

Quantitative Microbial Risk Assessment of Human Illness from Exposure to Marine Beach Sand

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ABSTRACT: Currently no U.S. federal guideline is available for assessing risk of illness from sand at recreational sites. The objectives of this study were to compute a reference level guideline for pathogens in beach sand and to compare these reference levels with measurements from a beach impacted by nonpoint sources of contamination. Reference levels were computed using quantitative microbial risk assessment (QMRA) coupled with Monte Carlo simulations. In order to reach an equivalent level of risk of illness as set by the U.S. EPA for marine water exposure (1.9×10^{-2}), levels would need to be at least about 10 oocysts/g (about 1 oocyst/g for a pica child) for *Cryptosporidium*, about 5 MPN/g (about 1 MPN/g for pica) for enterovirus, and less than 10^6 CFU/g for *S. aureus*. Pathogen levels measured in sand at a nonpoint source recreational beach were lower than the reference levels. More research is needed in evaluating risk from yeast and helminth exposures as well as in identifying acceptable levels of risk for skin infections associated with sand exposures.



INTRODUCTION

Regulatory agencies monitor recreational water based on fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*) and enterococci because epidemiologic studies have shown that concentrations of FIB in recreational water impacted by effluent from publically owned treatment works (POTW) (i.e., point sources of sanitary sewage) were correlated with recreational water illnesses (RWIs). The acceptable levels of risk of gastrointestinal illness from exposure to marine recreational water per visit is established in the U.S. at 1.9×10^{-2} (or 19 cases per 1000 swimmers) which then leads to the reference level of 35 CFU per 100 mL for enterococci. This differs from fresh water criteria which established an acceptable level of 33 CFU per 100 mL for enterococci and 126 for *E. coli* resulting in a risk of 8×10^{-3} .¹

Although rare, direct sewage spills onto beach sands can represent a concentrated source of pathogenic microbes to sand.² More common scenarios is contamination of beach sand from nonpoint sources even without impacts from POTW.^{3–5} Few studies link potential human health outcomes to the microbe levels found in sand.⁶ Whitman et al., 2009,⁷ who evaluated hand-mouth transfer of *E. coli* from beach sand within the freshwater Great Lakes region found that 11 individuals per thousand would likely develop gastrointestinal symptoms if all *E. coli* on the fingertip were ingested, which is close to the acceptable level of 8 per 1000 for impacts from recreational swimming at freshwater beaches. A recent large prospective cohort study showed that beachgoers, who were playing with sands by digging or being buried in the sand, had elevated risks

of RWIs, particularly gastrointestinal (GI) illness and diarrhea⁸ and illness was found to be associated with levels of enterococci.⁹ Although there are potential human health implications no guidelines have been established for FIB or pathogens levels in beach sands. So when a regulator is faced with a decision about the potential health impacts from beach sands, there is no benchmark available with which to decide whether or not the measured levels of microbes in the sand are cause for concern.

Studies that relate levels of microbial contaminants in sands in relation to acceptable levels of human health risks are few. Heaney et al.⁹ found an association between indicator microbe levels in sand and GI illness and diarrhea among beach goers who dig or bury themselves in sand. This study is very significant as it documents the need to further evaluate sand contact activities as a cause of illness at beach sites. The next step is to link the measured microbe levels in sand with risk. This link can be established through additional epidemiologic studies which are costly, difficult to conduct, and often show lack a clear relationship between FIB and GI illnesses. In the interim, quantitative microbial risk assessment (QMRA) may be a useful alternative to epidemiologic data to predict potential human health risks.^{6,10,11} Although QMRA has been developed for evaluating risks from swimming at recreational marine

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Table 1. Values, Assumptions and References for Corresponding Parameters Used in the Monte Carlo Simulation

| parameter | values used, assumptions, and references |
|-----------------------------|---|
| r | 4.202×10^{-3} for <i>Cryptosporidium parvum</i> . ¹⁰ dose–response parameters for Poliovirus I were substituted for enterovirus, with 9.102×10^{-3} for Poliovirus I. ¹⁰ 7.63×10^{-8} for <i>S. aureus</i> (number-days/cm ²) ^{(38, day 1 was used in this study).} |
| ED (hr) | Normal distribution. Mean (1.66) and standard deviation (0.247) ^(39 - time out of water) |
| SA (cm ²) | triangular: minimum (17), Likeliest (20), maximum (24)). twenty is the recommended value for child exposure assessment. ⁴⁰ seventeen and 24 correspond to 5th and 95th percentiles assuming that 20 was the median for children between 1 to <5 yrs old ⁴¹ |
| SS (g/cm ²) | for hands: log-normal distribution, geometric mean (4.9×10^{-4}) and geometric standard deviation (8.2×10^{-3}). For skin: uniform distribution for the whole body exposure, minimum (1.7×10^{-4}) to maximum (2.1×10^{-2}) based on hands, arms, legs, and feet. ^{41–44} |
| HM (hr ⁻¹) | normal distribution: Mean (8.4) and standard deviation (10.6) ^{41,45} |
| TE | triangular: minimum (0.14), Likeliest (0.16), Maximum (0.18). Based on average of thumb sucking, figure mounting, and palm licking ⁴² |
| IR (g/h) | Single value: 0.02 g/h for a child; 1 g/h for a pica child ⁴⁶ |
| ρ (g/cm ³) | ρ = bulk density = (grain density)·(1-Porosity). Uniform for the grain density: minimum (2.5) to maximum (2.8). ⁴⁷ normal for the porosity: mean (0.46) and standard deviation (0.06) ⁴⁸ |
| D (cm) | normal distribution: Mean (0.035) and standard deviation (0.005) ⁴⁸ |
| PL | uniform: <i>Cryptosporidium</i> (0.005 – 0.12 oocysts/g), Enterovirus (0.005 – 0.014 MPN/g), <i>S. aureus</i> (0.2 – 42 CFU/g). ¹⁸ used to estimate risk from case study beach site. Substitute PL for RPL into the equations listed in the main text. |

