Letter

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Risk-based threshold of gull-associated fecal marker concentrations for recreational water

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Abstract

A sensitive and specific marker of gull fecal contamination, *Catellicoccus* (CAT), has been used to conduct microbial source tracking in surface waters throughout the world. Yet, there are no guidelines for interpreting measured concentrations. Here, we use quantitative microbial risk assessment (QMRA) to evaluate CAT concentrations within a risk-based framework and develop a threshold at which the USEPA illness benchmark (∼3 illness/100 swimmers) is exceeded. We modeled illness risk from exposure to different concentrations of CAT in bathing waters using a Monte Carlo approach that considered densities of CAT and infectious zoonotic pathogens *Salmonella* and *Campylobacter* in gull feces, volume of water ingested during bathing, and dose-response relationships. We measured CAT densities in 37 fresh gull fecal droppings from six California beaches. Log_{10} densities ranged from 4.6-9.8 log_{10} copies CAT/g wet feces. When CAT exceeds 4×10^6 copies/100 ml water, median predicted illness exceeds 3 illness/100 swimmers.

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Introduction

Microbial source tracking (MST) has been employed at beaches around the world to determine sources of fecal pollution[1–6]. MST often utilizes molecular assays that target bacterial genes ("MST markers") found in the intestinal microflora of particular animal hosts. Identifying pollution sources is not only key to designing remediation strategies, but is also useful for gauging the health risks of swimming in recreational water. Feces from different animals may contain different pathogens with varying potential for infecting humans[7].

Many beaches host large gull populations, and beach managers often suspect that gulls are to blame for coastal water microbial contamination. Gull feces may contain *Salmonella* and *Campylobacter*[8] and the presence of these zoonotic bacterial pathogens in coastal waters has been associated with the presence of gulls[9]. A limited number of epidemiological studies has sought to determine whether non-point source fecal contamination from birds is associated with increased risk of swimmer illness[10,11]. These studies found increased risk of mild illness in swimmers compared to non-swimmers in water believed to be contaminated by bird feces. However, establishing a clear link between the presence of animal feces and human illness using an epidemiology study can be difficult due to factors such as low expected rates of illness associated with exposure to zoonotic pathogens[12].

In response to the need to identify gull-related contamination, several MST markers that are associated with gull feces (gull markers) have been developed[13–15]. Gull markers that target the 16S rRNA gene of *Catellicoccus marimammalium* (CAT)[16–18] have demonstrated sensitivity (73-96%) and specificity (86-96%) to gull and pigeon feces in laboratory studies[14,15]. CAT has been measured in a variety of surface waters and maximum concentrations from 10^4 to 10^6 copies/100 mL have been reported[5,9,18–20]. In some settings, CAT concentrations in bathing waters correlate to gull presence along the shoreline[9,18,19]. However, interpreting the measured concentrations remains confusing as there is no threshold to compare against. This represents a major obstacle to the application and interpretation not only of CAT concentrations, but also to nearly all MST markers that have been developed

to date.

This study uses a risk-based approach to establish a threshold value of CAT in coastal waters. A quantitative microbial risk assessment (QMRA)[21] is used to model illness risk from exposure to bathing waters contaminated with different levels of CAT. The QMRA utilizes Monte Carlo simulations[22–24] that sample from distributions including CAT concentrations in gull feces, pathogen concentrations in gull feces, and ingested water volumes.

QMRA has been used previously to model illness risk associated with swimming in gull feces-contaminated water[7,8], but those studies related a traditional fecal indicator for marine water, culturable *Enterococcus* (ENT), to modeled risk rather than CAT. The QMRA approach we used has been recommended by USEPA[25,26], harmonized with an epidemiology study[23], and applied to model risk from exposure to a range of bathing waters[8,22,24,27,28].

Methods

Feces collection. Thirty-seven gull (*Larus californicus* and *L. occidentalis*) fecal samples were collected at six Californian beaches (Figure S1) using methods described in the SI. After weighing individual fecal samples, each was added to 200 ml of deionized (DI) water and the water/feces mixture was shaken vigorously to create a slurry. Fecal slurries were filtered within 6 hours of collection.

CAT quantification. Between 10 and 200 ml (depending on turbidity) of the slurries were filtered through polycarbonate 0.4-μm pore size filters (EMD Millipore, Billerica, MA)[15]. One filtration blank, consisting of sterile DI water, was filtered every 12 samples. Filters were stored at -80°C (in a freezer or a cooler on dry ice) until DNA extraction. DNA was extracted from filters using a DNA-EZ ST1 kit (Generite, North Brunswick, NJ), previously shown to have good DNA recovery and limited co-extraction of inhibitors[29]. One filterless extraction blank was processed alongside the sample extractions. Extracted DNA was stored for a maximum of 30 days at -20°C before analysis.

