This is an open access article published under an ACS AuthorChoice License, which permits copying and redistribution of the article or any adaptations for non-commercial purposes.

Environmental Science & Technology

Article

pubs.acs.org/est

Human and Bovine Viruses and Bacteria at Three Great Lakes Beaches: Environmental Variable Associations and Health Risk

Steven R. Corsi,[*,†] Mark A. Borchardt,[‡] Rebecca B. Carvin,[†] Tucker R. Burch,[^] Susan K. Spencer,[‡] Michelle A. Lutz,[†] Colleen M. McDermott,[§] Kimberly M. Busse,[§] Gregory T. Kleinheinz,[§] Xiaoping Feng,[⊥] and Jun Zhu[⊥]

[†]U.S. Geological Survey, Wisconsin Water Science Center, 8505 Research Way, Middleton, Wisconsin 53562, United States

[‡]U.S. Department of Agriculture, Agricultural Research Service, 2615 Yellowstone Dr., Marshfield, Wisconsin 54449, United States

[§]Department of Biology and Microbiology, University of Wisconsin Oshkosh, 800 Algoma Boulevard, Oshkosh, Wisconsin 54901, United States

[⊥]Department of Statistics, University of Wisconsin−Madison, 1300 University Avenue, Madison, Wisconsin 53706, United States

[^]U.S. Geological Survey, Wisconsin Water Science Center, 2615 Yellowstone Drive, Marshfield, Wisconsin 54449, United States

Supporting Information

ABSTRACT: Waterborne pathogens were measured at three beaches in Lake Michigan, environmental factors for predicting pathogen concentrations were identified, and the risk of swimmer infection and illness was estimated. Waterborne pathogens were detected in 96% of samples collected at three Lake Michigan beaches in summer, 2010. Samples were quantified for 22 pathogens in four microbial categories (human viruses, bovine viruses, protozoa, and pathogenic bacteria). All beaches had detections of human and bovine viruses and pathogenic bacteria indicating influence of multiple contamination sources at these beaches. Occurrence ranged from 40 to 87% for human viruses, 65−87% for pathogenic bacteria, and 13−35% for bovine viruses. Enterovirus, adenovirus A, *Salmonella spp.*, *Campylobacter jejuni*, bovine polyomavirus, and bovine rotavirus A were present most frequently. Variables selected in multiple regression models used to explore environmental factors that influence pathogens included wave direction, cloud cover, currents, and water temperature. Quantitative Microbial Risk Assessment was done for *C. jejuni*, *Salmonella spp.*, and enteroviruses to estimate risk of infection and illness. Median infection risks for one-time swimming events were approximately 3×10^{-5}, 7×10^{-9}, and 3×10^{-7} for *C. jejuni*, *Salmonella* spp., and enteroviruses, respectively. Results highlight the importance of investigating multiple pathogens within multiple categories to avoid underestimating the prevalence and risk of waterborne pathogens.

INTRODUCTION

Disease transmission through fecal contamination of recreational waters has long been documented as a public health concern.[1] Studies of waterborne pathogens in recreational waters have demonstrated that fecal indicator bacteria (FIB) can be poor surrogates for pathogens.[2−6] As a result, some recent research has placed more emphasis on direct detection of pathogens. Analytical techniques have evolved, ranging from occurrence (presence or absence), to a quantitative outcome for a limited number of pathogens per analysis, to the current state where multiple pathogens from multiple classes of microorganisms can be quantified in each collected sample.[7−13]

Recreational waters are often influenced by complex watersheds that have multiple sources of fecal contamination. Human sources of pathogens include treated wastewater from municipal treatment systems, partially treated wastewater during periods when flow exceeds capacity ("blending" events), overflows of sanitary or combined sewers (SSOs and CSOs, respectively), failing or misconnected sanitary sewer lines, properly functioning or failing septic systems, land-surface application associated with septic maintenance, as well as direct bather shedding in recreational waters.[14−18] Nonhuman sources of pathogens include leakage from livestock manure holding ponds and storage areas, runoff from pastures and land-applied manure, and wildlife.[3,19−22] Once released into the environment, pathogens can be transported to surface waters through multiple pathways, including overland flow and shallow groundwater; which sources are present and the manner in which pathogens are transported to recreational waters are site specific. Differences in transport mechanisms determine the resulting survival, occurrence, and magnitude of waterborne pathogens.[23] Some pathogens have the capability to survive for long periods or even grow in the beach environment.[24,25]

Received: September 14, 2015
Revised: December 4, 2015
Accepted: December 14, 2015
Published: December 31, 2015

 © 2015 American Chemical Society

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

Combining current analytical capabilities with the increased availability of environmental data provides opportunities to investigate the effect of environmental factors such as fecal source, storm events, water temperature, suspended solids, turbulence, sunlight intensity, water nutrient content, and predation[3,26] on pathogen occurrence. These environmental conditions also affect occurrence and survival of co-occurring FIB. In recent years, a number of predictive models have been developed that link environmental conditions to FIB concentrations at beaches.[12,27] However, the development of predictive models relating environmental conditions directly to pathogen occurrence has been limited; those that do exist have been distributed among different microorganisms that have potentially different sources, influencing factors and hydrologic settings.[7,13,28−31]

The objectives of this study were (1) to quantify concentrations of multiple pathogens within four microbial categories (human viruses, bovine viruses, pathogenic bacteria, and protozoa) at three Lake Michigan beaches, (2) to identify environmental conditions that influence pathogen presence and variability, and (3) to estimate health risks for swimmers at the three beaches by conducting quantitative microbial risk assessments.

MATERIALS AND METHODS

Study Sites. Three beaches along the Wisconsin shore of Lake Michigan were selected for study based on land cover characteristics of contributing watersheds (Figure 1, Table S1 in Supporting Information). Land cover in the Clay Banks beach watershed is dominated by forested wetland (63%) and pasture/hay (17%). Possible sources of pathogens within the watersheds included wastewater treatment effluent, impervious runoff, agricultural runoff, and rural septic systems. Longshore currents may also deliver water from the Sturgeon Bay Ship Canal to the beach area, 3.4 km distant. The Sturgeon Bay Ship Canal connects Green Bay to Lake Michigan and receives wastewater effluent from the city of Sturgeon Bay located 6.3 km inland from Lake Michigan. Land cover in the Point Beach watershed is dominated by 29.9% pasture/hay, 28.0% cultivated crops and 28.2% forested wetland. The beach is 4.3 km from the nearest tributary (Molash Creek). The Red Arrow beach watershed has two potentially influential subwatersheds: a storm sewer that drains directly on the south boundary of the beach and the Manitowoc River 2.5 km north of the beach. The immediate drainage area of the beach has 99.8% urban land cover, and the Manitowoc River is dominated by 69.5% combined agricultural land cover. In addition, effluent from the City of Manitowoc wastewater treatment facility is located offshore approximately 1 km from the beach.

Data Collection. *Pathogen Sampling.* Pathogens were sampled from lake water by glass wool filtration[33,34] using a custom portable filtration system and prefilter (Figure S1). The sample inlet was anchored in water 60 cm deep at a depth of 30 cm above the lake bottom. Mean sample volume was 204.4 L (range 66.8−250.5 L, n = 71). Within 24 h of sampling, filters were shipped on ice to the analytical laboratory. The prefilter and glass wool filter were immediately eluted and the eluates further concentrated by polyethylene glycol.[33] Final concentrated sample volumes (FCSV) (mean = 4.2 mL, range 1−55 mL) were stored at −80 °C until analysis. The two FCSVs from a prefilter and glass wool filter pair were carried separately through subsequent analytical steps and the final results summed to yield final pathogen concentrations.