beach sites,^{12,13} it has not yet been used to evaluate microbial risks from beach sands. This study aims to fill this information gap by assessing risks from beach sands. The objective of this study was to compute a reference level guideline for pathogens via marine beach sand exposures and to compare these reference levels with pathogen levels measured at a beach impacted by nonpoint sources of contamination. Specifically the QMRA was coupled with Monte Carlo simulations to provide a range of uncertainty in acute infectious illness risks to human health, in particular to children, from beach sands.

MATERIALS AND METHODS

This study followed a QMRA framework, which consists of four steps including hazard identification, dose–response, exposure assessment, and risk characterization. For this current paper we utilized this QMRA approach to compute reference pathogen level (RPL) guidelines which essentially required identifying which pathogens to evaluate (hazard identification) and then determining an acceptable risk of illness for those pathogens (risk characterization). From the acceptable risk level an acceptable dose is computed (dose–response), and ultimately the corresponding level of pathogen in the sands is computed given an exposure scenario (exposure assessment). Results were compared with actual measured pathogen levels at a nonpoint source-impacted sub/tropical marine beach located in Miami, FL (Hobie Cat Beach) and the computation procedure was reinvoked to compute risks at the case study beach. Uncertainty of the estimations was evaluated through Monte Carlo simulations (Oracle Crystal Ball, Redwood Shores, CA). One million simulations were run for each scenario/equation combination.

Hazard Identification. Microbes from all major groups (bacteria, protozoa, viruses, fungi/yeasts, and helminthes) can be transmitted through waterborne routes and, as such, are organisms that are of concern in the beach environment. QMRA requires knowledge of pathogen levels in order to evaluate risk. Studies are numerous that measure levels of fecal indicator bacteria in beach sands.^{14–16} However FIB are not the critical pathogens of concern; they indicate the possible presence of more infectious pathogens. Establishing ratios of FIB to pathogen levels is particularly cumbersome as these ratios depend upon the source and fate of both the indicators and pathogens in the environment.¹⁷ Moreover, little is known about the fate of FIB and pathogens in beach sands which would further compound difficulties in establishing ratios of

fecal indicators to pathogen levels. For these reasons, we chose to develop reference guidelines levels for direct pathogen measures as opposed to FIB. The pathogens we chose to evaluate are those that were found at the study beach¹⁸ for which dose–response relationships are available. Although studies have also measured pathogenic yeasts^{18–20} or helminthes^{18,21} in sands, no dose–response data is available for these classes of microbes and so we were unable to compute RPLs for these groups of microbes.

Risk Characterization. The reference risk level used for computation purposes is the acceptable level of risk from gastrointestinal illness for swimming in marine recreational waters as specified by the U.S. Environmental Protection Agency (1.9×10^{-2} illnesses per visit). This value was used for both the pathogens that cause gastrointestinal illness (*Cryptosporidium* and enterovirus) and those that cause skin ailments (*S. aureus*). Typically acceptable risk levels are related to the severity of the illness; however, these differences were not incorporated as part of the current paper. Moreover, another assumption was necessary as dose response relationships do not relate the probability of illness to exposure levels but rather relate it to the probability of infection. For the infection versus illness discrepancy we made a conservative assumption that the probability of illness, ($P_{ill} = 1.9 \times 10^{-2}$), is equivalent to the probability of infection, P_{inf} . In the case of evaluating potential illness risks to children a more conservative assumption would be appropriate as studies show that children have a higher illness risk than adults from beach sand exposures.⁸

Dose–Response. An exponential model (eq 1) was used to compute probability of infection (P_{inf}) from *Cryptosporidium*, enterovirus, and *S. aureus* exposures.

$$P_{inf} = 1 - \exp(-rN) \quad (1)$$

Where: P_{inf} = probability of infection, N = number of pathogens per exposure, and r = exponential dose–response relationship. The units of N are in absolute numbers for oral exposures and in numbers per unit surface area of skin for dermal exposures. Values of r used for modeling purposes are given in Table 1. Rearranging eq 1 results in:

$$N = \frac{\ln(1 - P_{ill})}{r} \quad (2)$$

Exposure Assessment. The computation of the reference pathogen level in sands, RPL, corresponding to the assumed risk level is based upon the following equation.

