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Human-Associated Fecal Quantitative Polymerase Chain Reaction Measurements and Simulated Risk of Gastrointestinal Illness in Recreational Waters Contaminated with Raw Sewage

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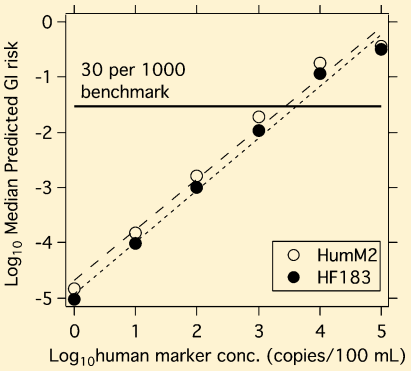
Supporting Information

ABSTRACT: We used quantitative microbial risk assessment to simulate the risk of gastrointestinal (GI) illness associated with swimming in waters containing different concentrations of human-associated fecal markers from raw sewage, HF183 and HumM2. The volume/volume ratio of raw sewage to ambient water was determined by comparing marker concentrations in recreational water to concentrations in raw sewage from 54 geographic locations across the United States. Concentrations of reference GI pathogens in raw sewage, volumes ingested by swimmers, dose−response functions, and fractions of infected that become ill were adopted from previous studies. Simulated GI risk increased with concentration of the human quantitative polymerase chain reaction markers in recreational waters. A benchmark illness rate of 30 GI illnesses per 1000 swimmers occurred at median concentrations of 4200 copies of HF183 and 2800 copies of HumM2 per 100 mL of recreational water. This study establishes a risk-based approach for interpreting concentrations of human fecal markers in ambient waters.



INTRODUCTION

Fecal indicator bacteria (FIB) such as *Enterococcus* spp. serve as recreational water quality indicators worldwide.[1] Their concentrations in recreational waters impacted by treated wastewater effluent are linked to adverse health outcomes in swimmers through epidemiology studies.[2,3] The U.S. Environmental Protection Agency (EPA) recently published revised recreational water quality criteria for enterococci that aim to keep the risk of gastrointestinal illness (GI) in swimmers below approximately 30 illnesses per 1000 swimmers.[4]

Remediating waterbodies impaired by fecal pollution has proven to be difficult because FIB are found in a variety of different animal fecal sources[5] and nonfecal environments such as sand, sediments, and soils,[6−9] as well as lacustrine and marine vegetation.[10−12] Microbial source tracking (MST) methods have been developed to link fecal contamination to the feces from specific animal hosts. MST markers are mostly genetic and target human-, gull-, ruminant-, and dog-associated fecal bacteria using quantitative polymerase chain reaction (qPCR).[13,14] While MST markers are used in a variety of applications to help pinpoint fecal pollution sources,[14] there is still a great deal of uncertainty in interpreting their concentrations.[15]

This study aims to relate human-associated qPCR marker concentrations in recreational waters to swimmer health risk using quantitative microbial risk assessment (QMRA). We employ a QMRA framework used extensively by researchers for simulating health risks from exposure in recreational waters[16−20] to report the estimated concentration of HF183 and HumM2 qPCR markers that corresponds to the EPA-adopted health target of ∼30 GI illnesses per 1000 swimmers.[4,21−23] In addition, we investigate the simulated health risk associated with human qPCR marker measurements approaching the limit of quantification (LOQ). The goal is to establish a risk-based approach to interpreting concentrations of MST markers.

METHODS

A static QMRA was used to estimate GI illness risk from swimming in recreational waters with varying concentrations of human qPCR markers from raw sewage using Matlab version R2015a (The Mathworks, Natwick, MA); the influence of immunity and secondary transmission was not considered.[24] Monte Carlo simulations (n = 10000 for each scenario) were used to estimate the risk of GI illness from swimming in recreational water with each considered concentration of qPCR marker incorporating uncertainties associated with (1) the

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Table 1. Sewage Concentrations, Dose−Response Relations, and P_{illinf} Values for Reference Enteric Pathogens Considered in This Study[a]

organism	C_{sewage} range (log_{10} per liter)	P_{inf}	P_{illinf} (distribution)	refs
Salmonella spp.	[0.5, 3]	$1-(1+\mu/2884)^{-0.3126}$	0.17−0.4 (uniform)	51−53
Campylobacter	[2, 5]	$1-{}_1F_1(0.024, 0.024+0.011, -\mu)$	$1-(1+\nu\mu)^{-r}$	54
E. coli O157:H7	[−1, 3.3][b]	$1-(1+\mu/48.8)^{-0.248}$	0.2−0.6 (uniform)	55−58
Cryptosporidium	[−0.3, 2.6]	$1-\exp(-0.09\mu)$	0.3−0.7(uniform)	59
Giardia	[0.8, 4]	$1-\exp(-0.0199\mu)$	0.2−0.7 (uniform)	43, 60
norovirus	[3, 6]	$1-{}_1F_1(0.04, 0.04+0.055, -\mu)$	0.6	61