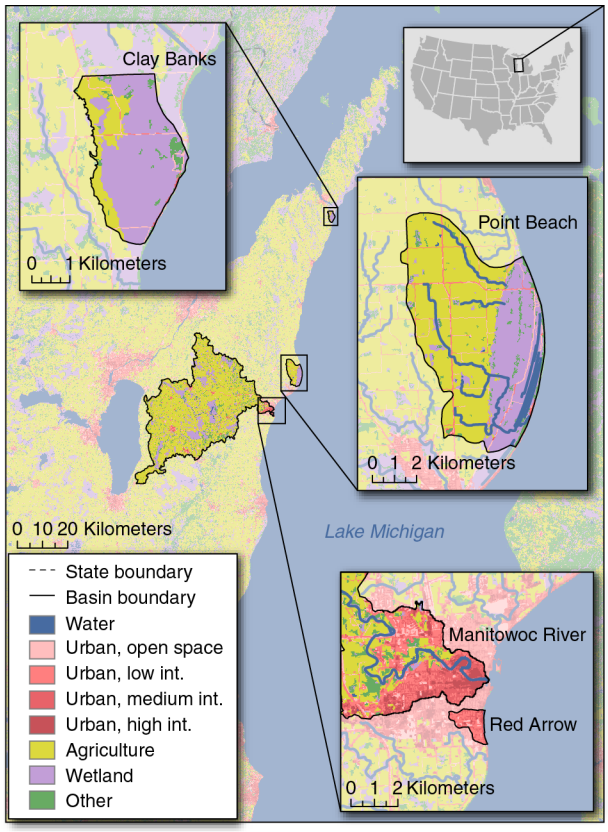

Figure 1. Location of watersheds and sample locations on three public beaches in Wisconsin. Base layer from National Land Cover Data set.[32] Watershed boundaries were manually defined onto 1:24 000 (7.5 min) USGS topographic quadrangle digital base map. Other category includes open water, forest, shrub, grassland, and barren land. Wetland category includes herbaceous wetland and forested wetland.

Environmental Data. Water samples and manually measured physicochemical parameters were collected at the three beaches and nearby tributaries 4 days per week for 14 weeks from the end of May to the beginning of September, 2010. Measured parameters included specific conductance, water temperature, turbidity, estimated wave height, air temperature, and water level or streamflow for tributaries (Table S2). All water samples were collected at the center of the beach, 30 cm below the surface in water with a depth of about 60 cm. Samples were analyzed quantitatively for the FIB, *E. coli* and enterococci, using Colilert and Enterolert (IDEXX Corp., Portland, ME). Environmental data were collected using protocols outlined in the Great Lakes Beach Sanitary Survey User Manual (2008).

Additional data collected included continuous time series of precipitation from on-site USGS monitoring stations, hydrometeorological modeling results from the NOAA Great Lakes Coastal Forecasting System,[35] daily measurement of lake water level from NOAA, and radar-indicated rainfall from the US National Weather Service North Central River Forecast Center (Table S2).

Microbiological Analysis. *Bacterial Culture.* *Campylobacter jejuni* and *Salmonella* spp. colony-forming units (CFU) were enumerated by plating 100 μL FCSV on CVA media (Cefoperazone, Vancomycin, and Amphotericin B) and MacConkey media, respectively, and incubated following conventional procedures for these bacteria.[36,37] Colonies

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

identified morphologically as *C. jejuni* and *Salmonella* spp. were confirmed by quantitative polymerase chain reaction (qPCR).

qPCR Analyses. qPCR was performed for genes specific to 21 waterborne pathogens in three categories. (1) Human enteric viruses: adenovirus A, adenovirus B, adenovirus C, D, and F, norovirus genogroups I and II, enterovirus, hepatitis A virus, and rotavirus; (2) Bovine enteric viruses: bovine coronavirus, bovine polyomavirus, bovine rotavirus group A, bovine rotavirus group C, bovine adenovirus, bovine enterovirus, bovine viral diarrhea virus Types 1 and 2; and (3) Pathogens not specific to humans or cattle: *Campylobacter jejuni*, *Salmonella* spp., enterohemorrhagic *E. coli*, *Mycobacterium avium* subsp. paratuberculosis, and *Cryptosporidium parvum*.

PCR and reverse transcription (RT) procedures were identical to those described in Borchardt et al. (2012, Supporting Information) except the reverse transcriptase SuperScript II was replaced with SuperScript III (Life Technologies, Grand Island, NY). Nucleic acid extraction procedures and measurement of PCR inhibition are described in Supporting Information. No-template controls for PCR, RT, and extraction steps were performed for every qPCR assay; all controls were negative (i.e., no Cq value). Equipment blanks were performed in the field at three beaches on one date and two beaches a month later; all five equipment blanks were negative. Quality assurance measures for the qPCR standard curves and sources and enumeration of the positive controls are reported in the Supporting Information, Table S3.

Data Analysis. Results were explored using pairwise Spearman correlation analysis (at $p = 0.05$ significance level) and multiple linear regression with pathogens as the response variables and the water quality and hydro-meteorological variables as predictors (Table S2). Data were combined for the three sites with a predictor variable term to differentiate among sites. The number of pathogen observations per site was not enough to warrant regression models for each individual site except for the sum of human viruses at Clay Banks beach. Regression analyses were attempted only for pathogens and pathogen classes with greater than 40% occurrence. This included enterovirus, the sum of human viruses, *Campylobacter jejuni*, *Salmonella* spp., the sum of pathogenic bacteria, sum of human pathogens (i.e., human virus and bacteria), and the sum of all pathogens as response variables.

Predictor variables for the full data set were screened in three stages for selection in the final regression models. The first stage examined the utility of predictor variables that had missing values using the least absolute shrinkage and selection operator (LASSO).[38] Predictors with missing values included *E. coli*, specific conductance, turbidity, water temperature, and manual wave-height estimate. These predictors were not valuable enough in describing pathogen variability to warrant exclusion of observations and were eliminated from further consideration. The second stage included selection among the remaining predictors using LASSO. For cross validation to minimize spurious relations in variable selection, the LASSO routine was run 100 times, using a different 10-fold cross validation each time. Predictor variables that were present in at least 25% of the models were retained for the final stage of variable selection using stepwise left-censored maximum likelihood estimation. For the individual data sets, there were not sufficient observations to use the variable selection technique described above, so heuristic models fit by left-censored maximum likelihood estimation were explored with variable selection based on prior knowledge of the individual beaches. Data analyses were done using the R project for statistical computing with core functionality and the glmnet, censReg packages.[39−41] The censored value regression was based on the standard Tobit model using maximum likelihood estimation.[42] The rationale for this data analysis approach is described in the Supporting Information.

