Lake Erie Protection Fund (SG 405-11)

Final Report

Does Enterococci qPCR Predict Pathogen levels?

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ABSTRACT

Current approaches for assessing and communicating risk associated with recreational water contact are inadequate for protecting public health. New approaches for rapidly and practically assessing water quality are needed. Accordingly, this project was designed around the evaluation of real-time molecular methods and practical measures for rapidly assessing infectious disease risk at two of Ohio's Lake Erie beaches. In this study, we present a baseline understanding of the human health risk attributed to infectious enteric viruses and *Arcobacter* as human pathogens that may be impacting public health through exposure to Lake Erie beach waters. Using rapid molecular methods and traditional water quality monitoring instruments, our objectives were: 1) to investigate the significance of these microorganisms as important emerging pathogens in Lake Erie beach water; 2) to determine whether the current fecal indicator (cultured *E. coli*) and the most likely new method that US EPA will adopt in 2012 (enterococci qPCR) can predict the presence of human pathogens; and 3) to develop statistical models using sanitary survey data (previously collected) and environmental determinants in explaining pathogen levels. Through accomplishing our objectives, we intended to provide a more complete understanding of fecal contamination and human health risks at these Lake Erie beaches.

INTRODUCTION

Microbial water quality is routinely monitored by using culture methods for bacterial indicators such as *E. coli* and enterococci. However, these indicators are thought to have limited value in assessing the presence and the level of human pathogens such as enteric viruses and bacteria, such as *Arcobacter*. In addition, present culture methods for detecting fecal indicators take 18 to 24 hours to obtain results (U.S. EPA 2006). This delay permits at-risk beaches to stay open until indicator results are available and has been demonstrated to make beach closures occur too late or occur unnecessarily when indicator densities have already returned to safe levels. Therefore, EPA and researchers are striving to provide useful rapid methods for identifying undesirable conditions in advance of contact recreation exposure.

The current recreational water quality criteria for fecal indicators are scheduled for revision no later than October 15, 2012 per a consent decree stemming from *Natural Resources Defense Council v. Johnson and U.S. EPA* (2008). The consent decree mandates the development of a rapid alternative to the culture-based method for the determination of the single-day maximum, mandating a useful result be obtained in under six hours of sample collection. Among the potential rapid methods being considered, quantitative polymerase chain reaction (qPCR) assays have obtained the most attention, whereby the targeting genetic material (DNA or RNA) is quantified. To date, the most promising qPCR assay among EPA researchers for new criteria development appears to be qPCR for *Enterococcus*, which has been demonstrated to be useful in predicting gastrointestinal illness following beach exposure (Wade et al. 2008). The

impacts of switching the single-day maximum fecal indicator criteria on the basis of this epidemiological study and the consent decree are still unknown.

The beaches of Lake Erie frequently experience advisory conditions due to point and nonpoint sources of fecal contamination, which contributes to possible health risks and economic losses through illness and beach closure. Recent reports from the Centers for Disease Control and Prevention (CDC) indicate that viruses are the most significant cause of recreational waterborne diseases from surface waters with viral outbreaks being observed consistently every year for over the last decade. Among the enteric viruses, noroviruses (NoV), adenoviruses (AdV), and enteroviruses (EntV) are the most prevalent agents responsible for these recreational waterborne outbreaks. NoVs are responsible for the most cases of acute gastroenteritis worldwide, and AdVs are frequently associated with diarrhea, respiratory illnesses and epidemic conjunctivitis. EntVs are also known to cause illnesses including gastroenteritis to pericarditis and aseptic meningitis. Despite their worldwide existence and reputation in the public health community, the occurrence of these viruses in Lake Erie water has not been studied, largely due to technological limitations. The bacteria, *Arcobacter*, has also been recognized as an important enteropathogens and as a potential zoonotic agent (link the diseases from animals to humans). *Arcobacter*-contaminated water may serve as a route of disease transmission to humans. In 2004, it was reported that groundwater-associated outbreaks at an island of Lake Erie was attributed to the transport of microbiological agents from wastewater treatment facilities and septic tanks to Lake Erie and the subsurface (Fong et al. 2007). The outbreak affected approximately 1,450 residents and visitors of South Bass Island, Ohio. The researchers found high prevalence of *Arcobacter* in water samples and suggested that environmental water should be further monitored for this bacterium (Fong et al. 2007).