[a] μ is the dose. P_{inf} is the probability of infection. P_{illinf} is the probability of becoming ill after infection. ${}_1F_1$ is the hypergeometric function. When specified, P_{illinf} values are represented by a range of parameters, as indicated, drawn from a uniform distribution. P_{illinf} for *Campylobacter* is dose-dependent with $r = 2.44 \times 10^8$ and $\nu = 3.63 \times 10^{-9}$. References for P_{inf} and P_{illinf} are provided in the last column. References for sewage concentration range are refs 27 and 28. [b] The lower range is not detected, and −1 is used as a lower bound.

probability of illness upon infection, (2) the volume of water ingested during swimming, and (3) concentrations of human qPCR markers and reference pathogens in sewage.

We conducted the analysis for two different human qPCR markers: HumM2[22] and HF183Taqman.[23] These two markers have been evaluated in a number of studies and been shown to be highly specific to human feces,[13,22,25,26] with little to no cross reactivity with other host feces. Each model scenario was started by assuming the concentration of the human qPCR marker in recreational waters was constant at a concentration of 10^0, 10^1, 10^2, 10^3, 10^4, or 10^5 copies (cp)/100 mL.

A previous study[26] documented the concentrations of HF183 and HumM2 in raw sewage at 54 treatment plants around the United States. In aggregate, the HF183 and HumM2 marker concentrations were log-normally distributed with log_{10} means (log_{10} standard deviations) of 5.212 (0.566) and 4.978 (0.475) in units of copies per milliliter, respectively (see the Supporting Information for details of how data from ref 26 were manipulated from copies per nanogram of total nucleic acid to copies per milliliter of sewage). The volume/volume fraction (F_{sewage}) of sewage present in recreational waters with a specific concentration of human qPCR marker was determined as follows:

$$F_{sewage} = C_{ambient}/C_{sewage} \quad (1)$$

where $C_{ambient}$ is the assumed concentration of the marker in the ambient water as listed above and C_{sewage} is the concentration of the marker in sewage. C_{sewage} is randomly drawn from the corresponding log-normal distribution during each model iteration.

The concentration ranges of the reference pathogens norovirus, *Salmonella enterica*, *Campylobacter jejuni*, *Cryptosporidium*, *Giardia*, and *Escherichia coli* O157:H7 in raw sewage were obtained from a literature review,[27] except concentrations of *Campylobacter*, which were updated with more recent information[28] (Table 1). It is acknowledged that concentrations are expected to vary widely due in part to the different prevalence of disease in the population; the ranges reported in Table 1 are intended to be representative estimations to reflect the expected variability. The concentrations of reference pathogen *j* present in the ambient water with human qPCR marker were determined as follows:

$$C_{ambient\ pathogen\_j} = C_{sewage\ pathogen\_j} F_{sewage} \quad (2)$$

where $C_{sewage\ pathogen\_j}$ is the concentration of pathogen j in raw sewage. $C_{sewage\ pathogen\_j}$ was randomly drawn from a log-uniform distribution bounded by the values provided in Table 1 during each model iteration.

The distribution of water volume ingested during swimming (V) is based on a study of pool swimmers that determined that the volume of water ingested (in milliliters) during a 45 min swimming event by a group of adults and nonadults was best represented by a log_e-normal distribution with a mean of 2.92 and a standard deviation of 1.43.[29] A number was randomly drawn from this distribution to represent V during each model iteration.

The dose (μ) of each reference pathogen was determined by multiplying the volume ingested by the concentration of pathogen j in the ambient water ($VC_{ambient\ pathogen\_j}$). The dose was used as input to the reference pathogen dose−response functions (Table 1) to determine the probability of infection (P_{inf_j}). The probability of illness (P_{ill_j})was calculated by multiplying the probability of infection by the probability of illness given infection P_{illinf_j} (Table 1). For some reference pathogens, P_{illinf_j} was randomly drawn from a uniform distribution for each model iteration (Table 1). The cumulative probability of GI illness from each of the j pathogens was calculated as $P_{ill} = 1 - \prod_j(1 - P_{ill\_j})$.

The simulation was run 10000 times for each human qPCR marker at six different $C_{ambient}$ values for a total of 120000 iterations. The median, interquartile range, and 10th and 90th percentiles of the probability of illness for each qPCR marker concentration were calculated from the respective 10000 model realizations.