$$RPL = \frac{N}{S} \quad (3)$$

Where S corresponds to the mass of sands involved in the exposure. Combining eqs 2 and 3 results in

$$RPL = \frac{\ln(1 - P_{ill})}{r \cdot S} \quad (4)$$

Here the only unknown variable is S . Two different sets of S were computed, one for oral routes, S_{oral} , and another for dermal routes, S_{skin} . The values of S_{oral} , in units of sand mass, was estimated using two simulation models (eqs 5 and 6) that were dependent upon the exposure duration (ED) for a single visit to the beach. Equation 5 is based upon explicitly accounting for hand to mouth transfer of sand and includes the following factors: surface area of skin mouthed (SA), sand to skin adherence (SS), hand-to-mouth frequency (HM), and transfer efficiency from hand to mouth (TE). Equation 6 is based upon an overall sand ingestion rate (IR) which differs depending upon two distinct categories of children, children with normal eating behaviors and children with an eating disorder known as pica. Pica is characterized by persistent cravings to eat nonfood items. Pica is most common in people with developmental disabilities and in children between the ages of 2 and 3.²²

$$S_{oral-a} = SA \cdot SS \cdot HM \cdot TE \cdot ED \quad (5)$$

$$S_{oral-b} = IR \cdot ED \quad (6)$$

For dermal routes, the mass of sand exposed per unit area of skin (S_{skin}) was estimated using two other models (eqs 7 and 8). Equation 7 uses a sand to skin adherence factor (SS) which assumes that some sand falls from skin after exposure to sand. Equation 8 assumes a maximum limit on skin exposure by assuming that sand is evenly distributed on skin within a layer that is one grain size thick. This equation is thus based upon the bulk density (ρ) and particle size of sand (D). When computing dermal exposure (total number of pathogens), the S_{skin} values are multiplied by the surface area exposed. No difference in skin exposures were assumed for a pica versus a normal child.

$$S_{skin-a} = SS \quad (7)$$

$$S_{skin-b} = \rho \cdot D \quad (8)$$

Table 1 provides an explanation of each parameter used to estimate the S values along with their assumed values and distributions as incorporated into the Monte Carlo simulations.

Background for a Study Beach Used for Comparison.

Epidemiological studies along with beach water quality monitoring showed that people who were exposed to the water at this study site had higher risks of RWIs (e.g., GI, skin, and respiratory illnesses^{23,24} even though the site was without influence of a POTW.²⁵ Incidence of skin illnesses in the study site showed significant relationships with FIB, especially enterococci using the conventional membrane filter method.²⁶ Earlier studies showed that levels of enterococci in the water in this study site were associated with the beach sand quality^{25,27-29} and that the primary mechanisms of enterococci release from the sand to the water column was through tidal

action and rainfall-runoff.³⁰ Recently studies detected pathogens (e.g., *Cryptosporidium* spp.—0.12 oocysts per gram of dry sand, enterovirus—0.014 MPN/g, *Staphylococcus aureus*—42 CFU/g, *Candida tropicalis*—143 CFU/g, and nematode larvae—0.74 per gram of dry sand) in the beach sand at the study site.^{18,31} There has been no epidemiologic study evaluating levels of pathogens in the beach sand and RWIs up to date. However, it is well documented that these microorganisms could pose a threat to public health.³²⁻³⁷

RESULTS

For the pathogens evaluated that are transmitted via fecal oral routes (*Cryptosporidium* and enterovirus), the computed reference pathogen levels (RPLs) in the sand that would be necessary to result in 1.9×10^{-2} probabilistic illness risk differed depending upon the exposure simulation model and the percentile of simulated exposures below the threshold risk level. The RPLs (25th and 50th percentiles) for *Cryptosporidium* are on the order of 10–1000 oocysts/g for a normal child and on the order of single digits for a pica child. For enterovirus the computed range was from 5 to 500 MPN/g for a normal child and in the single digits for the pica child. The RPLs that may cause 50% of child beachgoers (without pica behavior) to develop GI illnesses from *Cryptosporidium* and enterovirus from the eq 5 was five times larger than based on eq 6 (Table 1). On the other hand, the RPLs for 25th percentiles based on the eq 5 were about $1/10$ of the 25th percentiles based on eq 6. Such inconsistent differences were created by the fact that eq 6 has only one variable (i.e., ED , note IR is constant) while eq 5 has five variables (i.e., SA , SS , HM , and TE in addition to ED). The RPLs for the pica child were more than fifty times lower (for the geometric mean) than normal child based on eqs 5 and 6, respectively.

A sensitivity analysis showed that for eq 5 the soil-skin adherence factor (SS) contributed the most (60%) to the variance followed by hand-to-mouth frequency factor (HM , 39%). The exposure duration (ED) contributed only 0.2% of variance, skin surface area (SA) only 0.1%, and transfer efficiency (TE) (<0.1%). For eq 6 the only variable was exposure duration and as a consequence it contributed 100% of the variance.

For the pathogen transmitted via skin (*S. aureus*), the computed RPL (25th and 50th percentiles) in the sands that would be necessary to result in 1.9×10^{-2} probabilistic illness risk was on the order of 10^6 to 10^7 CFU/g. A sensitivity analysis showed that the results from eq 8 were most sensitive to the diameter of the sand grains (accounted for 61% of the variance), followed by the porosity of the sand (36%) and grain density (3%).

Comparing the measured levels of gastro-intestinal pathogens to the RPLs computed in this study (last row of Table 2), the RPLs were orders of magnitude higher than the measured levels of pathogens, thereby suggesting that the risk from *Cryptosporidium*, enterovirus, and *S. aureus* are less than 1.9×10^{-2} . For the pathogen levels measured at the chosen study site, risks from *Cryptosporidium* and enterovirus were less than 10^{-5} for a normal child and less than 10^{-4} for a pica child. For the skin pathogen, the RPL was many orders of magnitude higher than the measured levels. The estimated risk of illness from *S. aureus* at the study beach was estimated at less than 10^{-7} (Table 3).

