89–2.09)	28 (5)	1.25 (0.77–2.04)
Test for trend[f]		*P*=0.08		*P*=0.10
tertile 1: ≥0 – 1723 CCE/g[e]	23 (6)	1.04 (0.62–1.75)	18 (5)	1.35 (0.74–2.49)
tertile 2: >1723 – 3579 CCE/g[e]	50 (11)	1.62 (1.06–2.48)	40 (9)	2.02 (1.30–3.16)
tertile 3: >3579 CEE/g[e]	39 (9)	1.53 (0.96–2.44)	23 (5)	1.47 (0.84–2.55)
Test for trend[f]		*P*=0.02		*P*=0.01
Buried in sand	(n= 4675)		(n = 4709)	
Not buried [d]	274 (6)	1.00	181 (4)	1.00
< median 2468 CCE/g[e]	13 (12)	1.57 (0.69–3.56)	12 (11)	2.22 (0.92–5.38)
≥median 2468 CCE/g[e]	7 (7)	1.12 (0.50–2.49)	3 (3)	0.73 (0.22–2.38)
Test for trend[f]		*P*=0.40		*P*=0.50
tertile 1 ≥0 – 1723 CCE/g[e]	4 (6)	0.80 (0.22–2.87)	4 (6)	1.28 (0.35–4.70)
tertile 2 >1723 – 3579 CCE/g[e]	9 (17)	2.17 (0.78–6.01)	8 (15)	2.80 (0.90–8.70)
tertile 3 >3579 CEE/g[e]	7 (8)	1.30 (0.58–2.90)	3 (3)	0.84 (0.26–2.78)
Test for trend[f]		*P*=0.21		*P*=0.32

[a] The numbers are participants reporting new symptoms, among those without baseline symptoms.

[b] Robust variance estimates clustering on household.

[c] The numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

[d] Reference category.

[e] CCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.

[f] *P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

TABLE 8

Relationship Between a Presence-Absence Index of All Six Fecal Indicators in Sand and Enteric Illness by Sand Contact Type.

	GI Illness		Diarrhea	
	No. (%)[a]	aOR (95% CI)[b]	No. (%)[a]	aOR (95% CI)[b]
Digging in sand	(n= 4672)		(n = 4706)	
Not digging [d]	182 (5)	1.00	115 (3)	1.00
Digging				
fecal index				
tertile 1	38 (9)	1.32 (0.83–2.10)	27 (6)	1.48 (0.89–2.47)
tertile 2	41 (9)	1.59 (1.01–2.50)	28 (6)	1.76 (1.07–2.90)
tertile 3	33 (8)	1.33 (0.82–2.13)	26 (7)	1.69 (0.98–2.93)
Test for trend[e]		*P*=0.06		*P*=0.01
Buried in sand	(n= 4675)		(n = 4709)	
Not buried [d]	274 (6)	1.00	181 (4)	1.00
Buried				
fecal index				
tertile 1	4 (5)	0.59 (0.21–1.65)	2 (2)	0.44 (0.11–1.74)
tertile 2	7 (11)	1.89 (0.81–4.41)	5 (8)	2.14 (0.72–6.34)
tertile 3	9 (16)	2.16 (0.75–6.19)	8 (14)	2.92 (0.91–9.37)
Test for trend[e]		*P*=0.12		*P*=0.07

[a] The numbers are participants reporting new symptoms, among those without baseline symptoms.

[b] Robust variance estimates clustering on household.

[c] The numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

[d] Reference category.

[e] *P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.