CAT concentrations were quantified using quantitative polymerase chain reaction (qPCR) following Lee et al.[18], with the modification that Taqman®Environmental Master Mix 2.0 (Applied Biosystems, Foster City, CA) was used to decrease the possibility of inhibition[30]. This assay was chosen as it was one of the best performing CAT assays in a multi-laboratory method evaluation study[15]. Inhibition was tested using the spike-and-dilute method[30]. Information on primer and probe sequences, standard curves, and negative controls are given in the SI. CAT copies/reaction measured by qPCR were converted to copies/g of wet feces. The concentrations of CAT in gull feces were $\log_{10}$-transformed and a probability density function was fitted to the data using MATLAB (Natick, MA).

QMRA

QMRA was conducted to predict the probability of gastrointestinal illness from a single swimming event in recreational water with varying concentrations of CAT from gull feces. Using the concentration of CAT in the recreational water, the model calculates reference pathogen doses, and then the probabilities of infection and illness associated with those doses. MATLAB was used to run Monte Carlo simulations (n=10,000 trials for each CAT concentration). Each trial drew from distributions of the input variables to incorporate their inherent uncertainty and variability. It was assumed that (1) CAT comes from fresh gull feces, and (2) only gulls, not pigeons or other animals, are the source of CAT.

Estimating reference pathogen dose. The expected reference pathogen dose, μ_{rp}, from non-dietary ingestion of gull-contaminated water was estimated by equation 1[8]:

$$\mu_{rp} = \frac{C_{CAT}}{F_{CAT}} \times R_{rp} \times p_{rp} \times V \quad (1)$$

where C_{CAT} is the concentration of CAT in ambient seawater [copies/100 ml], F_{CAT} is the concentration of CAT in wet gull feces [copies/g], R_{rp} is the concentration of pathogen species in wet gull feces [colony forming units (CFU)/g], p_{rp} is the fraction of human-infectious

pathogenic species or serotypes in gull feces[8], and V is the volume of seawater ingested [ml].

μ_{rp} was calculated for two reference pathogens, *Campylobacter* and *Salmonella*[7]. For each discrete order-of-magnitude value of C_{CAT} ranging from $10^3 - 10^7$ copies/100 ml, a distribution of μ_{rp} was generated with Monte Carlo trials by drawing values for parameters in equation 1 (Table 1).

The ratio $f = C_{CAT}/F_{CAT}$ represents the amount of gull feces present per volume of ambient water [g feces/100 ml]. An upper constraint of $f = 10$ g feces/100 ml seawater was applied as an upper limit, as this amount of contamination is extreme ($\sim 10\%$ by mass). If during any particular trial the draw from the F_{CAT} distribution was low enough to result in a violation of that constraint, then a new value was drawn from the F_{CAT} distribution until f was less than 10 g/100 ml. The number of times F_{CAT} was re-drawn per value of C_{CAT} is shown in Table S3.

Estimating probability of illness. The probability of illness for one reference pathogen, $P_{ill,rp}$ as a function of μ_{rp} was calculated following the method described by Teunis et al.[31]. This method estimates $P_{ill,rp}(\mu_{rp})$ with a series of two dose-response functions: the first estimates the probability of infection from one reference pathogen, $P_{inf,rp}$, and the second estimates the probability of illness given infection for one reference pathogen, $P_{ill|inf,rp}$. The choice of dose-response relationships is discussed in the SI.

Teunis et al.[31] and Teunis et al.[32] used pooled data from campylobacterosis and salmonellosis outbreaks, respectively, to develop hypergeometric $P_{inf,rp}$ dose-response relations. The hypergeometric equations arise from integrating over a distribution of ingested doses, as is necessary when only the mean dose ingested by a population is estimated or known. The corresponding conditional dose-response relationship that applies to cases, such as QMRA, when the exact dose is calculated, is given by Equation 2[33,34]:

$$P_{inf,rp}(\mu_{rp}) = 1 - \frac{B(\alpha, \beta + \mu_{rp})}{B(\alpha, \beta)} \tag{2}$$

where B is the standard beta function, and α and β are parameters for beta-distributed mean host sensitivities[33]. The second dose-response function, for $P_{ill|inf,rp}$, is given by Equation 3:

$$P_{ill|inf,rp}(\mu_{rp}) = 1 - (1 + \eta\mu_{rp})^{-\rho} \quad (3)$$

where η and ρ are parameters describing the distribution of duration of infection[32]. The model parameters α, β, η, and ρ for *Salmonella*[32] and *Campylobacter*[8,31] are shown in Table 2. It is assumed that all exposed hosts are susceptible to illness.