Table 2. Estimated Reference Pathogen Levels (RPLs) in the Sand That Would to Result in 1.9×10^{-2} Illness Risk to Children. All units are in per gram of dry sand^a

| sand exposure assumption ^b | <i>Cryptosporidium</i> (oocysts/g) | | enterovirus (MPN/g) | | <i>S. aureus</i> (CFU/g) | |
|---------------------------------------|------------------------------------|-------------------|---------------------|-------------------|--------------------------|-------------------|
| | 25th | 50th | 25th | 50th | 25th | 50th |
| eq 5 or 7 | 14 | 1.1×10^3 | 6 | 5.1×10^2 | 1.6×10^7 | 2.4×10^7 |
| eq 6 or 8 | 1.3×10^2 | 1.4×10^2 | 58 | 63 | 4.5×10^6 | 5.1×10^6 |
| eq 6 (pica) | 2 | 3 | 1 | 1 | NA ^b | NA |
| PL (study site) | 0.005–0.12 oocysts/g | | 0.005–0.014 MPN/g | | 0.2–42 CFU/g | |

^aThe RPLs for *Cryptosporidium* and enterovirus were based on sand exposure eqs 5 and 6 and *S. aureus* were based on the eqs 7 and 8. RPLs were based on 25th and 50th percentiles from the Monte Carlo simulations ($\times 1\,000\,000$). ^bNA, not applicable.

Table 3. Probability of Illness from Pathogens Detected at the Study Beach^a

| sand exposure assumption | <i>Cryptosporidium</i> | | enterovirus | | <i>S. aureus</i> | |
|--------------------------|------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| | 50th | 75th | 50th | 75th | 50th | 75th |
| eq 5 or 7 | 1.0×10^{-7} | 1.0×10^{-6} | 4.4×10^{-8} | 3.9×10^{-7} | 1.3×10^{-8} | 2.6×10^{-8} |
| eq 6 or 8 | 8.5×10^{-6} | 1.3×10^{-5} | 2.8×10^{-6} | 3.5×10^{-6} | 7.8×10^{-8} | 1.2×10^{-7} |
| eq 6 (pica) | 4.3×10^{-4} | 6.3×10^{-4} | 1.4×10^{-4} | 1.8×10^{-4} | NA ^b | NA |

^aIllness risks for *Cryptosporidium* and enterovirus were based on sand exposure eqs 5 and 6 and *S. aureus* were based on the eqs 7 and 8 ($\times 1\,000\,000$ Monte Carlo simulations). ^bNA, not applicable.

DISCUSSION

The RPLs for *Cryptosporidium*, enterovirus, and *S. aureus* were estimated for illness risk levels of 1.9×10^{-2} . The RPLs are high in comparison to values observed in the sand at the beach site used for comparison and evidence suggests that they are high in comparison to other benchmarks. For example, common sources of indicator bacteria and pathogens at beach sites include dogs and birds. Dog feces have been reported at 24 *Cryptosporidium* oocysts per gram on average with a maximum of 98 oocysts per gram,⁴⁹ indicating that undiluted dog feces are on the same order of magnitude of the estimated RPL. Birds on the other hand have been documented to carry much higher levels of *Cryptosporidium* (on the order of up to 1000 to 10 000 oocysts in their feces)^{50,51} suggesting that bird feces would need to be diluted with the sand by a factor of 10 or more in order to fall below the RPL. These arguments inherently assume that the species of *Cryptosporidium* found in dogs and birds is the species that could also cause illness in humans.

Enterovirus is similar to *Cryptosporidium* in that both are characterized by a low infective dose.^{34,52} Although mammals, in particular ruminants, have been known to carry bovine forms of the virus,⁵³ enterovirus infections are generally thought to be species specific, in particular the human forms. For this reason, animal feces are generally not considered to be a source of human infections. We hypothesize that the low levels of enterovirus observed within the study site may be due to wash off from individuals as they exit the beach water and/or from adsorption from the beach water and/or contaminated runoff during storm events. Regardless, for the nonpoint source beach evaluated, it appears as though insufficient enterovirus were found in the sediment to cause illness and without a concentrated animal or sewage source of the illness, transmission of enterovirus-related disease through beach sand seems unlikely.

The results showed that skin infectious risk from *S. aureus* was lower than GI infectious risk from *Cryptosporidium* and enterovirus in the sand. This finding is supported by Heaney et al. (2009)⁸ who showed that children had higher risks of GI illness than skin illness associated with sand exposures. For the beach site evaluated in this study, although *S. aureus* was

detected in both recreational water⁵⁴ and beach sand³¹ at the site, both epidemiologic study for recreational water^{23,26} and QMRA for beach sand at this site were consistent. The epidemiologic study did not find a correlation between skin ailments and *S. aureus* levels and it appears that the levels of *S. aureus* in sand were too low, limiting power to investigate associations with reports of skin infection. Apparently there is another pathogen at the study beach that is causing skin ailments during water exposure.

