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# Assessing Pathogen Risk to Swimmers at Non-Sewage Impacted Recreational Beaches

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The risk of gastrointestinal illness to swimmers from fresh sewage and non-sewage fecal sources at recreational beaches was predicted using quantitative microbial risk assessment (QMRA). The QMRA estimated the probability of illness for accidental ingestion of recreational water with a specific concentration of fecal indicator bacteria, here the geometric mean enterococci limit of 35 cfu 100 mL<sup>-1</sup>, from either a mixture of sources or an individual source. Using seagulls as an example non-sewage fecal source, the predicted median probability of illness was less than the illness benchmark of 0.01. When the fecal source was changed to poorly treated sewage, a relatively small difference between the median probability of illness and the illness benchmark was predicted. For waters impacted by a mixture of seagull and sewage waste, the dominant source of fecal indicator was not always the predicted dominant source of risk.

## Introduction

At recreational beaches in the United States, a sign may be posted warning swimmers when fecal indicator bacterial concentrations in the waters exceed the U.S. EPA recommended standards. The U.S. EPA fecal indicator bacterial standards are based on the relationships reported between fecal indicator concentration and human gastrointestinal illness at recreational beaches impacted by effluent from publicly owned (sewage) treatment works (POTW) (1). The health effects associated with POTW and sewage impacted beaches are likely from human infectious pathogens such as *Norovirus*, *Giardia*, and *Cryptosporidium* spp. (2) found in wastewaters. There is concern, however, that non-sewage impacted beaches may receive a different range and magnitude of pathogens and may require an alternative standard to be equally protective of human health (3).

Little epidemiologic work exists to justify an alternative water quality standard for non-sewage impacted beaches; planned epidemiologic studies cover only a small combination of possible non-human sources; and there are minimal resources to plan additional studies for the large number of combinations of possible fecal sources (4). Of the existing body of work, there is no clear relationship between illness and any fecal indicator for non-sewage impacted beaches (5, 6). The high cost and impracticality in undertaking many epidemiologic studies, along with the lack of any clear

relationship between indicator and health outcome at non-POTW impacted beaches has prompted the need to find an alternative way of estimating the conditions under which human health may be impacted (4). Such an alternative approach is implemented here to predict the pathogen risks from non-sewage sources at recreational beaches, using quantitative microbial risk assessment (QMRA) (7–9).

QMRA has been used to predict the public health outcome from exposure to recreational waters in multiple studies (10–16). All but one of these studies focus on waters dominated by human sources; and to our knowledge, only one study focused specifically on the risks from animal sources. Till et al. calculated the probability of illness from bird dominated recreational waters in New Zealand using observed densities of *Campylobacter*. This study, which considered other sources of contamination in addition to avian sources, motivated a revision to New Zealand's water quality guidelines for freshwater recreational areas (13).

The work described here also aims to inform future policy decisions where non-sewage sources dominate the contamination at recreational waters. The primary objective was to predict and prioritize the pathogen risk from non-sewage and sewage sources for a waterbody at the U.S. EPA recommended water quality limit. The second objective was to predict when a non-sewage source of pathogens may dominate the illness risk in a waterbody impacted by a mixture of sources.

## Method

A QMRA approach (7, 8) was constructed to calculate the probability of gastrointestinal (GI) illness for a healthy adult resulting from the accidental ingestion of recreational water impacted by fresh fecal contamination. The probability of GI illness was estimated separately for non-sewage and sewage sources of fresh fecal contamination. For both the non-sewage and sewage sources, the concentration of fecal indicator bacteria (FIB) was held constant at the geometric mean enterococci (ENT) limit of 35 cfu 100 mL<sup>-1</sup>. This approach allowed the predicted probability of illness for each source to be compared to the corresponding health benchmark of 0.01 (17) to prioritize source risk. In addition, the probability of illness from mixtures of sewage and a non-sewage (seagull) fecal source was calculated to determine when a non-sewage source of pathogens may dominate the illness risk.