Quantitative Microbial Risk Assessment. A quantitative microbial risk assessment (QMRA) was conducted for each of three organisms (enteroviruses, *Campylobacter jejuni*, and *Salmonella* spp.) Each QMRA consisted of three steps: (1) simulate concentrations, (2) calculate dose using simulated ingestion volumes, and (3) calculate probability of infection or illness using dose−response relationships. The computational approach included a two-dimensional Monte Carlo simulation with each simulation consisting of 10 000 iterations in the variability dimension (e.g., swimming ingestion rate) and 1000 iterations in the uncertainty dimension (e.g., dose−response parameters; Tables S4−S6). Risk was defined as the probability of infection per swimming event for enteroviruses and the probability of illness per swimming event for *C. jejuni* and *Salmonella* spp. Analysis was done in the R project for statistical computing with the mc2d package.[39,43]

Enterovirus and *C. jejuni* gene copy concentrations were predicted using the Tobit regression models described above (Tables S4−6). Model input values were simulated based on the distribution of values for each variable throughout the summer of 2010. Enterovirus values were then converted to plaque-forming units (PFU) using an assumed PFU to gene copy ratio of 0.01[44] and *C. jejuni* concentrations were converted to CFU using a study-specific CFU to gene copy ratio (Table S5, Figure S2). *Salmonella* spp. concentrations were simulated by random sampling from the empirical distribution of measured *Salmonella* spp. CFU concentrations (Table S6) since a Tobit model was not available.

Ingestion volumes were calculated as the product of simulated distributions of swimming time and ingestion rate based on the previously published data.[45] The log-normal distribution parameters for both variables were provided by Laura Suppes (University of Wisconsin−Eau Claire). These parameters, which are in the Supporting Information as Tables S4−S6, have not been previously published. However, they were derived from data for which statistical summaries have been previously published.[41] Doses were calculated by multiplying simulated concentrations by simulated distributions of ingestion volume.

The probability of infection for enteroviruses was calculated using an echovirus beta-Poisson dose−response model.[46] The probability of illness for enteroviruses could not be estimated because illness dose−response and morbidity data were unavailable. Distributions of the echovirus dose−response parameters, alpha and N_{50}, were represented as mixtures of uniform distributions defined by the parameters' percentiles (Table S4). The probability of infection for *C. jejuni* was calculated using a beta-Poisson model;[47] distributions containing 10 000 pairs of alpha and beta for *C. jejuni* were provided by Philip Schmidt (Table S5). The probability of illness for *C. jejuni* was calculated by multiplying probability of infection by a morbidity ratio of 0.28.[48] The probability of illness for *Salmonella* spp. was calculated using a conditional dose−response model, including a beta-Poisson model predicting the probability of infection and a model predicting the conditional probability of illness given infection.[49] The *Salmonella* spp.

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

dose−response model used was derived from outbreak data, and distributions containing 5000 sets of the dose−response parameters were provided by Norval Strachan (University of Aberdeen; Table S6). Evaluations of all beta-Poisson models were obtained using the Kummer confluent hypergeometric function in the gsl package in R[50] to avoid overestimating risk at low doses.[51] Total risk for multiple pathogens combined was computed as $1 - \prod_{3\ \text{pathogens}}(1 - P_{ip})$ where P_{ip} was the probability of illness from an individual pathogen.[52]

RESULTS

Occurrence and Magnitude. Human pathogens (viruses and bacteria) were detected in 67 of 71 samples collected at the three beaches. Detection frequencies for pathogen categories included 66% for human viruses, 24% for bovine viruses, and 85% for pathogenic bacteria. Protozoa (*Cryptosporidium parvum*) were not detected. All three beaches had detections of human and bovine viruses and pathogenic bacteria indicating the influence of multiple contamination sources (Figure 2). Detection of human viruses and pathogenic bacteria were greater than that for bovine viruses at all sites.

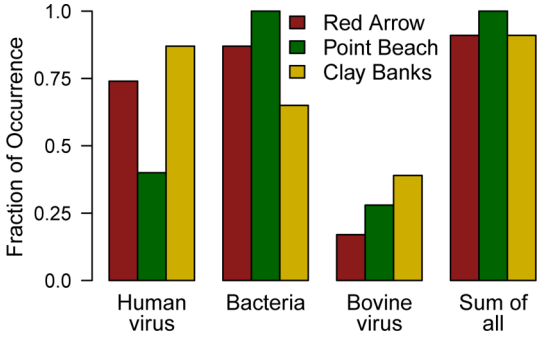

Figure 2. Occurrence of human viruses, pathogenic bacteria and bovine viruses at three Lake Michigan beaches during summer 2010.

Concentrations of total pathogens varied over 6 orders of magnitude, ranging from undetected to 81 000 genomic copies per liter (gc/L), with a mean of 1555 gc/L and median of 132 gc/L (Figure 3). Most samples resulted in total pathogen concentrations between 10 and 1000 gc/L (86%) with 8.5% of samples resulting in concentrations greater than 1000 gc/L. Human viruses and pathogenic bacteria were primarily responsible for the largest concentrations observed.

The most prevalent human viruses in samples were enterovirus (47% occurrence) followed by adenovirus A (27% occurrence) and adenovirus C, D and F (14% occurrence) with a maximum concentration of 6800 gc/L for enterovirus at Clay Banks beach. Adenovirus B, norovirus genogroup I, norovirus genogroup II, hepatitis A, and rotavirus were not detected during this study.

Among the bovine viruses tested, bovine polyomavirus and bovine rotavirus A were detected most frequently (14% and 10% occurrence respectively), and at the highest concentrations (Figure 4). Bovine enterovirus and bovine viral diarrhea virus Type 1 were each detected in one sample. Coronavirus, bovine rotavirus C, bovine adenovirus, and bovine viral diarrhea virus Type 2 were not detected.

Pathogenic bacteria were present most frequently (85% occurrence) and at the highest concentrations of all pathogen categories (Figures 3-4). *Salmonella* spp. and *Campylobacter jejuni* concentrations measured by qPCR were on the order of

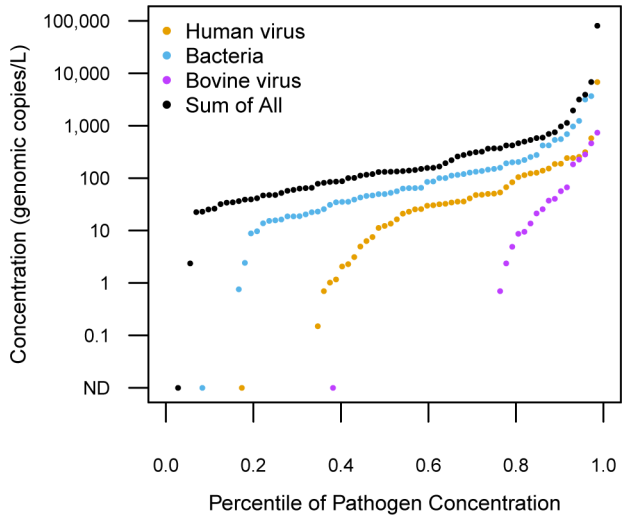

Figure 3. Cumulative distribution of human-specific virus, pathogenic bacteria, and bovine virus concentrations in beach samples at three Lake Michigan beaches during summer 2010.