PURPOSE AND SCOPE

To address the need for understanding the impact of qPCR as a tool for assessing human health risks at these Ohio Lake Erie beaches, our study was designed to answer following three questions: 1) Will enteric viruses (NoV, AdV and EntV) and the bacterial pathogen, *Arcobacter,* be detected by qPCR at the two Lake Erie beaches (Villa Angela and Euclid)? 2) Will culture-based methods for *E. coli* and enterococci and qPCR measurement of enterococci be predictive of pathogen densities (viruses and *Arcobacter*)*?* 3) Will rapid measures of environmental data coupled with qPCR be predictive of pathogen densities?

These research objectives were accomplished through the following three specific aims:

1) *Investigate presence and significance of pathogens and fecal indicators*
   We optimized and developed primers and probes for detecting fecal indicators, enteric viruses (NoV, AdV and EntV) and *Arcobacter* using qPCR. After the rapid methods were optimized, we employed the methods on the beach water samples from Villa Angela and Euclid Beaches (Cleveland Lakefront State Park, Cleveland, Ohio).

2) *Characterize statistical relationships between fecal indicators and pathogens.*
   Using the results from the Specific Aim #1, we utilized basic statistical tools to evaluate statistical associations and/or differences between the fecal indicators and pathogens.

3) *Development of multivariable models for predicting pathogen levels.*
   Predictive models relating water quality/environmental factors were considered for more advanced statistical analysis for model pathogen densities. Furthermore, determinants of water quality were evaluated with respect to their predictive potential for influencing results and/or enhancing pathogen density estimation.

METHODS

The study consisted of two components. The first component was the detection of fecal indicators and enteric pathogens via qPCR methods. The second component was to utilize the previously collected water quality data (environmental conditions, fecal indicator measured with culture-based methods) and link those results with the qPCR results from the first component. More details on study methods can be found in Lee et al. (2012).

Study Sites

The samples were obtained from Euclid (41° 35' 9" N ; 81° 34' 1" W) and Villa Angela (41° 35' 2" N ; 81° 34' 9" W) beaches, which are located at Cleveland Lakefront State Park (Cleveland, Ohio). The beaches are known to be impacted by Euclid Creek which is immediately adjacent to these two beaches and impaired by storm and sanitary sewer overflows (Francy et al., 2006). Water samples were collected 35 times from each beach with financial support from U.S. EPA GLRI. These samples were collected once-per-day three or four times per week from July 13 through September 16, 2010. The samples were collected in waters approximately three feet in depth in the approximate center of each beach as recommended by Ohio beach sampling protocol (Ohio Department of Health, 2009).

Previously Sponsored Water Quality Measurements

With the U.S. EPA GLRI grant, water quality was evaluated on-site and within two-hours at a field laboratory at the Ohio State University-Lake County Extension Office to obtain data for water temperature, dissolved oxygen, conductivity, pH, chlorophyll a, phycocyanin, and turbidity. Within two hours of field sampling, the culturing of *E. coli* and enterococci began. *E. coli* were cultured on modified m-TEC agar and enumerated within 24 hours in accordance with EPA Method 1603 (U.S. EPA, 2002). Enterococci were cultured on mEI agar and enumerated in the same time frame as *E. coli* in accordance with EPA Method 1600 (U.S. EPA, 2006). Also, hydrological and meteorological data were obtained from the NOAA-National Weather.

Quantification of Fecal Bacteria via qPCR

Using DNA extracts from membrane filters obtained from 35 Villa Angela and 35 Euclid Beach samples, the OLEC grant enabled qPCR assays to be performed by amplifying targeted DNA of

fecal indicator bacteria in accordance with the assays listed in Table 1. The DNA extracts (200ml water was filtered with0.45 µm pore size filter membrane) used in this study were obtained from the previously described water samples collected during the 2010 swimming season. DNA was extracted via a QIAamp® DNA stool kit.

Table 1. The fecal indicators quantified via qPCR in this study with supporting references for the primers and probes used for DNA amplification in this study.

Target Fecal Bacteria	Assay Name	References
Arcobacter	Arco	Bastyns et al. (1995) and González et al. (2010)
Bacteroides/Prevotella	HuBac	Bernhard and Field (2000) and Okabe et al. (2007)
E. coli	*uid*A	Chern et al. (2009)
Enterococcus	ent23	Ludwig and Schleifer (2000)

Quantification of Enteric Viruses via qPCR

Given the low densities of viruses in water, composite samples were made by combining every three or four samples by mixing equal portions from each sample and generating 1 L for RNA extraction and 500 mL for DNA extraction. The enteric viruses in each composite sample were concentrated using the cation-coated filtration method (Haramoto et al., 2005; Katayama et al., 2002). Using the concentrates, reverse transcription-qPCR (RT-qPCR) for EntV and NoV and qPCR assays for AdV were performed via an ABI 48-well StepOne™ Real Time System using the assays listed in Table 2.