**Problem Formation.** The sources of pathogens in this QMRA included the non-sewage source of seagull feces and poorly treated sewage. Pathogens representing different microbial groups (i.e., viruses, bacteria, and parasitic protozoa) commonly associated with each individual source were selected as reference pathogens. The seagulls were assumed to contribute two reference pathogens commonly reported in their feces, *Campylobacter jejuni* and *Salmonella enterica* (18–21). The sewage source was assumed to contribute four reference pathogens including those commonly reported in primary POTW effluent, *Norovirus*, *Giardia intestinalis*, *Cryptosporidium* spp., and *S. enterica* (22).

**Characterization of Exposure.** The pathogen dose was derived from the concentration of the fecal indicator in the water from a specific source. The dose  $\mu_{rp}^S$  of each reference pathogen (*rp*) in units of cfu, (oo)cysts, or genomes from each source (*S*) was calculated using the density of indicator and pathogens in the feces:

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$$P_{rp}^S = \frac{C_{ENT}^S F^S}{R_{ENT}^S 100} * R_{rp}^S * p^{S*} V \quad (1)$$

where S is the FIB source of sewage (Sw) or seagull (G),  $C_{ENT}$  is the surfzone concentration of ENT using a culture method (cfu 100 mL<sup>-1</sup>),  $F^S$  is the fraction of total ENT from source S,  $R_{ENT}^S$  is the ratio of the count of ENT to the wet mass of a composite gull fecal sample (cfu g<sup>-1</sup>) or to the volume of sewage (cfu L<sup>-1</sup>),  $V$  is the volume of water ingested (mL),  $R_{rp}^S$  is the ratio of the count of reference pathogen to the wet mass of a composite gull fecal sample or to the volume of sewage, and  $p^S$  is the fraction of human-infectious pathogenic strains for the reference pathogen from source S.

For seagulls, the ratio of the count of pathogens to the mass of seagull feces  $R_{rp}^G$  was estimated from 22 composite samples, representing a population of possible infected and non-infected individual gulls (23). For sources where a composite sample is not available, the rate of infection in the source population should be incorporated into eq 1.

The calculation of pathogen dose in eq 1 was used in two ways. First, the pathogen dose was calculated for accidental ingestion of water with an individual source contributing all of the pathogens and fecal indicator (i.e.,  $F^S = 1.0$ ) for the selected ENT concentration of  $C_{ENT} = 35$  cfu 100 mL<sup>-1</sup>. Second, given a mixture of two sources (i.e., sewage and seagull excreta) contributing ENT so that  $\sum F^S = 1.0$  and  $C_{ENT} = 35$  cfu 100 mL<sup>-1</sup>, the dose of pathogens from each source was calculated for each possible source combination.

Because many of the parameters used in estimating dose have natural variability (excluding the fixed  $C_{ENT}$  and  $F^S$ ), the QMRA process incorporated this variability using a Monte Carlo simulation. The Monte Carlo simulation consisted of 10,000 trials to create a distribution of pathogen dose for each pathogen and source. The log-uniform distribution was selected to capture the range reported in the literature for an input parameter when values spanned many orders of magnitude. The parameters and distributions used in the Monte Carlo simulation of dose are shown in Table 1.

Of the variables involved in estimating dose, the least studied and most uncertain is the fraction of human-infectious strains from seagulls  $p^G$ . Fenlon et al. (20) compared the serotypes of *Salmonella* isolated from gulls with the serotypes isolated from sewage near Aberdeen, Scotland and reported that both shared the top three most frequently isolated serotypes (i.e., *S. enterica* serotypes Agona, Panama, and Typhimurium). The top three most frequently isolated serotypes comprised 40% of all serotypes isolated from gulls (20). For *Campylobacter* spp., Moore et al. reported that 25% of the serotypes isolated from gulls were known as human infectious in Northern Ireland (24) while Quessy et al. suggested approximately 62% in Montreal (18). Of these 62% of serotypes from gulls, 30% had biotypes that matched human biotypes giving a rough estimate of the fraction of human infectious strains as  $(62\% * 30\%) / 100 = 0.2$  (18). Noting, however, that the fraction of human-infectious strains is likely to be site specific and related to the feeding and roosting patterns of the seagulls as well as the pathogen incidence rates in the surrounding community (25). Using the available data, the fraction of human-infectious strains from seagulls was modeled using a uniform distribution with a lower limit of 0.01 and an upper limit of 0.4 and subsequently examined through sensitivity analysis.

