Q-522

Microcystin Toxin and Enterococci Indicator Levels Associated with Freshwater Recreational Waterborne Illness

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Abstract

A prospective pilot study of beach users at three Iowa Beaches was conducted in the summer of 2005 to determine the number of gastrointestinal (GI) illness and skin irritation/rash symptoms in swimmers and non-swimmers and to correlate those symptoms with water quality indicators in beach water. One-thousand and thirty-nine people were enrolled in the study with 261 participants completing on-line weekly follow-up surveys over four weeks which included questions about water activities, time spent at the beach, occurrence of GI illness or skin irritation, etc. Composite water samples were collected daily and analyzed for *E.coli*, enterococci, and total microcystin toxin levels using EPA methods 1603, 1600, and immunoassay, respectively. Spearman correlation coefficients were calculated for bacteria indicator exposures and GI illness or skin irritation with the following significant associations: enterococci level and diarrhea at Beach 1, enterococci level and skin irritation/rash symptoms at Beach 2, and microcystin toxin level and skin irritation/rash at Beach 1. Enterococci levels at Beach 1 exceeded the EPA recommended geometric mean standard (33 CFU/100mL) 29 times while these levels at Beach 2 exceeded the EPA standard 24 times. The most significant variable related to the risk for skin irritation with all factors included using a stepwise regression analysis was microcystin level because the correlation was so strong at Beach 1 (p<0.0001). This association was unexpected since the microcystin levels at this beach were relatively low during the study with all values <1 µg/L and no algal blooms identified. The results of this pilot study indicate statistical association between illness and microcystin toxin presence and enterococci levels. More research is needed to determine if these associations exist on a larger scale at other inland beaches and to further explore the positive correlation between low levels of microcystin toxin and skin irritation/rash at Beach 1.

Figure 1. Iowa Beach Study Beaches located in Johnson County in the Iowa River watershed

Introduction

The Iowa Department of Natural Resources has been monitoring Iowa's beach water quality since 2000 utilizing fecal coliforms, *E.coli*, and enterococci bacterial indicators. High levels of these fecal indicators after rainfall events and no point sources identified during intensive water sampling and sanitary survey investigations indicate the fecal contamination was primarily non-point source. Epidemiological studies performed by EPA report a direct relationship between the density of *E.coli* and enterococci in surface waters and an increase in swimming-associated diarrhea. However, these studies were completed in coastal marine environments (included only two fresh water beaches) where swimming beaches were located near point sources of human contamination, such as pipes discharging sewage effluent (US EPA 1986). These studies contributed to the establishment of recreational water-quality guidelines for *E.coli* and enterococcus to predict the likelihood of gastrointestinal illness in marine and freshwater settings. Freshwater guidelines are 126 CFU/100mL and 33 CFU/100mL for *E.coli* and enterococci, respectively (geometric mean of five samples over 30 days). It is not known whether these traditional fecal bacterial indicators would be predictive when nonhuman sources predominate, especially in freshwater where limited studies have been performed (Calderón 1991). Since 2003, IDNR has been monitoring the occurrence of cyanotoxins in Iowa lakes and beach water because of the potential adverse health effects in humans and animals. Exposure to high levels of microcystins has been associated with skin rashes and animal deaths in the Midwest (USGS 2006). To evaluate the efficacy of EPA's bacterial standards and of microcystin monitoring to protect Iowans from water-borne illness, an epidemiologic study was conducted at three freshwater beaches in Iowa. The number of self-reported gastrointestinal (GI) illness and skin irritation/rash symptoms in swimmers and non-swimmers was correlated with water quality indicator levels *(E. coli,* enterococci and microcystin) in beach water.

Methods

Subject Recruitment: Eight University of Iowa students were hired and trained to collect water samples and enroll beach users from June 15 through July 20, 2005, at each beach. Flyers describing the purpose of the project were distributed to beach users. Students distributed water bottles and offered t-shirts after follow-up questionnaire completion as incentives to participate. Participants were required to read and sign an informed consent statement as part of the enrollment procedure. All study instruments and protocols were approved by the Institutional Review Board at the University of Iowa.

Follow-up questionnaires: Participants were contacted weekly by e-mail to complete follow-up questionnaires over a 4 week period following enrollment. Questionnaires asked about GI illness and skin irritation/rash history, and collected information on beach indicator variables including in-the-water activities; time spent at the beach, food ingestion, previous medical history, etc. Questionnaires were accessed on-line through a University of Iowa Department of Epidemiology website using Telesform, a Windows-based software for survey data collection and analysis. Reminders were sent by e-mail on Fridays to participants who had not yet completed the questionnaire for that week.

Water sampling and analysis: One composite water sample was taken daily at each beach from three locations across the beach and at three water depths (ankle-, knee-, and chest-deep). Water from these nine points was combined into one sample and returned the same day to the University Hygienic Laboratory for analysis. *E. coli* and enterococci were determined by EPA Methods 1603 and 1600, respectively. Total microcystin levels were determined by immunoassay (AbraXis LLC, Warminster, PA). Quality control procedures were performed as appropriate and specified in the methods referenced.

Data analysis: Spearman correlation coefficients were calculated to compare various beach indicator variables to GI illness symptoms and to skin irritation/rash symptoms. Multiple regression analyses ranked beach variables, bacterial levels in the water, and various interactions as risk factors for GI illness episodes and skin irritation/rash episodes.

Figure 2.

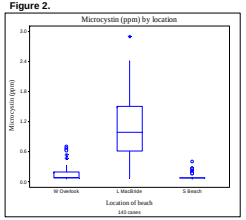
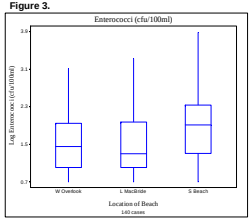


Figure 3.