Table 2. The enteric viruses evaluated in this study via RT-qPCR and qPCR with references for the primers and probes used for viral DNA/RNA amplification.

Target Enteric Virus	Assay Name	References
Human Adenovirus (AdV)	HAdV	Heim et al. (2003)
Human Enterovirus (EntV)	HEV	Monpoeho et al. (2000)
Human Norovirus (NoV)	HNoV GII	Kageyama et al. (2003)

Statistical Analysis

A variety of statistical methods were used in this study. Summary statistics and scatterplots were generated. T-tests, Pearson correlation analysis, logistic regression and multivariable linear regression were all performed. All analyses were performed using the Stata 11.0 (StataCorp, College Station, TX) statistical software package.

RESULTS AND DISCUSSION

E. coli and Enterococci Plate Counts

The beaches of Villa Angela and Euclid demonstrated the presence of significant fecal contamination at these two adjoining beaches as illustrated through the *E. coli* and enterococci plate counts (Table 3). Given the skewed nature of the distribution for *E. coli* and enterococci, median and geometric mean results are more useful for understanding daily conditions. The geometric means for *E. coli* and enterococci (not illustrated in Table 3) are 55.6 and 42.3 CFU/100 mL, respectively. If Ohio used the EPA freshwater criteria for geometric mean sampling of enterococci, the beaches in this study would exceed the 33 CFU/100 mL threshold and be deemed impaired due to fecal indicators. Furthermore, by reviewing the ranges illustrated in Table 3, it is apparent that both *E. coli* exceeded the 235 CFU/100 mL single-day threshold for *E. coli.* In fact 21% (14 of 68) of all *E. coli* samples in this study exceeded the *E. coli* single-day maximum demonstrating the known water quality problems at these beaches was still apparent in our study.

Table 3. Summary statistics for the parameters measured in this study.

Parameter	N	Mean	Median	Range
Ent qPCR, ent23 (log copies/100 mL)	66	3.11	3.30	DL - 4.98
E. coli Plate (CFU/100 mL)	68	155.91	58.67	0.33 - 1033.33
Enterococci Plate (CFU/100 mL)	68	86.95	44.17	0.33 - 520
E. coli qPCR , uidA (log copies/100 mL)	66	2.68	2.31	DL - 3.74
HuBac qPCR (log copies/100 mL)	66	3.35	2.60	DL - 5.93
Arcobacter qPCR (log copies/100 mL)	65	4.04	4.05	DL - 5.48
pH	66	8.48	8.49	7.81 - 8.98
Sp. Conductivity (uS)	66	291.65	308.50	149 - 341
Dissolved Oxygen (ppm)	54	11.42	11.03	6.27 - 14.80
Wave Height (cm)	64	26.38	13.50	0.5 - 120
Avg. Wind Speed (mph)	70	7.35	6.60	3.6 - 15.2
Daily Precip. (in.)	70	0.09	0.00	0 - 0.88
Previous Precip. (in.)	70	0.08	0.00	0 - 0.88
Total Geese (individuals)	70	7.14	1.00	1 - 94
Total Gulls (individuals)	70	41.93	14.00	0 - 245
Total People (individuals)	70	1.24	1.00	0 - 15
Chlorophyll a (ppb)	69	6.58	5.06	2.04 - 17.76
Turbidity (NTU)	70	8.52	2.70	1.1 - 41.7
Water Temp. (°C)	66	24.11	25.09	19.51 - 27.03
Phycocyanin (ppb)	70	12.52	12.02	5.81- 38.09

Additionally, among water quality and qPCR parameters, we see significant variability, which is ideal for evaluating the various relationships of the fecal indicators under differing conditions.