**Characterization of Human Health Effects.** Based on the objective to calculate the risk attributable to an individual source at the FIB water quality limit, a static QMRA approach was implemented with no secondary transmission modeled (33). A distribution of the probability of GI infection was calculated for each pathogen from source S using dose-response relationships from the literature for a healthy adult with best parameter estimates provided in Table 2 and the

10,000 Monte Carlo samples of pathogen dose. The resulting 10,000 estimates of probability of infection for each pathogen and source combination were then multiplied by the best estimate conditional probability of illness provided in Table 2 to calculate the probability of GI illness. The distribution of illness risk captures the natural variability in pathogen dose for the selected FIB waterbody concentration and does not incorporate the uncertainty from the dose-response relationships. The possible change in predicted risk due to the dose-response model selection is discussed later for each source. Statistical analysis and simulations were implemented in R (34) and Mathematica v. 5.2.

The probability of infection from *C. jejuni* was estimated using a Beta-Poisson dose-response model for infection (35, 36). Two alternative parameterizations were implemented, for healthy adults and for a mixed population of adults and children aged 3-13 (37). The ID<sub>50</sub>, i.e., the dose required to produce infection in 50% of the experimental subjects, for the adult *C. jejuni* model was 800 cfu and 2 cfu for the mixed population. *Salmonella enterica* is known to have a wide range of virulence across strains (38). Here, the probability of illness from the highly virulent strain shared by gulls and humans, *S. enterica* Bareilly, was estimated using the Gompertz model (38-41). The probabilities of illness from *Cryptosporidium parvum* and *Giardia intestinalis* were estimated using the exponential dose-response model for infection (ID<sub>50</sub> of 8 oocysts and 35 cysts, respectively) and conditional probabilities of illness (42, 43). The infectivity for *C. hominis*, although not modeled here, falls within the range of the three *C. parvum* isolates used for parameterization (43).

The probability of infection from *Norovirus* was estimated using a single hit model with a Poisson stopped logarithmic series distribution for aggregated virus genomes in suspension. The selected dose-response relationship incorporates the aggregation state of *Norovirus* through the aggregation parameter  $a$  ( $0 \leq a < 1$ ). The dose-response parameters ( $\alpha$ ,  $\beta$ ) were estimated from a combined data set of aggregated and non-aggregated inocula (i.e., "8fIIa + 8fIIb) resulting in the best estimate of the *Norovirus* infectivity. The aggregation parameter was set to simulate a disaggregated dose with ID<sub>50</sub> of 21 genomes for  $a = 0.0001$  (vs. ID<sub>50</sub> of 1018 genomes when aggregated with  $a = 0.9997$ ) as no additional data were available for the aggregation of *Norovirus* in environmental mixtures. A constant conditional probability of illness was selected over the dose-dependent form due to the small environmental doses (44).

**Risk Characterization.** The risk from each source was characterized as the total probability of GI illness after one exposure to recreational water through accidental ingestion of water while swimming. The total probability of illness  $P_{GI}^S$  from a specific source was calculated using the probability of illness from each source-specific reference pathogen as  $P_{GI}^S = 1 - \prod_{rp} (1 - P_{ill, rp}^S)$ .