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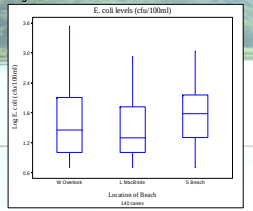

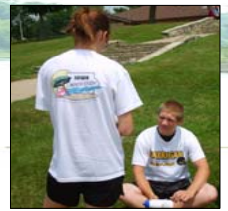

Results

Subject recruitment: A total of 1039 people were initially enrolled in the study during the "at the beach" recruitment effort. Of that number, 261 persons completed on-line follow-up questionnaires.

Contaminant levels by beach: Bacterial levels of *E. coli* and enterococci in beach water are presented in Figures 2-4. Lake Macbride beach samples exceeded the *E. coli* standard once and the enterococci standard 24 times; West Overlook Beach samples exceeded the *E. coli* standard 3 times and the enterococci standard 29 times. Observations from the third beach (Sandy Beach) were excluded from analysis due to the low number of questionnaires completed (6).

Correlation Analysis: Spearman correlations between bacteria exposures and GI illness episodes or skin irritation/rash episodes are presented in Table 1, with significant correlations ($p < .05$) in red (skin rash) and blue (diarrhea) print. The enterococci level is correlated with diarrhea episodes at West Overlook Beach. Enterococci are also correlated with skin irritation/rash episodes at Macbride Beach, while microcystin level is correlated with skin irritation/rash episodes at West Overlook Beach.

Results for a stepwise regression analysis for the number of diarrhea episodes are presented in Table 2. Variables related to the risk for diarrhea episodes were, in rank order, the Macbride Beach variable, the interaction between swallowing lake water and the Macbride beach variable, the interaction between the amount of time spent in the water and the beach variable, immersing one's head in the water, and the enterococci level (borderline).

Results of a stepwise regression analysis for the number of skin irritation/rash episodes are presented in Table 3. Variables related to the risk for skin irritation/rash episodes were, in rank order, microcystin level, the interaction between each of the microbiological contaminants and the beach variable, immersing one's head in the water, being male, and the interaction between wading and the beach variable. There was a larger effect of the microcystin level and skin irritation/rash at West Overlook than Lake Macbride (demonstrated by negative t-value) and this association was still very significant when adjusted with the other parameters in this regression.

Table 1. Spearman Correlation for Bacterial Indicators at each Beach

	Macbride Beach		West Overlook Beach	
	Diarrhea episodes	Skin rash episodes	Diarrhea episodes	Skin rash episodes
E. coli / 100 mL	0.0104 0.9371 60	-0.0654 0.6194 60	0.1900 0.1459 60	0.1052 0.4237 60
Enterococci / 100 mL	-0.0223 0.8655 60	0.2910 0.0241 60	0.3564 0.0052 60	0.0026 0.9844 60
Microcystin (ppb)	0.0831 0.5277 60	0.1260 0.3374 60	0.1291 0.3255 60	0.5237 <0.0001 60

Top number: Spearman correlation coefficient; Middle number: Prob > |r| under H0:Rho=0;Bottom number: number of observations

Table 2. Stepwise Regression Analysis for Diarrhea Episodes

115 participants	t-value	Pr > |t|
Macbride (MB)	3.06	0.0028
Swallow lake H20	1.08	0.2835
Amount H20 swallowed	1.45	0.1491
Time in water	0.29	0.7718
Enterococci	1.92	0.0577
Immersed head	-2.10	0.0385
Swallow H20 * MB	-2.51	0.0135
Time in water * MB	-2.31	0.0226

Table 3. Stepwise Regression Analysis for Skin irritation/rash episodes

116 participants	t-value	Pr > |t|
Macbride (MB)	0.31	0.7566
Wading	-1.11	0.2685
Microcystin	4.53	<0.0001
Immersed head	-2.04	0.0437
Gender	-2.02	0.0456
Wading * MB	1.91	0.0594
Microcystin * MB	-3.61	0.0005
Enterococci	-0.82	0.4146
E. coli	1.98	0.0508
Enterococci * MB	2.15	0.0341
E. coli * MB	-2.48	0.0146

Conclusions

The results of this pilot study show a positive association between reported illness and fecal indicator bacteria (*E.coli* and enterococci) and microcystin toxin presence at two freshwater beaches in Iowa. Statistically significant associations were found for enterococci level and diarrhea at West Overlook Beach, enterococci level and skin irritation/rash symptoms at Lake Macbride Beach, and microcystin toxin level and skin irritation/rash at West Overlook Beach. Stepwise regression analysis showed a highly significant association between microcystin level and skin irritation at West Overlook Beach (p<0.0001). This was an unexpected finding; microcystin levels at this beach were relatively low during the study with all values <1 µg/L and no algal blooms identified. Lake Macbride Beach had higher microcystin levels but no association with skin rash. The significant association of microcystin level and skin irritation/rash at West Overlook may be due to some other interaction (e.g. a different cyanotoxin or activity) that was not captured or sampled in this study. More research is needed to determine if these associations exist on a larger scale at other inland beaches and to further explore the positive correlation between low levels of microcystin toxin and skin irritation/rash at West Overlook Beach.

Acknowledgements

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References

Calderon, Rebecca, E. Mood, and A. Dufour, 1991. Health Effects of Swimmers and Nonpoint Sources of Contaminated Water, International Journal of Environmental Health Research 1:21-31.

United States Environmental Protection Agency, Ambient Water Quality Criteria for Bacteria –EPA440/5-84-002. Washington, DC: USEPA, 24p.

United States Geological Survey, HAB Brief: June 2006. Lawrence KS: USGS, 2p.