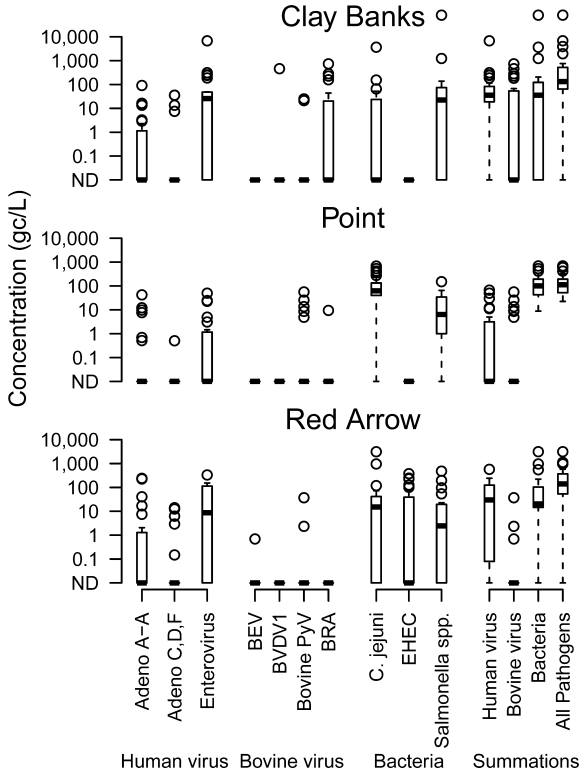

Figure 4. Boxplots of concentrations of human viruses, bovine viruses, and pathogenic bacteria in samples from three Lake Michigan beaches during summer 2010. Adeno, adenovirus; BEV, bovine enterovirus; BVDV1, bovine viral diarrhea virus Types 1 and 2; PyV, polyomavirus; BRA, bovine rotavirus A; *C. jejuni*, *Campylobacter jejuni*.

10 to 1000 gc/L; *Salmonella* spp. was detected in one sample from Clay Banks beach site at 81 000 gc/L, which was the highest individual pathogen concentration of the study. The concentration medians and ranges of *Salmonella* spp. and *C. jejuni* measured by culture (n = 87) were 0.2 (0−1,033) and 0 (0−117) CFUs/L, respectively. Enterohemorrhagic *E. coli* was detected in 6 of 23 samples collected from Red Arrow beach and was not detected in samples from other beaches.

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

Mycobacterium avium subsp. *paratuberculosis* IS900 was detected in four samples with at least one detection at each beach. None of these detections were confirmed as *Mycobacterium avium* subsp. *paratuberculosis* using the 251 genetic marker.[53]

Fecal indicator bacteria results varied from less than 1 MPN/100 mL to >2420 MPN/100 mL with medians of 24 and 62.4 MPN/100 mL and means of 171.1 and 342 MPN/ml for *E. coli* and enterococci respectively (Figure S3). There were no consistent correlations of *E. coli* or enterococci to human pathogens or bovine viruses, and pathogens were present in samples that ranged from less than detection limits to greater than detection limits for *E. coli* and enterococci concentrations.

Relation to Environmental Conditions. Pairwise analyses showed a number of environmental parameters were correlated with pathogen concentrations (Table S7). Multiple linear regression models were developed to examine these relations further. Exploration of models for individual beaches resulted in only one model that explained significant variability: human virus concentrations at Clay Banks beach. For this model, the most important predictor was the water surface slope between Menomonee, Michigan, in Green Bay, to the east end of the Sturgeon Bay Shipping Channel, near Clay Banks beach (Table 1). This predictor provided an indication of flow direction in the Sturgeon Bay Shipping Channel.

Multiple linear regression modeling yielded models for six pathogen categories when the data from the three beaches were combined (Table 1). Wave direction variables were important in five models, cloud cover variables were important in four models, and currents and water temperature variables were important in one model each. Three of the six regression models indicated that Point Beach had either higher concentrations (*Campylobacter* and sum of pathogenic bacteria) or lower concentrations (human viruses) under conditions similar to the other beaches. Regression models for *Salmonella spp.* and bovine viruses did not result in selection of predictive variables that explained significant variability.

QMRA. The one-time swimming event risk was estimated for exposures to enterovirus, *C. jejuni*, and *Salmonella* spp. at the three beaches (Figure 5). At the median uncertainty level, median probabilities of *C. jejuni* illness, *Salmonella* spp. illness, and enterovirus infection are 2×10^{-5}, 8×10^{-6}, and 3×10^{-7}, respectively (Figure 5). These values are all lower than the EPA recreational water benchmark of 32 illnesses per 1000 primary contact recreators per event.[54] Enterovirus risk estimates never exceeded the EPA benchmark at the median uncertainty level. For *C. jejuni* and *Salmonella* spp., the benchmark was exceeded for 0.1% and 6.4% of cases at the median uncertainty level, respectively. For *Salmonella* spp., 25% of cases exceeded the benchmark at the 75th percentile of uncertainty.

Risk estimates varied considerably in the variability and uncertainty dimensions, although risk estimates for all three pathogens were more variable than uncertain (Table S8). In other words, QMRA inputs such as water ingestion rate and pathogen concentration contributed more to the variation in the risk estimates than model coefficients (water temperature, waves, currents, the sample the beach was collected at, and cloud cover). Ingestion rate was the strongest individual factor determining risk for enteroviruses and *C. jejuni*, and pathogen concentration was the strongest individual factor determining risk for *Salmonella* spp. (Figure 5).

Table 1. Predictors Selected in Multiple Linear Regression for Pathogen Concentrations at Three Lake Michigan Beaches in Wisconsin during Summer, 2010[a]

Beach	Pathogens	Predictor variables (sign of coefficient)	Standardized Coefficient
All beaches combined	Enterovirus	maximum 1-h parallel current	−167
		mean 12-h long-shore wave component	−4.50
		Mean 12-h off-shore wave component	3.71
		10-d water temperature	−0.78
	Human Virus	Ag/forest beach	−6.86
		Mean 12-h off-shore wave component	2.90
	Campylobacter	Maximum 12-h cloud cover	7.26
		Ag/forest beach	5.44
	Salmonella	No model selected	--
	Sum of pathogenic bacteria	Maximum 24-h cloud cover	22.9
		Ag/forest beach	2.74
	Sum of Human viruses and pathogenic bacteria	Maximum 12-h cloud cover	6.04
		mean 12-h long-shore wave component	1.04
	Sum of all pathogens	Maximum 12-h cloud cover	5.38
		mean 12-h long-shore wave component	0.91
Clay Banks	Human viruses	Slope from Green Bay[b] to Lake Michigan	320
		24-h mean long-shore wave component	1.27
		6-h mean cloud cover	5.73

[a]Individual pathogens and pathogen classes with greater than 40% occurrence were chosen for regression modeling. [b]24 h mean water surface slope from measurements at Menomonee Michigan in Green Bay to the east end of the Sturgeon Bay Shipping Channel at Lake Michigan.

DISCUSSION

Human virus occurrence in the present study was greater than the 24−53% reported in previous studies of human viruses at Lake Michigan beaches.[14,29,55] The maximum human virus concentrations observed in the present study (6800 gc/L) fell within the wide range previously reported using PCR-based methods (3800 virus particles/L-169 000 gc/L;.[14,55] Similar to results from previous studies, human virus occurrence was dominated by enterovirus and adenovirus.