## Results and Discussion

**Predicted Risk of GI Illness from Sewage and Non-Sewage Impacted Waters.** The pathogen risk to adult swimmers from sewage and seagull fecal impacted recreational water was compared to the health benchmark of 0.01 using the dose inputs in Table 1 and the adult dose-response relationships in Table 2. The boxplot of total predicted illness risk attributable to ingestion of water contaminated by fresh seagull fecal matter or fresh primary POTW effluent for a recreational waterbody with a fecal indicator bacterial concentration at the water quality limit (35 cfu 100 mL<sup>-1</sup> ENT) is presented in Figure 1. The GI illness risks from the individual reference pathogens are also presented for each source. The resulting median and average predicted reference

**TABLE 1. Parameters Used for Calculation of Pathogen Dose from Sewage (Sw) and Seagull Feces (Gull)**

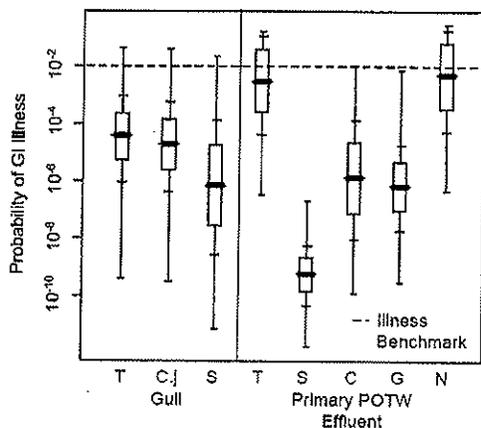
| parameter   | source | units                   | distribution parameters <sup>a</sup> | reference |
|---|--------|-------------------------|--------------------------------------|-----------|
| density of ENT in fecal waste ( $R_{ENT}^f$ )                 | Sw     | cfu L <sup>-1</sup>     | $a = 10^7$ $b = 10^8$                | (22)      |
|   | Gull   | cfu g <sup>-1</sup>     | $a = 6.0$ $b = 8.0$                  | (26, 27)  |
| density of <i>Campylobacter</i> in fecal waste ( $R_{Cj}^f$ ) | Sw     | cfu L <sup>-1</sup>     | NA                                   | NA        |
|   | Gull   | cfu g <sup>-1</sup>     | $a = 3.3$ $b = 6.0$                  | (23)      |
| density of <i>Salmonella</i> in fecal waste ( $R_S^f$ )       | Sw     | cfu L <sup>-1</sup>     | $a = 0.5$ $b = 3.0$                  | (28)      |
|   | Gull   | cfu g <sup>-1</sup>     | $a = 2.3$ $b = 9.0$                  | (23)      |
| density of <i>Cryptosporidium</i> in fecal waste ( $R_C^f$ )  | Sw     | oocysts L <sup>-1</sup> | $a = -0.3$ $b = 4.6$                 | (29)      |
|   | Gull   | oocysts g <sup>-1</sup> | NA                                   | NA        |
| density of <i>Giardia</i> in fecal waste ( $R_G^f$ )          | Sw     | cysts L <sup>-1</sup>   | $a = 0.8$ $b = 4.0$                  | (29)      |
|   | Gull   | cysts g <sup>-1</sup>   | NA                                   | NA        |
| density of <i>Norovirus</i> in fecal waste ( $R_N^f$ )        | Sw     | genomes L <sup>-1</sup> | $a = 3.0$ $b = 7.5$                  | (30, 31)  |
|   | Gull   | genomes g <sup>-1</sup> | NA                                   | NA        |
| volume of water ingested ( $V$ )                              | NA     | mL                      | $\mu = 2.92$ $\sigma = 1.43^b$       | (32)      |
| human-infectious fraction of pathogen strains ( $p^2$ )       | Sw     | NA                      | 1.0                                  | NA        |
|   | Gull   | NA                      | $a = 0.01$ $b = 0.4$                 | (20)      |

<sup>a</sup>  $a$  and  $b$  are the upper and lower bounds of a uniform distribution;  $a$  and  $b$  are the upper and lower bounds of a log<sub>10</sub>-uniform distribution;  $\mu$  and  $\sigma$  are the parameters of a log-normal distribution. <sup>b</sup> Estimated from a combined population of children and adults. NA - not available.