A limitation of this study was that risk characterizations were not performed for groups of pathogenic microbes (e.g., yeast and helminths) known to inhabit beach sediments at the study site used for comparative purposes. *Candida tropicalis* and nematodes were detected at the study beach. However due to lack of dose–response information, RPLs could not be computed for these microorganisms. Identification of *Candida* species in beach sand is consistent with other studies conducted in South Florida¹⁹ and in Portugal.²⁰ Both of these studies found that among the yeasts evaluated, *Candida* was the most common with *C. tropicalis* found in highest numbers in South Florida. Both Vogel et al. (2007)¹⁹ and Sabino et al. (2011)²⁰ speculate that the presence of these yeast species have a bearing on human health for those exposed to sand. Based upon a comparison of beaches studied in Portugal, Sabino et al. (2011)²⁰ goes further and recommends an upper limit of 15 CFU/g for yeasts to minimize health impacts. This value is lower than the 143 CFU/g value observed for the study site evaluated in the current study. In order to improve the link between sand measurements to human health, dose response relationships should be established for common yeast species observed in beach sands in particular *C. tropicalis*. Once a dose–response relationship is established, QMRA can be implemented to estimate reference pathogen levels of yeast in beach sand.

Nematodes represent a very possible risk especially in areas that are open to dogs and feral cats. Various types of nematodes have been identified in soils at public parks^{55–57} and beach sands,²¹ with evidence of transmission to humans via sands suspect to be contaminated with cat feces.⁵⁸ More work is needed developing dose response curves for nematodes and to identify acceptable levels of nematodes within beach sands in

light of the fact that documented cases have shown that sands have served as the vehicle of transmission.

In addition to yeast and nematodes, which have been detected in beach sands, studies should also be conducted to evaluate the potential presence of norovirus in beach sands and its potential role in transmitting gastrointestinal illness. Norovirus has been implicated as the most likely etiologic agent in recreational waters⁵⁹ and because of the proximity of the sand to water, there is a possibility that sand can also serve as a vehicle of transmission for norovirus. Due to the lack of culture based method for human forms of norovirus, measurements in sand would need to rely on qPCR technology which is generally limited due to high detection limits and patchy distribution of microbes in beach sands. Future work should focus on comparing norovirus levels in sand to its corresponding RPLs.

Another limitation was that the dose–response data utilized in this risk assessment for *S. aureus* infection was based upon a human exposure study that utilized intact skin.⁶⁰ The infectious risks for *S. aureus* reported in this study would not be applicable for individuals with cuts and wounds. This is of particular concern as the sand itself can cause abrasions allowing for more direct contact between microbes in the sand and subcutaneous skin.

QMRA is also limited by the fact that it models infection rates as opposed to illness rates. The EPA reference risk of 1.9×10^{-2} is an illness rate and thus the reference level guidelines estimated as part of this study are conservative because not all infections result in illness. Moreover the QMRA approach utilized in this study would benefit from an evaluation of acceptable skin illness rates as the 1.9×10^{-2} is benchmarked against gastrointestinal (GI) illness as opposed to skin ailments. The 1.9×10^{-2} GI illness rate also represents the summation of all GI illnesses by numerous potential pathogens, so evaluating individual pathogens against this risk should recognize that GI can be caused by multiple microbes and the summation of risk from all GI microbes should be considered when evaluating beach sand safety.

This study computed pathogen concentrations that result in 1.9×10^{-2} infection risk, which is consistent with the EPA's acceptable illness risk for marine water. Since children are more susceptible to RWIs,⁸ 1.9×10^{-2} infection risk could be used as a preliminary reference pathogen level guideline to protect beach users, especially children, from potential infectious risks from beach sands. Further development of QMRA and surveys for beach sand quality along with an epidemiologic study, including children, will be necessary to address relationships between beach sand contamination and public health in order to protect children from exposures to beach sand. The focus of children at the beach environment is especially important, due to play behavior at beach sites that would increase a child's exposure.

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Notes

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REFERENCES

- (1) U.S. Environmental Protection Agency (EPA). *Ambient Water Quality Criteria for Bacteria—1986*, U.S. EPA A440/5-84–002; U.S. EPA: Washington, DC, 1986.
- (2) Mika, K. B.; Imamura, G.; Chang, C.; Conway, V.; Fernandez, G.; Griffith, J. F.; Kampalath, R. A.; Lee, C. M.; Lin, C.-C.; Moreno, R.; Thompson, S.; Whitman, R. L.; Jay, J. A. Pilot- and bench-scale testing of faecal indicator bacteria survival in marine beach sand near point sources. *J. Appl. Microbiol.* **2009**, *107*, 72–84.
- (3) Beversdorf, L. J.; Bornstein-Forst, S. M.; McLellan, S. L. The potential for beach sand to serve as a reservoir for *Escherichia coli* and the physical influences on cell die-off. *J. Appl. Microbiol.* **2007**, *102* (5), 1372–81.
- (4) Yamahara, K. M.; Layton, B. A.; Santoro, A. E.; Boehm, A. B. Beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. *Environ. Sci. Technol.* **2007**, *41* (13), 4515–4521.
- (5) 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), 214–225.
- (6) World Health Organization (WHO). *Guideline for Safe Recreational Water Environments*, Coastal and fresh waters; WHO: Geneva, Switzerland, 2003; Vol. 1.
- (7) Whitman, R. L.; Przybyla-Kelly, K.; Shively, D. A.; Nevers, M. B.; Byappanahalli, M. N. Hand-mouth transfer and potential for exposure to *E. coli* and F+ coliphage in beach sand, Chicago, Illinois. *J. Water Health* **2009**, *7* (4), 623–629.
- (8) Heaney, C. D.; Sams, E.; Wing, S.; Marchall, S.; Brenner, K.; Dufour, A. P.; Wade, T. J. Contact with beach sand among beachgoers and risk of illness. *Am. J. Epidemiol.* **2009**, *170* (2), 164–172.
- (9) Heaney, C. D.; Sams, E.; Dufour, A. P.; Brenner, K. P.; Haugland, R. A.; Chern, E.; Wing, S.; Marshall, S.; Love, D. C.; Serre, M.; Noble, R.; Wade, T. J. Fecal Indicators in Sand, Sand Contact, and Risk of Enteric Illness Among Beachgoers. *Epidemiology* **2012**, *23* (1), 95–106.
- (10) Haas, C.; Rose, J.; Gerba, C. *Quantitative Microbial Risk Assessment*; John Wiley and Sons: New York, 1999.
- (11) Ashbolt, N. J.; Schoen, M. E.; Soller, J. A.; Roser, D. J. Predicting pathogen risks to aid beach management: the real value of quantitative microbial risk assessment (QMRA). *Water Res.* **2010**, *44* (16), 4692–4703.
- (12) Schoen, M. E.; Ashbolt, N. J. Assessing pathogen risk to swimmers at non-sewage impacted recreational beaches. *Environ. Sci. Technol.* **2010**, *44*, 2286–2291.
- (13) Viau, E. J.; Lee, D.; Boehm, A. B. Swimmer Risk of Gastrointestinal Illness from Exposure to Tropical Coastal Waters Impacted by Terrestrial Dry-Weather Runoff. *Environ. Sci. Technol.* **2011**, *45* (17), 7158–65, DOI: 10.1021/es200984b.
- (14) Alm, E. W.; Burke, J.; Spain, A. Fecal indicator bacteria are abundant in wet sand at freshwater beaches. *Water Res.* **2003**, *37* (16), 3978–3982.