Pathogenic bacteria occurrence in the present study was also slightly greater than reported in previous studies at Great Lakes beaches of 0−69%.[21,24,25,56−60] The most prevalent organisms detected in recreational waters from these studies varied from pathogenic *E. coli*, *Campylobacter*, and *Salmonella*, but many of the studies focused on a single species. Pathogenic bacteria are not informative for source identification because the origin may include human, agricultural, or wildlife sources.[22,61,62]

Pathogenic bacteria occurrence had the greatest influence on the overall human pathogen occurrence levels in this study. This has not consistently been shown to be the case in other studies where multiple pathogen categories have been investigated.[7,11,12]

These results highlight the importance of investigating multiple pathogens within multiple categories to avoid

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

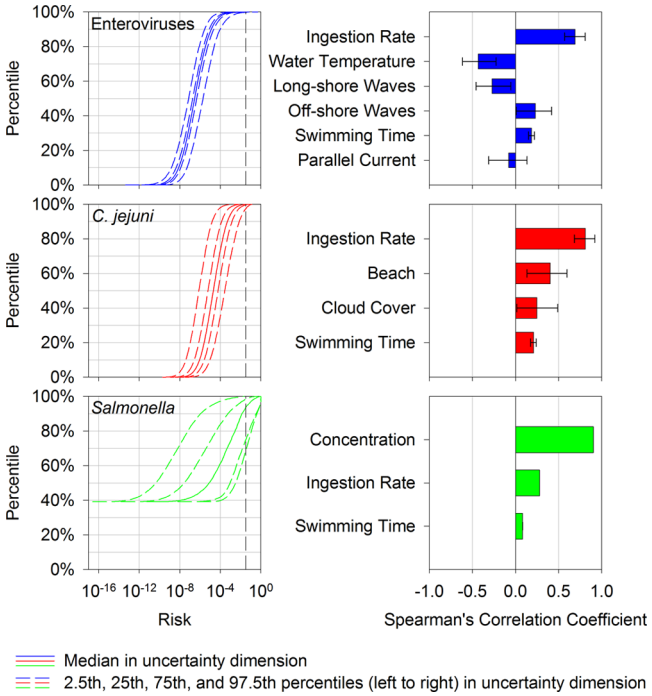

Figure 5. Cumulative distributions (left) and tornado plots (right) for risk estimates. The vertical dashed line represents a health risk of 32 illnesses per 1000 primary contact recreators per event (EPA's 2012 recreational water quality criteria). Curves for the cumulative distributions represent the 2.5, 25, 50, 75, and 97.5 percentiles along the combined distribution of those parameters that contribute uncertainty to the risk estimates (i.e., uncertainty of the statistical model coefficients and uncertainty of the dose−response parameters). Bars on tornado plots represent the median estimate of Spearman's correlation coefficient between risk estimates and the simulation inputs indicated on the vertical axes. Error bars represent the 2.5th and 97.5th percentiles of those estimates. Error bars are absent for *Salmonella* spp. because the only uncertain simulation inputs were dose−response parameters.

underestimating the prevalence of human pathogens in these waters. For example, monitoring of only one of the two most frequently detected human viruses in the present study would have resulted in 47% (enterovirus), or 27% (adenovirus A) occurrence which is within the range of previous studies. Similarly, monitoring of only one of the two most frequently detected pathogenic bacteria in the present study would have resulted in 69% (C. jejuni) or 62% (*Salmonella* spp.) occurrence which is also in the range of previous studies.

Another potential influence on the higher prevalence of pathogens in the present study compared to previous studies could be the relatively high precipitation amounts during the study period leading to increased runoff. June and July had 2.3 and 2.2 times the amount of precipitation indicated for those months than the mean monthly precipitation for the previous 40 years at Manitowoc, WI (data from http://www.ncdc.noaa.gov/, accessed October 29, 2015).

Bovine virus occurrence at the study beaches was lower than that of human viruses and pathogenic bacteria, lower than bovine virus occurrence in riverine environments (37−55.6%) and the mean concentration (31 gc/L) was on the low end of the range reported in previous research 1.2−306 gc/L;.[8−10,13,63] Dilution and dispersion and possible survival differences in the Great Lakes environment may contribute to these differences compared to samples from river systems. Bovine virus sources are limited primarily to dairy operations in this study area. Groundwater and surface water are potential transport pathways to the beach areas.

At all three beaches, septic systems in the contributing watersheds, bather shedding, and longshore transport from remote sources along the lakeshore are potential sources of human waste that could contain human viruses. At Red Arrow beach, other potential human waste sources included possible leaking sanitary sewer systems in the surrounding urban area and treated wastewater that discharged into Lake Michigan about 1 km from the beach. At Clay Banks beach, human fecal sources in the nearby Sturgeon Bay Ship Canal included possible sanitary discharges from watercraft in the marinas and wastewater effluent discharge from the City of Sturgeon Bay. Sanitary sewer leakage has been documented to be a common source of human waste contamination in receiving waters,[64] and septic systems have previously been shown to contaminate receiving waters during periods of failure as well as periods of normal operation.[65−67] Human viruses have been shown to be present in wastewater effluent and can even survive state-of-the-art wastewater treatment processes.[68]

Predictors identified in the statistical modeling process provide insight into potentially important fecal sources and environmental conditions. Modeling results for Clay Banks beach indicated that the water level slope from Green Bay to Lake Michigan was by far the most important predictor for explaining variability of human viruses. Human virus concentrations increased with increasing slope toward Lake Michigan. A slope in this direction induces a current toward Lake Michigan from the Sturgeon Bay Ship Canal that has several potential sources of human waste contamination.

In addition, previous work indicates that groundwater discharge near this beach travels through a fractured rock system beneath numerous farmsteads and a suburban development of more than 50 homes.[69] This indicates potential for groundwater transport from septic systems as another source of human viruses at this beach.

Modeling results indicated that wave direction and cloud cover were important in explaining the variability of pathogens at the three study beaches. Cloud cover was used in this case as a surrogate for sunlight exposure, an abiotic factor known to reduce survival of microorganisms.[70,71] A positive estimate of the regression coefficient for cloud cover indicating greater pathogen concentrations as cloud cover increases, thereby reducing exposure to ultraviolet radiation, is consistent with this concept. Wave directions influence the currents in the nearshore environment and have potential to indicate movement of water from a pathogen source to the beach. The model for enterovirus from the combined beach data included longshore current, suggesting that transport from sources other than the beach area could have been important. Inclusion of 10 day water temperature in this model is consistent with previous work that has shown reduced survival as temperature increased.[72] Modeling results indicated that Point beach had lower human virus concentrations but higher pathogenic bacteria concentrations than the other two beaches under the same environmental conditions suggesting that Point beach is more influenced by contamination from agricultural and/or wildlife sources than from human waste in comparison with the other two beaches. This is consistent with the dominance of agriculture and forested land cover in the Point beach watershed.

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

The QMRAs addressing swimming-related risk for recreational water quality that are available for comparison with the present study[48,52,73] use different approaches because they were intended to achieve different objectives. These previous QMRAs used an assumed, constant level of fecal contamination (equal to EPA's recreational water quality criteria) to estimate the abundance of pathogens that might be expected in recreational water for several sources of fresh fecal pollution. In contrast, the present QMRA approach is based on direct pathogen measurements at three specific recreational water sites, which means the risk estimates reflect the possibility of relatively good water quality with very few or no pathogens. Despite this major difference, comparison with these previous QMRAs is warranted given the abundance of fecal indicators at the three study beaches. Calculating geometric means and statistical threshold values (STV) for enterococci and *E. coli* for the study period, Clay Banks beach did not exceed recreational water quality criteria[54] for *E. coli* (sufficient data was not available for enterococci) and Point Beach slightly exceeded only the STV for enterococci (STV = 145). On the other hand, Red Arrow beach was the only study beach that exceeded the criteria for geometric mean and STV for enterococci (44 and 1,791, respectively) and *E. coli* (160 and 2420, respectively).