**TABLE 2. Pathogen Dose-Response Relationships and Parameter Values for Adults**

| pathogen                                     | dose ( $\mu$ ) unit | dose-response relationship  | dose-response parameter values  | probability of illness conditional on infection                                   | parameter values reference |
|--|---------------------|---|---|---|----------------------------|
| <i>Campylobacter jejuni</i>                  | cfu                 | $P_{inf} = 1 - {}_1F_1(\alpha, \alpha + \beta, -\mu)$                         | $\alpha = 0.145$ , $\beta = 7.59$ ;                                   | $P_{ill inf} = 0.2$ ; mixed population;   | (35-37)                    |
| <i>Salmonella enterica</i> serotype Bareilly | cfu                 | $P_{inf} = 1 - \exp(-\exp(-\ln(a) + \ln(\mu)))$                               | mixed population <sup>a</sup> ;<br>$\alpha = 0.024$ , $\beta = 0.011$ | $P_{ill inf} = 1 - (1 + \eta\mu)^{-r}$ ;<br>$\eta = 10^{-8.44}$ ; $r = 10^{8.36}$ |                            |
| <i>Cryptosporidium parvum</i>                | oocysts             | $P_{inf} = 1 - e^{-E(r)\mu}$  | $\ln(a) = 11.68$ , $b = 0.82$   | NA  | (38-41)                    |
| <i>Giardia intestinalis</i>                  | cysts               | $P_{inf} = 1 - e^{-E(r)\mu}$  | $E(r) = 0.09$   | $P_{ill inf} = 0.7$   | (43)                       |
|  |                     | $P_{inf} = 1 - {}_2F_1(\alpha, \mu(1 - a)/(a), \alpha + \beta, (-a)/(1 - a))$ | $E(r) = 0.0199$   | $P_{ill inf} = 0.9$   | (42)                       |
| <i>Norovirus</i> <sup>b</sup>                | genomes             |   | $\alpha = 0.04$ ; $a = 0.0001$ ;<br>$\beta = 0.055$                   | $P_{ill inf} = 0.68$  | (44)                       |

<sup>a</sup> Alternative parametrization for a mixed population of adults and children ages 3-13 (37). <sup>b</sup> Susceptible portion of population is estimated as 80% (44, 45). NA = not applicable.



**FIGURE 1. Predicted GI illness by reference pathogen (median, interquartile range, 10th and 90th percentiles, minimum, and maximum) for adults following a single accidental ingestion of recreational water containing fresh fecal contamination at 35 cfu 100 mL<sup>-1</sup> enterococci contributed by seagulls or primary POTW effluent (T = total GI risk, C<sub>j</sub> = *C. jejuni* risk; S = *Salmonella* risk; C = *Cryptosporidium* risk; G = *Giardia* risk; N = *Norovirus* risk).**

pathogen concentrations in the recreational water are provided in Table 3.

When the measured surfzone fecal indicator level is 35 cfu 100 mL<sup>-1</sup> and gulls contribute 100% of the measured fecal indicator, the predicted median probability of GI illness attributed to gulls was  $3.6 \times 10^{-5}$  for adults. The predicted median illness risk from gulls and the 90th percentile risk

were less than the acceptable GI illness risk benchmark assumed to be 0.01. The median predicted illness risk was 2 log<sub>10</sub> units less than the illness benchmark ( $\log_{10}(0.01) - \log_{10}(3.6 \times 10^{-5}) = 2.4$ ). Therefore, when fecal indicator levels are at the limit of 35 cfu 100 mL<sup>-1</sup> and the dominated fecal contamination is fresh gull feces, the risk to adult swimmers from gulls appears to be substantially less than the illness benchmark based on the existing scientific knowledge on the proportion of human infectious strains from gulls.

The QMRA was rerun with alternative assumed ENT concentrations to determine when the predicted risk from gulls exceeded the illness benchmark. When the assumed ENT concentration was greater than 20,000 cfu 100 mL<sup>-1</sup> ENT, the median predicted risk from gulls exceed the illness benchmark of 0.01.