The probability of illness for each reference pathogen is then calculated as $P_{ill,rp} = P_{inf,rp} \times P_{ill|inf,rp}$. Finally, the total probability of illness due to the presence of either pathogen, P_{ill}, is calculated using Equation 4[7]. It is assumed that hosts are only infected with one pathogen at a time.

$$P_{ill} = 1 - \prod_{rp}(1 - P_{ill,rp}) \quad (4)$$

The final results of the Monte Carlo simulations were P_{ill} distributions. Distributions were compared to a threshold of 3 illnesses per 100 swimmers, the approximate illness threshold recommended by the EPA[26].

Sensitivity analysis. Sensitivity analyses were conducted following the method of Xue et al.[35] to test the effects of changing individual variables on P_{ill} (see SI).

Results and Discussion

Concentration distributions. All positive and negative controls for the CAT assay resulted as expected. No PCR inhibition was observed. CAT concentrations in gull feces ranged from 10^2 and 10^{10} copies/g, with most concentrations between 10^8 and 10^9 copies/g (Table S4, Figure S2). Data are described by a Weibull distribution with scale and shape parameters $\pm$ 95% confidence intervals of $a = 8.73 \pm 0.180$ and $b = 8.26 \pm 1.12$, respectively.

Probability of illness. P_{ill} increases with C_{CAT} (Figure 1). There is a linear relationship between the $\log_{10}$-transformed median probability of illness and the $\log_{10}$-transformed CAT concentration: $\log_{10}$ median P_{ill} = -10.2 + 1.3 $\times$ $\log_{10} C_{CAT}$, R^2 = 0.98 (Figure S3)[36]. Based on this regression, median P_{ill} equals 0.03 when $C_{CAT} = 4 \times 10^6$ copies/100 ml. For $C_{CAT} = 6 \times 10^5$ and $C_{CAT} = 2 \times 10^7$ copies/100 ml, the 75th and 25th percentiles of the P_{ill} distribution is 0.03, respectively (see SI).

The relative contributions of *Campylobacter* and *Salmonella* to P_{ill} vary depending on C_{CAT} (Figure S4). For $C_{CAT} = 10^6 - 10^7$ copies/100 ml, the probability of illness due to *Campylobacter* ($P_{ill,C}$) is greater than the probability of illness due to *Salmonella* ($P_{ill,S}$) by nearly an order of magnitude. CAT is a novel alternative indicator so data on environmental concentrations are limited. A mean ambient CAT concentration as high as 2.8×10^6 copies/100 ml has been reported for a Lake Ontario beach with high observed gull impact[19]. At a Lake Erie beach, a maximum CAT concentration of 5.5×10^6 copies/100 ml was measured[18]. Based on the results of this study, at those concentrations, illness rates might exceed the threshold of 0.03.

In a previous QMRA that considered gull fecal contamination, Schoen and Ashbolt[8] estimated P_{ill} from exposure to a seawater concentration of 35 ENT colony forming units (CFU)/100 ml from a gull fecal source. The authors found the risk to adult swimmers from gull feces at that concentration ($\sim 10^{-4.5}$) is substantially less than a risk threshold of 0.01. Because we expect gull feces to contain 10-100 copies of CAT per CFU ENT[14], a concentration of 35 ENT CFU/100 ml from gulls would correspond to a concentration of 350-3500 copies CAT/100 ml. For that CAT concentration range, the present study predicts a probability of illness much less than the threshold (at most $\sim 10^{-6}$), consistent with the previous results.

A previous study[36] estimated a risk-based threshold for human-specific fecal markers. They found a median risk of 0.03 when HF183 concentrations were $\sim 10^3$ copies/100 ml. The HF183 threshold is three orders of magnitude smaller than that CAT threshold, a a direct

result of the diverging concentrations of MST markers and pathogens in human versus gull feces.

Sensitivity analysis. The sensitivity analysis indicated the model is most sensitive to F_{CAT}, R_C, and V at concentrations of C_{CAT} near the threshold value 10^6 copies/100 ml. P_{ill} estimates, therefore, could be improved by reducing uncertainty in the distributions of those variables. Because to date there are few studies that characterize the F_{CAT} distribution, additional measurements would be particularly valuable. In contrast, although there is considerable uncertainty in p_{rp}[8], additional research to reduce that uncertainty is unlikely to improve estimates of P_{ill} in this model.

Study Limitations. An important consideration in estimating P_{ill} is the age of the gull feces, that is, the elapsed time between feces deposition and collection. This QMRA study specifically estimates the risk from exposure to unaged gull feces deposited in recreational water. The concentrations of both CAT and pathogens will decay over time in environmental matrices[37], and not necessarily at the same rates. The differential decay of CAT and pathogens therefore remains an important area for future research.