Risk estimates for *C. jejuni* illness (median = 2×10^{-5}) and *Salmonella* spp. illness (median = 8×10^{-6}) from the current study are consistent with the low end of previous median risk estimates for nonhuman sources of fecal pollution (livestock and wildlife), which were approximately 1×10^{-5} for *C. jejuni* and 1×10^{-6} for *Salmonella enterica*.[48,52] On the other hand, risk estimates for *C. jejuni* and *Salmonella* spp. are inconsistent with previous median risk estimates for human sources of fecal pollution, which were approximately 1×10^{-4} for *C. jejuni*[73] and varied between approximately 1×10^{-9} and 1×10^{-7} for *Salmonella enterica*.[52,73] These results might be explained by the combined land-use for the watersheds of the three study beaches, which consisted largely of livestock and wildlife inputs from agricultural and natural land cover with a smaller amount of influence from human fecal pollution from urban stormwater and wastewater treatment plant effluent.

These results also hold when considering total risk instead of pathogen-specific risk. Assuming that the three pathogens considered in this QMRA co-occur and that no other pathogens contribute significantly to total risk at these three beaches, the median total risk was estimated as 3×10^{-5}. Previous estimates of median total risk for nonhuman fecal sources were approximately 1×10^{-5} at the low end,[48,52] while previous estimates of total median risk for human fecal sources varied between approximately 1×10^{-3} and 1×10^{-1}.[48,52]

This study is unique for its comprehensive approach in using empirical measurements of multiple pathogens at three beaches to develop statistical models relating environmental predictors to pathogen concentrations, and using the empirical distributions of these environmental predictors over a swimming season to calculate pathogen dose and consequent illness risk. The risk estimates presented here are generally consistent with other QMRAs of swimming in recreational freshwater that relied on indicator:pathogen ratios in fecal wastes instead of empirical measurements. A key limitation of the present study is that sampling frequency may have been inadequate for capturing short-term spikes in pathogen concentrations that lead to higher illness risks. Relations of pathogen concentrations to environmental variables highlighted the influence of currents, waves, and cloud cover on illness risk. The lack of a consistent, dominant pathogen across results from this and other studies illustrates the importance of analyzing for multiple pathogens and pathogen categories when assessing overall pathogen prevalence and risk. Further, the inclusion of multiple pathogen categories allowed for a broader understanding of the contamination sources affecting these beaches.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04372.

Land cover and potential contamination sources at study beaches. Predictor variables used in regression model development. Diagram of portable pathogen sampling system. Sources and enumeration of qPCR positive controls. qPCR quality assurance information. Swimming time and ingestion rate information used for QMRA estimations. QMRA simulation input information. Correlations of environmental conditions to pathogen prevalence. Variability and uncertainty ratios for QMRA results (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (608) 821-3835; fax: (608) 821-3817; e-mail: srcorsi@usgs.gov.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Great Lakes Restoration Initiative and the USGS Ocean Research Priorities Plan for support. Sampling and laboratory assistance was provided by Linsi Whitman, Marvi Verma, Megan Giese, Dean Sanders, and Tyler Mickesh from University of Wisconsin-Oshkosh; Michael Lehr and Amanda Rader at University of Wisconsin-Manitowoc; and Jordan Gonnering, Hana Millen, and Austin Baldwin from the USGS Wisconsin Water Science Center. We thank Benjamin Siebers for providing original artwork for the Abstract. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

■ REFERENCES

(1) Stevenson, A. H. Studies of Bathing Water Quality and Health. *Am. J. Public Health Nations Health* **1953**, *43* (5_Pt_1), 529−538.

(2) Bosch, A. Human enteric viruses in the water environment: a minireview. *Int. Microbiol.* **1998**, *1* (3), 191−196.

(3) 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.

(4) Griffin, D. W.; Donaldson, K. A.; Paul, J. H.; Rose, J. B. Pathogenic human viruses in coastal waters. *Clin. Microbiol. Rev.* **2003**, *16* (1), 129−143.

(5) McQuaig, S. M.; Noble, R. T. Viruses as Tracers of Fecal Contamination. In *Microbial Source Tracking: Methods, Applications, and Case Studies*; Hagedorn, C., Blanch, A. R., Harwood, V. J., Eds.; Springer: New York, 2011; pp 113−135.

(6) Ishii, S.; Nakamura, T.; Ozawa, S.; Kobayashi, A.; Sano, D.; Okabe, S. Water Quality Monitoring and Risk Assessment by Simultaneous Multipathogen Quantification. *Environ. Sci. Technol.* **2014**, *48* (9), 4744−4749.

(7) Waschbusch, R.; Corsi, S.; Sorsa, K.; Walker, J.; Standridge, J.; Schneider, T. *Data Collection and Modeling of Enteric Pathogens, Fecal*