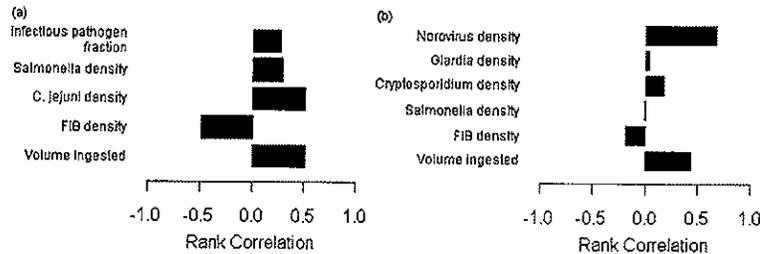
The predicted probability of GI illness attributable to seagulls for a recreational water at the water quality limit had large variability, represented by the range in Figure 1. This large variability in risk was due to the natural variability in the parameters used to estimate the dose, as presented in Table 1. A sensitivity analysis of the probability of GI illness to the dose parameters is presented in Figure 2 using the Spearman rank correlation coefficients of the Monte Carlo simulation for seagull feces and primary POTW effluent contamination. For the risk attributable to seagulls, the volume of water ingested ( $V$ ), the density of FIB in feces ( $R_{ENT}^f$ ), and the density of *C. jejuni* in feces ( $R_{Cj}^f$ ) were of relatively equivalent importance. The density of *Salmonella* in the feces and the fraction of human-infectious pathogens in the feces were relatively less important.

Due to the lack of data on the fraction of the human-infectious pathogen strains from gulls, changes in the

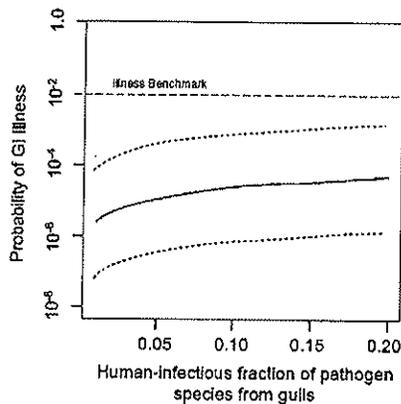
**TABLE 3. Predicted Median (Average) Pathogen Concentrations in Recreational Water Contaminated by Fecal Waste<sup>a</sup>**

| source                | <i>Campylobacter</i><br>(cfu 100 mL <sup>-1</sup> ) | <i>Salmonella</i><br>(cfu 100 mL <sup>-1</sup> )    | <i>Giardia</i><br>(cysts 100 mL <sup>-1</sup> )     | <i>Cryptosporidium</i><br>(oocysts 100 mL <sup>-1</sup> ) | <i>Norovirus</i><br>(genomes 100 mL <sup>-1</sup> ) |
|-----------------------|---|---|---|---|---|
| seagull               | 1.5 × 10 <sup>-1</sup><br>(1.2)                     | 1.6<br>(4.9 × 10 <sup>2</sup> )                     | NA  | NA  | NA  |
| primary POTW effluent | NA  | 4.3 × 10 <sup>-5</sup><br>(1.6 × 10 <sup>-4</sup> ) | 2.0 × 10 <sup>-4</sup><br>(1.2 × 10 <sup>-3</sup> ) | 1.0 × 10 <sup>-4</sup><br>(3.1 × 10 <sup>-3</sup> )       | 1.2 × 10 <sup>-1</sup><br>(2.5)                     |

<sup>a</sup> Predictions based on assumptions in Table 1 for 35 cfu ENT 100 mL<sup>-1</sup>; NA = data not available.



**FIGURE 2. Spearman rank correlation coefficient for dose parameter inputs to the predicted probability of GI illness from accidental ingestion of recreation water containing fresh fecal contamination at 35 cfu 100 mL<sup>-1</sup> ENT for (a) seagull feces and (b) primary POTW effluent.**



**FIGURE 3. Parametric sensitivity analysis of the predicted probability of gastrointestinal illness (median, 10th and 90th percentiles) for adults attributable to *Campylobacter jejuni* from accidental ingestion of recreational water containing fresh seagull fecal contamination at 35 cfu 100 mL<sup>-1</sup> ENT to changes in the assumed fraction of total *C. jejuni* strains from seagulls that are human infectious.**

probability of GI illness due to changes in the human-infectious fraction were examined using a parametric sensitivity analysis. The parametric sensitivity analysis of the probability of illness shows that the illness risk further decreases as the assumed fraction of total pathogen strains from gulls that are human infectious decreases below the best estimate of 0.2 (Figure 3).