RESULTS AND DISCUSSION

MST markers are being implemented around the world to better understand sources of fecal pollution, yet interpreting MST marker concentrations remains challenging. Our findings present new insights into the relationship between two human qPCR marker concentrations and simulated human health risk. We found that health risk increases as the concentration of human qPCR markers increases in waters contaminated with raw sewage (Figure 1). The median simulated GI illness rate per 1000 swimmers varied between 9.5×10^{-6} and 0.32 when the HF183 marker varied between 1 and 10^5 copies/100 mL in recreational water impacted by raw sewage. There was a linear relationship between log_{10}-transformed HF183 median concentrations (in units of copies per 100 mL) and simulated log_{10}-transformed GI illness rates [log_{10}(GI risk) = −4.93 + 0.94 × log_{10}HF183; RSQ = 0.99]. Thus, for every ∼1 log increase in HF183 concentration, there is a predicted 1 log increase in risk. On the basis of this regression relationship, a median illness rate of 30 per 1000 occurs when the median concentration is 4200 copies/100 mL.

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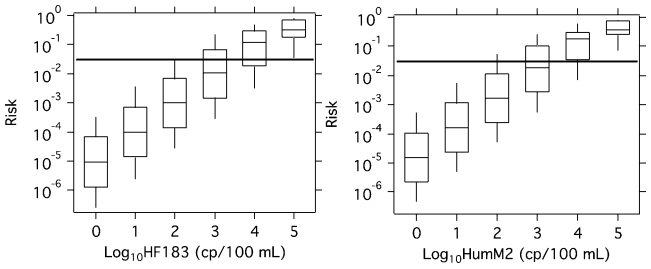

Figure 1. GI risk as a function of concentration of the HF183 marker in ambient water (left). GI risk as a function of concentration of HumM2 marker in ambient water (right). cp is copy. Box and whiskers indicate median (line in the middle of the box), 25th and 75th percentile (bottom and top of box, respectively), and 10th and 90th percentile (bottom and top of whisker, respectively). The horizontal line indicates a risk of 0.03, which is approximately the EPA benchmark risk of ~30 GI per 1000 swimmers.

The median simulated GI illness rate per 1000 swimmers varied between 1.5×10^{-5} and 0.36 when the HumM2 marker varied between 1 and 10^5 copies/100 mL in ambient water (Figure 1). There is a linear relationship between $\log_{10}$-transformed concentrations of HumM2 (in units of copies/100 mL) and $\log_{10}$-transformers GI illness risk [$\log_{10}$(GI risk) = $-4.69 + 0.92 \times \log_{10}$ HumM2; RSQ = 0.98]. As for HF183, every ~1 log increase in HumM2 concentration results in a predicted 1 log increase in GI risk. On the basis of the regression relationship, a median illness rate of 30 per 1000 occurs when the median concentration is 2800 copies/100 mL.

In sewage, the concentration of HF183 marker is significantly higher than that of HumM2 (mean difference is 0.2 $\log_{10}$ unit, paired t test, $p < 0.001$). Therefore, it is not surprising that the concentration of HF183 corresponding to the threshold GI illness level (4200 copies/100 mL) is approximately twice that of HumM2 (2800 copies/100 mL).

For both model simulations, the probability of contracting GI illness from norovirus contributes the majority to the cumulative probability of illness. Norovirus accounted for between 70 and 100% of the predicted GI illness, depending on the marker concentration (Figures S2–S7). This result is consistent with other QMRA studies that suggest norovirus drives recreational waterborne illness when the source of fecal contamination is human.[17] Only at the highest tested marker concentration (10^5 copies/100 mL) does another reference pathogen begin to contribute >1% of the risk, and that pathogen is *Campylobacter*, which contributes approximately a quarter of the simulated risk at the highest level of human qPCR markers.