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

Indicators and Real-time Environmental Data at Madison, Wisconsin Recreational Beaches for Timely Public Access to Water Quality Information, Technical Report for USEPA Project R-82933901-0; 2004.
(8) Fong, T.-T.; Griffin, D. W.; Lipp, E. K. Molecular assays for targeting human and bovine enteric viruses in coastal waters and their application for library-independent source tracking. *Appl. Environ. Microbiol.* **2005**, *71* (4), 2070−2078.
(9) Hundesa, A.; Maluquer de Motes, C.; Bofill-Mas, S.; Albinana-Gimenez, N.; Girones, R. Identification of human and animal adenoviruses and polyomaviruses for determination of sources of fecal contamination in the environment. *Appl. Environ. Microbiol.* **2006**, *72* (12), 7886−7893.
(10) Hundesa, A.; Bofill-Mas, S.; Maluquer de Motes, C.; Rodriguez-Manzano, J.; Bach, A.; Casas, M.; Girones, R. Development of a quantitative PCR assay for the quantitation of bovine polyomavirus as a microbial source-tracking tool. *J. Virol. Methods* **2010**, *163* (2), 385−389.
(11) Staley, C.; Reckhow, K. H.; Lukasik, J.; Harwood, V. J. Assessment of sources of human pathogens and fecal contamination in a Florida freshwater lake. *Water Res.* **2012**, *46* (17), 5799−5812.
(12) Francy, D. S.; Stelzer, E. A.; Duris, J. W.; Brady, A. M.; Harrison, J. H.; Johnson, H. E.; Ware, M. W. Predictive models for Escherichia coli concentrations at inland lake beaches and relationship of model variables to pathogen detection. *Appl. Environ. Microbiol.* **2013**, *79* (5), 1676−1688.
(13) Corsi, S. R.; Borchardt, M. A.; Spencer, S. K.; Hughes, P. E.; Baldwin, A. K. Human and bovine viruses in the Milwaukee River watershed: Hydrologically relevant representation and relations with environmental variables. *Sci. Total Environ.* **2014**, *490*, 849−860.
(14) Aslan, A.; Xagoraraki, I.; Simmons, F. J.; Rose, J. B.; Dorevitch, S. Occurrence of adenovirus and other enteric viruses in limited-contact freshwater recreational areas and bathing waters. *J. Appl. Microbiol.* **2011**, *111* (5), 1250−1261.
(15) Borchardt, M. A.; Chyou, P.-H.; DeVries, E. O.; Belongia, E. A. Septic system density and infectious diarrhea in a defined population of children. *Environ. Health Perspect.* **2003**, *111* (5), 742−748.
(16) Bower, P. A.; Scopel, C. O.; Jensen, E. T.; Depas, M. M.; McLellan, S. L. Detection of Genetic Markers of Fecal Indicator Bacteria in Lake Michigan and Determination of Their Relationship to Escherichia coli Densities Using Standard Microbiological Methods. *Appl. Environ. Microbiol.* **2005**, *71* (12), 8305−8313.
(17) Loge, F. J.; Lambertini, E.; Borchardt, M. A.; Başağaoğlu, H.; Ginn, T. R. Effects of Etiological Agent and Bather Shedding of Pathogens on Interpretation of Epidemiological Data Used to Establish Recreational Water Quality Standards. *Risk Anal.* **2009**, *29* (2), 257−266.
(18) U.S. Environmental Protection Agency. *Report to Congress: Impacts and Control of CSOs and SSOs*, EPA 833-R-04-001; Office of Water: Washington, D.C., 2004.
(19) Haack, S. K.; Fogarty, L. R.; Wright, C. Escherichia coli and enterococci at beaches in the Grand Traverse Bay, Lake Michigan: Sources, characteristics, and environmental pathways. *Environ. Sci. Technol.* **2003**, *37* (15), 3275−3282.
(20) U.S. Department of Agriculture. Natural Resources Conservation Service. *Introduction to Waterborne Pathogens in Agricultural Watersheds*; Nutrient Management Technical Note; Nutrient Management Technical Note No. 9; U.S. Department of Agriculture, 2012; p 84.
(21) Converse, R. R.; Kinzelman, J. L.; Sams, E. A.; Hudgens, E.; Dufour, A. P.; Ryu, H.; Santo-Domingo, J. W.; Kelty, C. A.; Shanks, O. C.; Siefring, S. D.; et al. Dramatic Improvements in Beach Water Quality Following Gull Removal. *Environ. Sci. Technol.* **2012**, *46* (18), 10206−10213.
(22) Kinzelman, J.; McLellan, S. L.; Amick, A.; Preedit, J.; Scopel, C. O.; Olapade, O.; Gradus, S.; Singh, A.; Sedmak, G. Identification of human enteric pathogens in gull feces at Southwestern Lake Michigan bathing beaches. *Can. J. Microbiol.* **2008**, *54* (12), 1006−1015.
(23) Ferguson, C.; Husman, A. M.; de, R.; Altavilla, N.; Deere, D.; Ashbolt, N. Fate and transport of surface water pathogens in watersheds. *Crit. Rev. Environ. Sci. Technol.* **2003**, *33* (3), 299−361.
(24) Byappanahalli, M. N.; Sawdey, R.; Ishii, S.; Shively, D. A.; Ferguson, J. A.; Whitman, R. L.; Sadowsky, M. J. Seasonal stability of Cladophora-associated Salmonella in Lake Michigan watersheds. *Water Res.* **2009**, *43* (3), 806−814.
(25) Vanden Heuvel, A.; McDermott, C.; Pillsbury, R.; Sandrin, T.; Kinzelman, J.; Ferguson, J.; Sadowsky, M.; Byappanahalli, M.; Whitman, R.; Kleinheinz, G. T. The Green Alga, Cladophora, Promotes Escherichia coli Growth and Contamination of Recreational Waters in Lake Michigan. *J. Environ. Qual.* **2010**, *39* (1), 333.
(26) Griffith, J. F.; Schiff, K. C.; Lyon, G. S.; Fuhrman, J. A. Microbiological water quality at non-human influenced reference beaches in southern California during wet weather. *Mar. Pollut. Bull.* **2010**, *60* (4), 500−508.
(27) Nevers, M. B.; Whitman, R. L. Policies and practices of beach monitoring in the Great Lakes, USA: a critical review. *J. Environ. Monit.* **2010**, *12* (3), 581.
(28) Lipp, E. K.; Kurz, R.; Vincent, R.; Rodriguez-Palacios, C.; Farrah, S. R.; Rose, J. B. The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. *Estuaries* **2001**, *24* (2), 266−276.
(29) Wong, M.; Kumar, L.; Jenkins, T. M.; Xagoraraki, I.; Phanikumar, M. S.; Rose, J. B. Evaluation of public health risks at recreational beaches in Lake Michigan via detection of enteric viruses and a human-specific bacteriological marker. *Water Res.* **2009**, *43* (4), 1137−1149.
(30) 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.
(31) Wilkes, G.; Edge, T. A.; Gannon, V. P. J.; Jokinen, C.; Lyautey, E.; Neumann, N. F.; Ruecker, N.; Scott, A.; Sunohara, M.; Topp, E. Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. *Water Res.* **2011**, *45* (18), 5807−5825.
(32) Fry, J.; Xian, G.; Jin, S.; Dewitz, J.; Homer, C.; Yang, L.; Barnes, C.; Herold, N.; Wickham, J. Completion of the 2006 National Land Cover Database for the Conterminous United States. *Photogramm. Eng. Remote Sens.* **2011**, *77* (9), 858−864.
(33) Millen, H. T.; Gonnering, J. C.; Berg, R. K.; Spencer, S. K.; Jokela, W. E.; Pearce, J. M.; Borchardt, J. S.; Borchardt, M. A. Glass wool filters for concentrating waterborne viruses and agricultural zoonotic pathogens. *J. Visualized Exp.* **2012**, No. No. 61, e3930.
(34) Abd-Elmaksoud, S.; Spencer, S. K.; Gerba, C. P.; Tamimi, A. H.; Jokela, W. E.; Borchardt, M. A. Simultaneous Concentration of Bovine Viruses and Agricultural Zoonotic Bacteria from Water Using Sodocalcic Glass Wool Filters. *Food Environ. Virol.* **2014**, *6* (4), 253−259.
(35) Schwab, D. J.; Bedford, K. W. Great Lakes forecasting. In *Coastal Ocean Prediction*; Mooers, C., Ed.; Amer. Geophys. Union Coastal and Estuarine Studies, 1999.
(36) Farmer, J. J. I. Enterobacteriaceae: Introduction and Identification. In *Manual of Clinical Microbiology*; Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A., Yolken, R. H., Eds.; ASM Press, 2003; pp 647−650.
(37) Nachamkin, I. Campylobacter and Arcobacter. In *Manual of Clinical Microbiology*; Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A., Yolken, R. H., Eds.; ASM Press, 2003; pp 905−908.
(38) Tibshirani, R. Regression Shrinkage and Selection via the Lasso. *J. R. Stat. Soc. Ser. B Methodol.* **1996**, *58* (1), 267−288.
(39) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2014.
(40) Friedman, J.; Hastie, T.; Tibshirani, R. Regularization paths for generalized linear models via coordinate descent. *J. Stat. Softw.* **2010**, *33* (1), 1.