An alternative dose-response relationship for *C. jejuni* (the dominant reference pathogen from seagull feces) was tested for a more sensitive mixed population of children and adults (Table 2) to evaluate the impact of model selection on risk using the original Monte Carlo estimates of dose. When the *C. jejuni* dose-response model for sensitive subpopulations was implemented for fresh gull fecal contamination at 35 cfu ENT 100 mL<sup>-1</sup>, the median predicted risk increased less than 1 log<sub>10</sub> unit.

When fresh POTW primary effluent contributes 100% of the measured fecal indicator in Figure 1, the predicted median probability of GI illness by accidental ingestion was 3.1 × 10<sup>-3</sup>, less than 1 log<sub>10</sub> unit from the illness benchmark. The small difference between the QMRA predicted probability of

illness from sewage and the health benchmark provides reassurance that the QMRA method employed yields results similar to those of epidemiology studies investigating POTW-impacted recreational beaches. The large difference between the two median predicted risks for gulls and sewage (i.e.,  $P_{ill}^{sew} = 3.6 \times 10^{-5}$  and  $P_{ill}^{gull} = 3.1 \times 10^{-3}$ ) at 2 log<sub>10</sub> units illustrates that a waterbody at the FIB recreational water quality limit may present a different risk to swimmers depending on the source of the fecal contamination.

The predicted probability of GI illness from fresh primary POTW effluent, like the risk attributable to seagulls, had large variability due to natural variation in the model parameters used in the estimation of dose. The density of *Norovirus* in the effluent ( $R_{N}^{sew}$ ) was identified as the most important parameter based on the rank order correlation coefficients (Figure 2b). *Norovirus* also dominated the total pathogen risk attributable to primary POTW effluent (Figure 1).

The predicted risk from *Norovirus* was based on the assumption that the conditional probability of illness did not decrease with decreasing dose. When a dose-dependent conditional illness for *Norovirus* described by Teunis et al. (44) was substituted, the median predicted probability of GI illness from poorly treated sewage was reduced to 1.3 × 10<sup>-8</sup>. This decrease in risk was due to the reduced conditional probability of illness calculated for the small environmental dose of *Norovirus* (i.e., < 1.0 genome). The dose-dependent conditional probability of illness is highly uncertain at the low dose range (44) and warrants additional research given the potential importance of *Norovirus* in recreational risks.

After *Norovirus*, *Cryptosporidium* and *Giardia* contribute to the total sewage risk. Both parasitic protozoa show a range of virulence with 95% credible intervals for the dose-response parameter  $r$  CI (0.007, 0.3) (43) and CI (0.0097, 0.036) (42), respectively. Sensitivity analysis of the total probability of illness from ingestion of water contaminated by primary POTW effluent to parametric changes in the dose-response parameters for *Cryptosporidium* or *Giardia* within the reported 95% CI showed only small changes in total risk with a difference less than 1 log<sub>10</sub> unit from best estimate parametrizations. However, *Giardia* cysts are likely to lose viability faster than *Cryptosporidium* oocysts, so as with other reference pathogens, the results presented only model conditions of fresh fecal contamination.

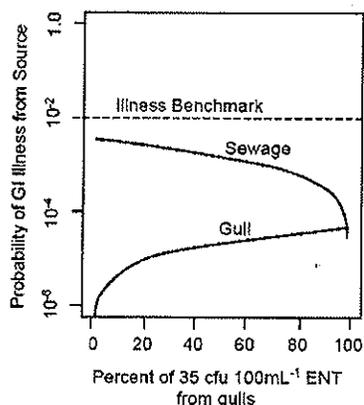


FIGURE 4. Comparison of median illness risk for adults when total ENT concentration (at 35 cfu 100 mL<sup>-1</sup>) is attributed to a mixture of primary POTW effluent (sewage) and seagull feces (gulls).