- (15) Whitman, R. L.; Nevers, M. B. Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. *Appl. Environ. Microbiol.* **2003**, *69* (9), 5555–5562.
- (16) Bonilla, T. D.; Nowosielski, K.; Cuvelier, M.; Hartz, A.; Green, M.; Esiobu, N.; McCorquodale, D. S.; Fleisher, J. M.; 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.
- (17) Soller, J. A.; Schoen, M. E.; Bertrand, T.; Ravenscroft, J. E.; Ashbolt, N. J. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Res.* **2010**, *44* (16), 4674–4691.
- (18) Shah, A. H.; Abdelzaher, A. M.; Mathew, P.; Hernandez, R.; Solo-Gabriele, H. M.; Kish, J.; Scortzetti, G.; Fell, J. W.; Diaz, M. R.; Scott, T. M.; Lukasik, J.; Harwood, V. J.; McQuaig, S.; Sinigalliano, C. D.; Gidley, M. L.; Wanless, D.; Ager, A.; Lui, J.; Stewart, J. R.; Plano, L. R. W.; Fleming, L. E. Indicator microbes correlate with pathogenic bacteria, yeast, and helminthes in sand at a subtropical recreational beach site. *J. Appl. Microbiol.* **2011**, *110*, 1571–1583.
- (19) Vogel, C.; Rogerson, A.; Schatz, S.; Laubache, H.; Tallman, A.; Fell, J. Prevalence of yeasts in beach sand at three bathing beaches in South Florida. *Water Res.* **2007**, *41*, 1915–1920, DOI: 10.1016/j.watres.2007.02.010.
- (20) Sabino, R.; Verissimo, C.; Cunha, M. A.; Wergikoski, B.; Ferreira, F. C.; Rodrigues, R.; Parada, H.; Falcão, L.; Rosado, L.; Pinheiro, C.; Paixão, E.; Brandão, J. Pathogenic fungi: An unacknowledged risk at coastal resorts? New insights on microbiological sand quality in Portugal. *Mar. Pollut. Bull.* **2011**, *62*, 1506–1511.
- (21) Silva, P. F. d.; Cavalcanti, I. M. D.; Irmao, J. I.; Rocha, F. J. S. Common beach sand contamination due to enteroparasites on the southern coast of Pernambuco State. *Brazil. Rev. Inst. Med. Trop. S. Paulo* **2009**, *51* (4), 217–218.
- (22) Rose, E. A.; Porcerelli, J. H.; Neale, A. V. Pica: common but commonly missed. *J. Am. Board Fam. Pract.* **2000**, *13* (5), 353–8.
- (23) Fleisher, J. M.; Fleming, L. E.; Solo-Gabriele, H. M.; Kish, J. K.; Sinigalliano, C. D.; Plano, L.; Elmir, S. M.; Wang, J. D.; Withum, K.; Shibata, T.; Gidley, M. L.; Abdelzaher, A.; He, G.; Ortega, C.; Zhu, X.; Wright, M.; Hollenbeck, J.; Backer, L. C. The BEACHES study: health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. *Int. J. Epidemiol.* **2010**, *39* (5), 1291–1298.
- (24) Abdelzaher, A. M.; Wright, M. E.; Ortega, C.; Hasan, A. R.; Shibata, T.; Solo-Gabriele, H. M.; Kish, J.; Withum, K.; He, G.; Elmir, S. M.; Bonilla, J. A.; Bonilla, T. D.; Palmer, C. J.; Scott, T. M.; Lukasik, J.; Harwood, V. J.; McQuaig, S.; Sinigalliano, C. D.; Gidley, M. L.; Wanless, D.; Plano, L. R. W.; Garza, A. C.; Zhu, X.; Stewart, J. R.; Dickerson, J. W. Jr.; Yampara-Iquise, H.; Carson, C.; Fleisher, J. M.; Fleming, L. E. Daily measures of microbes and human health at a non-point source marine beach. *J. Water Health* **2011**, *9* (3), 443–457.
- (25) Shibata, T.; Solo-Gabriele, H. M.; Fleming, L.; Elmir, S. Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. *Water Res.* **2004**, *38*, 3119–3131.
- (26) Sinigalliano, C. D.; Fleisher, J. M.; Gidley, M. L.; Solo-Gabriele, H. M.; Shibata, T.; Plano, L.; Elmir, S. M.; Wang, J. D.; Wanless, D.; Bartowiak, J.; Boiteau, R.; Withum, K.; Abdelzaher, A.; He, G.; Ortega, C.; Zhu, X.; Wright, M.; Kish, J.; Hollenbeck, J.; Backer, L. C.; Fleming, L. E. Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. *Water Res.* **2010**, *44* (13), 3763–3772.
- (27) Wright, M. E.; Solo-Gabriele, H. M.; Abdelzaher, A. M.; Elmir, S.; Fleming, L. E. The inter-tidal zone is the geographic location of elevated concentrations of enterococci. *Water Sci. Technol.* **2011**, *63*, 542–549.
- (28) Zhu, X.; Wang, J. D.; Solo-Gabriele, H. M.; Fleming, L. E.; Elmir, S. A microbial water quality model for recreational marine beaches. *Water Res.* **2011**, *45*, 2985–2995.