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995

(41) Henningsen, A. *censReg: Censored Regression (Tobit) Models*, 2013.
(42) Tobin, J. Estimation of Relationships for Limited Dependent Variables. *Econometrica* **1958**, *26* (1), 24−36.
(43) Pouillot, R.; Delignette-Muller, M.-L. Evaluating variability and uncertainty in microbial quantitative risk assessment using two R packages. *Int. J. Food Microbiol.* **2010**, *142* (3), 330−340.
(44) Husman, A. M. de R.; Lodder, W. J.; Rutjes, S. A.; Schijven, J. F.; Teunis, P. F. M. Long-Term Inactivation Study of Three Enteroviruses in Artificial Surface and Groundwaters, Using PCR and Cell Culture. *Appl. Environ. Microbiol.* **2009**, *75* (4), 1050−1057.
(45) Suppes, L. M.; Abrell, L.; Dufour, A. P.; Reynolds, K. A. Assessment of swimmer behaviors on pool water ingestion. *J. Water Health* **2014**, *12* (2), 269.
(46) Huang, Y. Echovirus: Dose Response Models - QMRAwiki. http://qmrawiki.canr.msu.edu/index.php/Echovirus:_Dose_Response_Models (accessed May 11, 2015).
(47) Schmidt, P. J.; Pintar, K. D. M.; Fazil, A. M.; Flemming, C. A.; Lanthier, M.; Laprade, N.; Sunohara, M. D.; Simhon, A.; Thomas, J. L.; Topp, E.; et al. Using Campylobacter spp. and Escherichia coli data and Bayesian microbial risk assessment to examine public health risks in agricultural watersheds under tile drainage management. *Water Res.* **2013**, *47* (10), 3255−3272.
(48) 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.
(49) Teunis, P. F. M.; Kasuga, F.; Fazil, A.; Ogden, I. D.; Rotariu, O.; Strachan, N. J. C. Dose−response modeling of Salmonella using outbreak data. *Int. J. Food Microbiol.* **2010**, *144* (2), 243−249.
(50) Hankin, R. K. S. Special functions in R: introducing the gsl package. *R News* **2006**, *6* (4).
(51) Teunis, P. F. M.; Havelaar, A. H. The Beta Poisson Dose-Response Model Is Not a Single-Hit Model. *Risk Anal.* **2000**, *20* (4), 513−520.
(52) Schoen, M. E.; Ashbolt, N. J. Assessing Pathogen Risk to Swimmers at Non-Sewage Impacted Recreational Beaches. *Environ. Sci. Technol.* **2010**, *44* (7), 2286−2291.
(53) Beumer, A.; King, D.; Donohue, M.; Mistry, J.; Covert, T.; Pfaller, S. Detection of Mycobacterium avium subsp. paratuberculosis in Drinking Water and Biofilms by Quantitative PCR. *Appl. Environ. Microbiol.* **2010**, *76* (21), 7367−7370.
(54) U.S. Environmental Protection Agency. *Recreational Water Quality Criteria*, EPA 820-F-12-058; Office of Water: Washington, D.C., 2012; p 69.
(55) Xagoraraki, I.; Kuo, D. H.-W.; Wong, K.; Wong, M.; Rose, J. B. Occurrence of human adenoviruses at two recreational beaches of the great lakes. *Appl. Environ. Microbiol.* **2007**, *73* (24), 7874−7881.
(56) Lauber, C. L.; Glatzer, L.; Sinsabaugh, R. L. Prevalence of Pathogenic Escherichia coli in Recreational Waters. *J. Great Lakes Res.* **2003**, *29* (2), 301−306.
(57) Chomeau, V.; Kleinheinz, G.; Kolberg, R.; McDermott, C.; Nevers, M. B.; Schuster, W.; Whitman, R. L. *Door County Beach Contamination Source Identification Interim Report*; Door County Soils & Water Conservation Department, 2006; p 78.
(58) Hamelin, K.; Bruant, G.; El-Shaarawi, A.; Hill, S.; Edge, T. A.; Bekal, S.; Fairbrother, J. M.; Harel, J.; Maynard, C.; Masson, L. A virulence and antimicrobial resistance DNA microarray detects a high frequency of virulence genes in Escherichia coli isolates from Great Lakes recreational waters. *Appl. Environ. Microbiol.* **2006**, *72* (6), 4200−4206.
(59) Kleinheinz, G.; McDermott, C.; Nevers, M. B.; Kolberg, R.; Whitman, R. L.; Schuster, W.; Chomeau, V. *Door County Beach Contamination Source Identification: Final report 2006−2007*; Door County Soils & Water Conservation Department, 2007; p 93.
(60) Haack, S. K.; Fogarty, L. R.; Stelzer, E. A.; Fuller, L. M.; Brennan, A. K.; Isaacs, N. M.; Johnson, H. E. Geographic Setting Influences Great Lakes Beach Microbiological Water Quality. *Environ. Sci. Technol.* **2013**, *47* (21), 12054−12063.
(61) Munroe, D. L.; Prescott, J. F.; Penner, J. L. Campylobacter jejuni and Campylobacter coli serotypes isolated from chickens, cattle, and pigs. *J. Clin. Microbiol.* **1983**, *18* (4), 877−881.
(62) World Health Organization. *Campylobacter*, Fact Sheet 255; 2011.
(63) Ley, V.; Higgins, J.; Fayer, R. Bovine enteroviruses as indicators of fecal contamination. *Appl. Environ. Microbiol.* **2002**, *68* (7), 3455−3461.
(64) Sauer, E. P.; VandeWalle, J. L.; Bootsma, M. J.; McLellan, S. L. Detection of the human specific Bacteroides genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water Res.* **2011**, *45* (14), 4081−4091.
(65) DeBorde, D. C.; Woessner, W. W.; Lauerman, B.; Ball, P. N. Virus occurrence and transport in a school septic system and unconfined aquifer. *Groundwater* **1998**, *36* (5), 825−834.
(66) Borchardt, M. A.; Bertz, P. D.; Spencer, S. K.; Battigelli, D. A. Incidence of Enteric Viruses in Groundwater from Household Wells in Wisconsin. *Appl. Environ. Microbiol.* **2003**, *69* (2), 1172−1180.
(67) Borchardt, M. A.; Bradbury, K. R.; Alexander, E. C.; Kolberg, R. J.; Alexander, S. C.; Archer, J. R.; Braatz, L. A.; Forest, B. M.; Green, J. A.; Spencer, S. K. Norovirus outbreak caused by a new septic system in a dolomite aquifer. *Groundwater* **2011**, *49* (1), 85−97.
(68) Xagoraraki, I.; Yin, Z.; Svambayev, Z. Fate of Viruses in Water Systems. *J. Environ. Eng.* **2014**, *140* (7), 04014020.
(69) Bradbury, K. R.; Cobb, M. K. *Delineation of Areas Contributing Groundwater to Springs and Wetlands Supporting the Hine's Emerald Dragonfly, Door County, Wisconsin*, Open-File Report WOFR2008-04; Wisconsin Geological and Natural History Survey: Madison, WI, 2008; p 33.
(70) Gerba, C. P.; Gramos, D. M.; Nwachuku, N. Comparative Inactivation of Enteroviruses and Adenovirus 2 by UV Light. *Appl. Environ. Microbiol.* **2002**, *68* (10), 5167−5169.
(71) Rzezutka, A.; Cook, N. Survival of human enteric viruses in the environment and food. *FEMS Microbiol. Rev.* **2004**, *28* (4), 441−453.
(72) Bosch, A.; Pinto, R. M.; Abad, F. X. Survival and transport of enteric viruses in the environment. In *Viruses in foods*; Springer, 2006; pp 151−187.
(73) Soller, J. A.; Schoen, M. E.; Bartrand, 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.

DOI: 10.1021/acs.est.5b04372
Environ. Sci. Technol. 2016, 50, 987−995