An additional consideration is that CAT has been detected not only in gull, but also in pigeon feces[14,15], and it may be present in other birds as well[13]. Pigeon feces contain the same reference pathogens[25] as gull feces: *Campylobacter*[38] and *Salmonella*[39]. However, the concentrations and fractions of infective species may differ among bird feces, resulting in a different prediction of P_{ill}. Further limitations of using the dose-response models and the Dufour et al.[40] estimates for V are described in the SI.

Supporting Information Available

Additional information on methods, as well as tables and figures. This material is available free of charge via the Internet at `http://pubs.acs.org/`.

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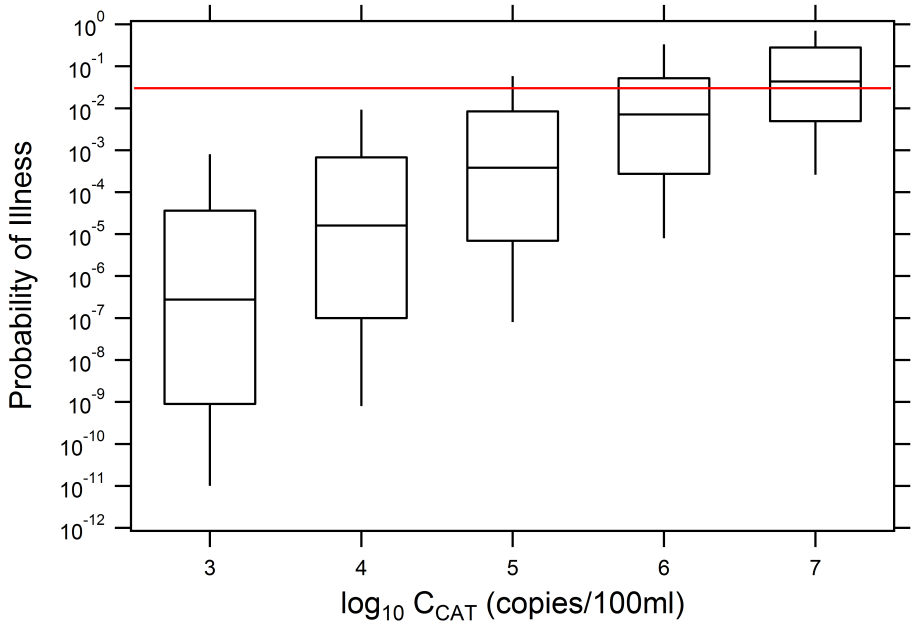

Figure 1: Probability of illness, P_{ill}, predicted when different concentrations of the gull marker, C_{CAT}, are present in ambient water. The midline of each box represents the median, the bottom and top of each box represent the first and third quartile, respectively, and the bottom and top whisker represent the 10th and 90th percentile, respectively. The red line indicates the threshold of 3 cases of illness/100 swimmers.

Table 1: Variable distributions used in Monte Carlo simulations to calculate the reference pathogen dose, μ_{rp}, from incidental ingestion of seawater. A and B are the scale and shape parameters of a Weibull distribution fit to $\log_{10}$-transformed F_{CAT} data; a and b are the upper and lower bounds of a $\log_{10}$-uniform distribution for R_S and R_C; c and d are the upper and lower bounds of a uniform distribution for p; C and D are the ln-mean and standard deviation of a natural-log normal distribution. The medians of the distributions (as defined in the table) are as follows: F_{CAT}: 8.35, R_S: 5.6, R_C: 4.6, p: 0.2, and V: 2.92.

variable	**units**	**distribution parameters**	**reference**
density of CAT in gull feces (F_{CAT})	copies/g wet feces	A=8.73, B=8.26	this study
density of *Salmonella* in gull feces (R_S)	CFU/g wet feces	$a = 2.3$ $b = 9.0$	Lévesque et al.[41]
density of *Campylobacter* in gull feces (R_C)	CFU/g wet feces	$a = 3.3$ $b = 6.0$	Lévesque et al.[41]
human-infectious fraction of pathogen strains (p)	–	c=0.01 d=0.4	Schoen and Ashbolt[8], Fenlon[42]
volume of water ingested	ml	$C = 2.92$ $D = 1.43$	Dufour et al.[40]

Table 2: Dose-response parameters used to calculate $P_{inf,rp}$ and $P_{ill|inf,rp}$ for *Salmonella* and *Campylobacter*. α and β are parameters for beta-distributed mean host sensitivities (Equation 2), and η and ρ are parameters describing the distribution of duration of infection (Equation 3).

pathogen	**parameter**				**reference**
	α	β	η	ρ	
Salmonella	8.53×10^{-3}	3.14	69.0	8.23	Teunis et al.[32]
Campylobacter	2.4×10^{-2}	1.1×10^{-2}	3.6×10^{-9}	2.4×10^{8}	Teunis et al.[31]

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