- (29) Phillips, M. C.; Solo-Gabriele, H. M.; Piggot, A. M.; Klaus, J. S.; Zhang, Y. Relationships between sand and water quality at recreational beaches. *Water Res.* **2011**, *45*, 6763–6769.
- (30) Enns, A. A.; Vogel, L. J.; Abdelzaher, A. M.; Solo-Gabriele, H. M.; Plano, L. R. W.; Gidley, M. L.; Phillips, M. C.; Klaus, J. S.; Piggot, A. M.; Feng, Z.; Reniers, A. J. H. M.; Haus, B. K.; Elmir, S. M.; Zhang, Y.; Jimenez, N. H.; Abdel-Mottaleb, N.; Schoor, M. E.; Brown, A.; Khan, S. Q.; Dameron, A. S.; Salazar, N. C.; Fleming, L. E. Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions (in review).
- (31) Abdelzaher, A.; Wright, M.; Ortega, C.; Solo-Gabriele, H.; Miller, G.; Elmir, S.; Newman, X.; Shih, P.; Bonilla, A.; Bonilla, T. D.; Palmer, C. J.; Scott, T.; Lukasik, J.; Harwood, V. J.; McQuaig, S.; Sinigalliano, C.; Gidley, M.; Plano, L.; Zhu, X.; Wang, J. D.; Fleming, L. E. Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. *Appl. Environ. Microbiol.* **2010**, *76* (3), 724–32.
- (32) Yoder, J. S.; Hlavsa, M. C.; Craun, G. F.; Hill, V.; Roberts, V.; Yu, P. A.; Hicks, L. A.; Alexander, N. T.; Calderon, R. L.; Roy, S. L.; Beach, M. J. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events—United States, 2005–2006. *Morbidity Mortality Weekly Rep.* **2008**, *57*, No. SS-9, pages 1 to 38.
- (33) Poh, C. L.; Tan, E. L. Detection of enteroviruses from clinical specimens. *Methods Mol. Biol.* **2011**, *665*, 65–77.
- (34) Fong, T.-T.; Lipp, E. K. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiol. Mol. Biol. Rev.* **2005**, *69* (2), 357–371.
- (35) Soge, O. O.; Meschke, J. S.; No, D. B.; Roberts, M. C. Characterization of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* spp. isolated from US West Coast public marine beaches. *J. Antimicrob. Chemother.* **2009**, *64* (6), 1148–55, DOI: 10.1093/jac/dkp368.
- (36) Elmir, S. M.; Wright, M. E.; Solo-Gabriele, H. M.; Abdelzaher, A.; Fleming, L. E.; Miller, G.; Rybolowik, M.; Shih, M-T P.; Pillai, S. P.; Cooper, J. A.; Quaye, E. A. Quantitative evaluation of bacteria released by bathers in a marine water. *Water Res.* **2007**, *41*, 3–10.
- (37) Plano, L. R. W.; Garza, A. C.; Shibata, T.; Elmir, S. M.; Kish, J.; Sinigalliano, C. D.; Gidley, M. L.; Miller, G.; Withum, K.; Fleming, L. E.; Solo-Gabriele, H. M. Shedding of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* from adult and pediatric bathers in marine waters. *BMC Microbiol.* **2011**, *11* (5), 1–10.
- (38) Rose, J. B.; Haas, C.N. A risk assessment framework for the evaluation of skin infections and the potential impact of antibacterial soap washing. *American Journal of Infectious Control* **1999**, *27* (6), S26–S33.
- (39) Wang, J. D.; Solo-Gabriele, H. M.; Abdelzaher, A. M.; Fleming, L. E. Estimation of enterococci input from bathers and animals on a recreational beach using camera images. *Mar. Pollut. Bull.* **2010**, *60*, 1270–1278.
- (40) Hemond, H. F.; Solo-Gabriele, H. M. Children's Exposure to Arsenic from CCA-Treated Wooden Decks and Playground Structures. *Risk Anal.* **2004**, *24* (1), 51–64.
- (41) U.S. EPA. *Exposure Factors Handbook*, 2011 ed. (Final), EPA/600/R-09/052F; U.S. Environmental Protection Agency: Washington, DC, 2011.
- (42) Kissel, J. C.; Shirai, J. H.; Richter, K. Y.; Fenske, R. A. Empirical investigation of hand-to-mouth transfer of soil. *Bull. Contam. Toxicol.* **1998**, *60*, 379–386.
- (43) Holmes, K. K. Jr.; Shirai, J. H.; Richter, K. Y.; Kissel, J. C. Field measurement of dermal soil loadings in occupational and recreational activities. *Environ. Res.*, **A** **1999**, *80* (2 pt 1), 148–157.
- (44) Shoaf, M. B.; Shirai, J. H.; Kedan, G.; Schaum, J.; Kissel, J. C. Child dermal sediment loads following play in a tide flat. *J. Expo. Anal. Environ. Epidemiol.* **2005**, *15* (5), 407–412.
- (45) Xue, J.; Zartarian, V.; Tulve, N.; Moya, J.; Freeman, N.; AuYeung, W.; Beamer, P.. A metaanalysis of children's object-to-