**Dominant Source in a Mixture of Sources.** Although comparisons of predicted risk to a health benchmark like those presented in Figure 1 are useful in identifying possible sources of pathogen risk from a human health perspective, recreational waters can be contaminated by a mixture of more than one pathogen source. The predicted illness risk for water with a mixture of fresh seagull and sewage waste is presented in Figure 4 to determine when a non-sewage source may dominate illness risk for various sewage/non-sewage mixtures. For each mixture, the pathogen dose was estimated from the dose parameters in Table 1 and the best estimate dose-response relationships for adults in Table 2. For example, when 100% of the ENT at 35 cfu 100 mL<sup>-1</sup> are from gulls, on the far right of Figure 4, the median predicted probability of GI illness from gulls matches that presented in Figure 1. Likewise, when 100% of the ENT at 35 cfu 100 mL<sup>-1</sup> are from sewage, on the far left of Figure 4, the median predicted probability of GI illness from sewage matches that presented in Figure 1. Considering all possible mixtures along the *x*-axis, the median predicted probability of GI illness from gulls was greater than that from sewage only when gulls represent greater than 98% of the fecal indicator load. Therefore, the dominant source of fecal indicator at a recreational beach may not be the source of dominant risk. Conversely, little fresh sewage contamination mixed with non-sewage source(s) may dominate risk.

The range of possible pathogen concentrations expected in a recreational water with a mixture of gull and sewage sources was also calculated. For example, the median predicted *Norovirus* concentration over the range of possible source mixtures containing sewage varied from  $1.2 \times 10^{-1}$  to  $2.3 \times 10^{-3}$  genomes 100 mL<sup>-1</sup>. The predicted distribution of an alternative indicator concentration such as total *Bacteroidales* genomes could also be calculated to aid in source tracking applications as long as the ratio between fecal mass (or volume) and the alternative indicator can be specified.

To extend the presented QMRA approach to non-fresh sources (including treated POTW effluent), additional research is necessary on the differences in persistence between indicators and pathogens over time in the environment. The relationships between indicator and human-associated pathogens in the environment are not well understood due to likely unique survival characteristics of each pathogen and potential growth or shorter persistence of fecal indicators. Generally, *Campylobacter*, *Giardia*, and *Salmonella* die quickly in seawaters exposed to sunlight, possibly less than 24 h (46, 47). It follows then that the risk from seagull feces is likely only relevant when the contamination is recent. This

may not be the case for other bird species or animals with a different set of reference pathogens.

Both *Norovirus* (48) and *Cryptosporidium* (47) have potential to persist in seawaters for days; thereby, changing the indicator to pathogen ratio over time as contamination ages. Furthermore, the use of *Norovirus* as measured by qPCR for non-fresh human sources (such as treated POTW effluent) must assume an aggregation state and a fraction of total to human infectious genomes. Teunis et al. noted that the ratio of total to infectious virions in the inoculum used for the dose-response parametrization was unknown and that the dose-response relationship is only applicable when this ratio is consistent (44). In the absence of information on the aggregation and infectivity of *Norovirus* in sewage, conservative assumptions of non-aggregated *Norovirus* and a fraction of total to human infectious genomes consistent with that from the inoculum were implemented.

Future research quantifying the fraction of total pathogen strains from animals could be important to help rule out sources as a potential human health risk. The sensitivity analysis of the predicted probability of illness from ingestion of water containing gull fecal material to the assumed fraction of total pathogen strains from gulls that are human infectious (Figure 3) showed that as the assumed human-infectious fraction decreased from 0.2, the median illness risk also decreased. This human-infectivity uncertainty is common with many zoonotic pathogen species in addition to secondary environmental sources like sand; hence, it is important that future research focus on specifying pathogen densities and genotypes to allow risk characterization from unstudied sources of fecal contamination at recreational beaches.

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