Laboratory protocols, qPCR instrument selection, and technician skill all contribute to a qPCR method LOQ. A typical protocol could be 100 mL of ambient water is filtered through a 0.2 μm pore size filter to capture bacteria, the DNA from those bacteria is extracted using a commercial extraction kit into 100 μL of elution buffer, and 2 μL of the extract template is combined with Mastermix and subjected to QPCR.[12] For HF183 and HumM2 qPCR methods, many laboratories report 10 copies per PCR as the LOQ (e.g., Russell et al.[12]). Using the example protocol, this is equivalent to a water concentration of 500 copies/100 mL, assuming a 100% DNA recovery efficiency. If recovery efficiency is 50%, then the LOQ is 1000 copies/100 mL. This range of LOQ values is below the median concentration thresholds corresponding to 30 GI illnesses per 1000 swimmers but is within the same order of magnitude. According to the regression equations, concentrations of 500 and 1000 copies/100 mL correspond to median GI illness rates of 4 and 8 per 1000 swimmers for HF183, respectively, and 6 and 12 per 1000 swimmers for HumM2, respectively. The risk output distribution for both qPCR markers at concentrations of 100 copies/100 mL spans a range that includes the risk threshold. These observations suggest that positive detections below a typical LOQ for human marker qPCR assays have potential to be health-relevant. Thus, the inclusion of all positive detection data, even those near or below the LOQ, in water quality interpretations is warranted. Further, LOQs for MST methods should be clearly reported, as they are likely to vary from study to study, and thus, the simulated risk level at the LOQ will also vary. Careful attention to method performance, changes in protocol, and laboratory proficiency with qPCR measurements near LOQ will be necessary to understand and interpret human marker qPCR measurements.

This study modeled the risk of GI illness in recreational waters contaminated with raw sewage. Raw sewage can enter recreational waters from broken sewer lines, during treatment plant failures, and during combined sewer overflows. The modeled scenario assumes that human qPCR markers and reference pathogens occur in recreational waters at the same ratio that they occur in raw sewage, no differential attenuation occurs between the points where sewage enters the environment (a creek, groundwater, or a storm drain) and enters the recreational swimming site, and raw sewage is the only source of pathogens. This scenario is chosen because of its simplicity and as a basis for examining more complex scenarios in the future.

A review of the literature indicates very little work on the simultaneous attenuation of human qPCR markers and reference pathogens. Human qPCR marker persistence in water can vary depending on a number of factors such as water type, cell state, predation, oxygen, temperature, sunlight, salinity, and sediment.[14,30,31] qPCR-measured human-associated *Bacteroides* have reported T_{90} values between 1 and 47 days depending on studied conditions.[32−37] There are more data about the decay of reference pathogens; however, like the human marker studies, research has been conducted under diverse experimental conditions, and results are reported in different formats. For example, infectious norovirus can persist for 61 days in groundwater microcosms[38] and has a T_{90} of 11 days in surface waters (using murine norovirus as a surrogate).[39] *Campylobacter* has a T_{90} of ~1 day in lake water incubated at different temperatures in the dark;[40] shorter T_{90} values are expected in sunlit waters.[41] *E. coli* O157:H7 T_{90} values in large mesocosms were 3 days.[42] The T_{90} values of many reference pathogens and human markers appear to overlap, although more research should examine the relative decay of human markers and reference pathogens under the same experimental conditions. Because norovirus accounts for most of the risk in this study, experiments that simultaneously study norovirus and human marker decay are particularly important.

The QMRA model used herein relies on a number of assumptions and simplifications, including the following: (1) immunity and person-to-person spread of GI illness do not substantially impact the risk results, (2) dose−response curves apply to the general population, and (3) exposure is well approximated by the values from ref 29. Even though a number of assumptions are made, previous work with a very similar

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model by others[17,19,21,27,43−46] shows this QMRA framework to be appropriate for estimating GI risks during exposure to recreational waters. In fact, the results of QMRA and epidemiology research at EPA study beaches can be harmonized,[17,46,47] lending credence to the results presented here.

This study modeled a scenario in which raw sewage contaminates recreational waters. However, there are many other potential sources of human fecal pollution such as illicit dumping, failing septic systems, open defecation, and treated wastewater. Because there is a smaller number of individual contributors, concentrations of human qPCR markers and pathogens in feces and septage are likely to be more variable than they are in raw sewage.[13] A literature review summarized the range of reference pathogen concentrations expected in treated wastewater,[27] but there are limited data about the concentrations of HumM2 and HF183 qPCR markers in treated wastewater. One study[48] examined treated wastewater effluents for HumM2 and HF183 qPCR markers and found concentrations below or near the reported laboratory LOQ (~10^4 copies/100 mL of effluent). Future efforts to explore the human MST qPCR marker−health relationships in recreational waters impacted by septage, treated effluents, and human feces are needed.

Few recreational water epidemiology studies have measured human qPCR markers in conjunction with FIB in water and attempted to relate them to swimmer GI illness.[49,50] No association between the qPCR markers used in this study (HF183 and HumM2) is reported in those studies that were conducted at beaches with nonpoint sources of contamination.[49,50] Future work that evaluates concurrently QMRA and epidemiological approaches to evaluate the relationship between human MST markers and human health risks at sewage-impacted beaches would be useful.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.5b00219.

Additional details on calculation of marker concentrations in sewage and Figures S1−S7 (PDF)

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Notes

The authors declare no competing financial interest.

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