mouth frequency data for estimating non-dietary ingestion exposure. *J. Expo. Sci. Environ. Epidemiol.* **2010**, *20*, 536–545.

(46) U.S. Environmental Protection Agency (EPA). *Exposure Factor Handbook*; U.S. EPA, National Center for Environmental Assessment: Washington, DC, 1997.

(47) Das, B. M. *Principles of Geotechnical Engineering*. PWS Engineering Publishers, Boston, MA, 1985.

(48) Phillips, M. C., Solo-Gabriele, H. M., Reniers, A. J. H. M., Wang, J. D., Kiger, R. T., Abdel-Mottaleb, N.. Pore water transport of enterococci out of beach sediments. *Mar. Pollut. Bull.* **2011a**, *62*, 2293-2298.

(49) Dubná, S.; Langrová, I.; Nápravník, J.; Jankovská, I.; Vadlejch, J.; Pekár, S.; Fechtner, J. The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic. *Vet. Parasitol.* **2007**, *145*, 120–128.

(50) Graczyk, T. K.; Majewska, A. C.; Schwab, K. J. The role of birds in dissemination of human waterborne enteropathogens. *Trends Parasitol.* **2008**, *24* (2), 55–59.

(51) Kuhn, R. C.; Rock, C. M.; Oshima, K. H. Occurrence of *Cryptosporidium* and *Giardia* in wild ducks along the Rio Grande River Valley in Southern New Mexico. *Appl. Environ. Microbiol.* **2002**, *68* (1), 161–165.

(52) DuPont, H. L.; Chappell, C. L.; Sterling, C. R.; Okhuysen, P. C.; Rose, J. B.; Jakubowski, W. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N. Engl. J. Med.* **1995**, *332* (13), 855–859.

(53) Jiménez-Clavero, M. A.; Escribano-Romero, E.; Mansilla, C.; Gómez, N.; Córdoba, L.; Roblas, N.; Ponz, F.; Ley, V.; Sáiz, J.-C. Survey of bovine enterovirus in biological and environmental samples by a highly sensitive real-time reverse transcription-PCR. *Appl. Environ. Microbiol.* **2005**, *71* (7), 3536–3543.

(54) Shibata, T.; Solo-Gabriele, H. M.; Sinigalliano, C. D.; Gidley, M. L.; Plano, L. R.; Fleisher, J. M.; Wang, J. D.; Elmir, S. M.; He, G.; Wright, M. E.; Abdelzaher, A. M.; Ortega, C.; Wanless, D.; Garza, A. C.; Kish, J.; Scott, T.; Hollenbeck, J.; Backer, L. C.; Fleming, L. E. Evaluation of conventional and alternative monitoring methods for a recreational marine beach with non-point source of fecal contamination. *Environ. Sci. Technol.* **2010**, *44* (21), 8175–8181.

(55) Avcioglu, H.; Balkaya, I. The relationship of public park accessibility to dogs to the presence of *Toxocara* species ova in the soil. *Vector Borne Zoonotic Dis.* **2011**, *11* (2), 177–180.

(56) Tiyo, R.; Guedes, T. A.; Falavigna, D. L.; Falavigna-Guilherme, A. L. Seasonal contamination of public squares and lawns by parasites with zoonotic potential in southern. **2008**, *82* (1), 1–6.

(57) Mandarino-Pereira, A.; Silva de Souza, F.; Lopes, C. W. G.; Pereira, M. J. S. Prevalence of parasites in soil and dog feces according to diagnostic tests. *Vet. Parasitol.* **2010**, *70*, 176–181.

(58) Centers for Disease Control and Prevention (CDC). Outbreak of Cutaneous Larva Migrans at a Children's Camp—Miami, Florida, 2006. *Morbidity Mortality Weekly Rep.*, **2007**, *56*(49), 1285–1287

(59) Soller, J. A.; Bartrand, T.; Ashbolt, N. J.; Ravenscroft, J.; Wade, T. J. Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. *Water Res.* **2010**, *44* (16), 4736–4747.

(60) Singh, G.; Marples, R. R.; Kligman, A. M. Experimental *Staphylococcus aureus* infections in humans. *J. Invest. Dermatol.* **1971**, *57*, 149–62.