As expected, the culture-based methods for *E. coli* and enterococci showed a significant positive relationship as demonstrated in the regression plot below (Figure 1) and through the

significant Pearson correlation (r = 0.635, *p* = 0.000). Beyond this significant positive relationship, the plate counts did not reveal any other significant positive associations with the other microbiological measures of water quality (Tables 4 & 5). This is not as surprising given that these other measures are molecular measures relying on qPCR and the amplification of genetic material as opposed to the culturing of viable colony forming units. It is concerning that a statistically significant negative association exists between the *E. coli* plate count and *E. coli* qPCR results (r = -0.279, *p* = 0.013) and the enterococci plate count and *E. coli* qPCR results (r = -0.292, *p* = 0.018). These statistical results suggest that for every increase in colony forming units observed on either an *E. coli* or enterococci plate, we anticipate the opposite effect in the qPCR data for the uidA gene (*E. coli* marker), suggesting less *E. coli*.

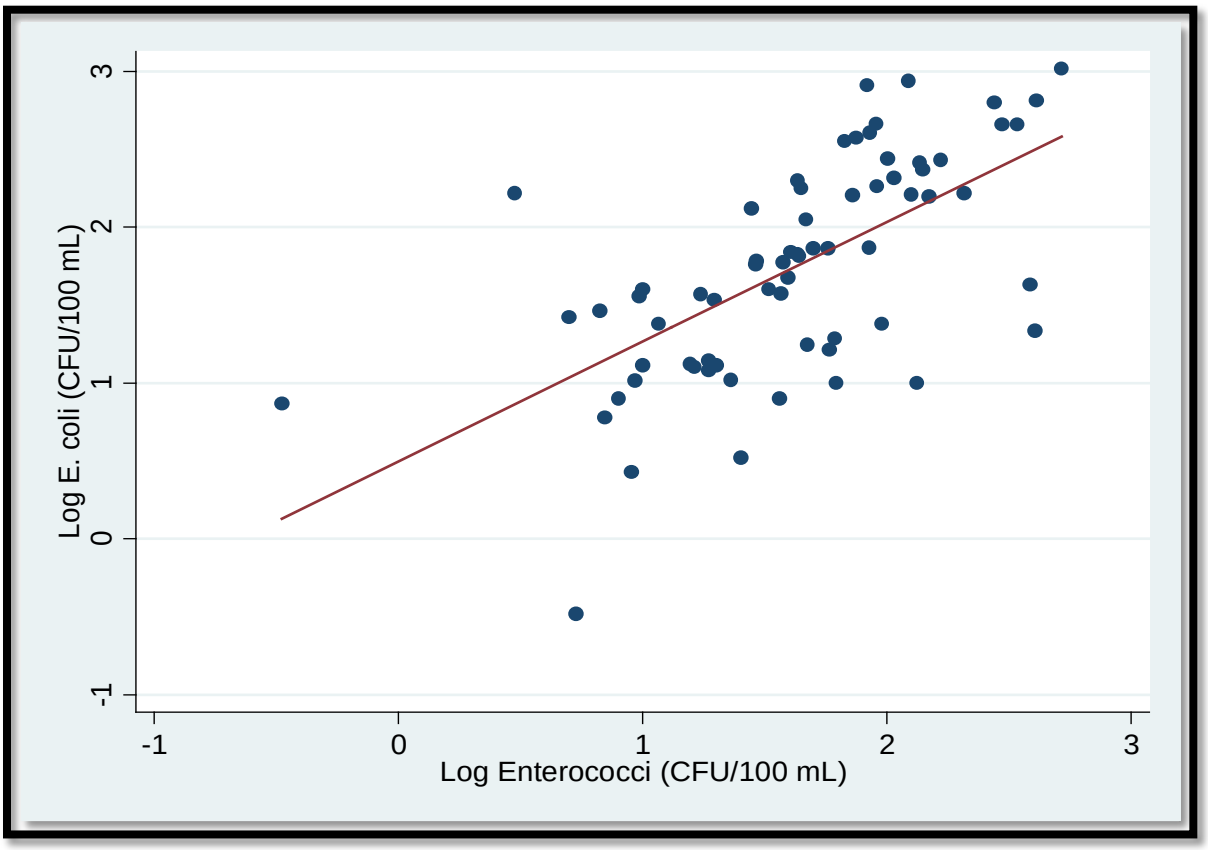

Figure 1. Scatterplot with trendline illustrating the positive association between densities of enterococci and *E. coli* as measured by culture-based methods from Euclid and Villa Angela beach samples (r = 0.635, regression *p* = 0.000).

Table 4. Pearson correlation coefficients evaluating associations among the microbiological parameters quantified in this study.

Fecal Indicator	Enterococci qPCR	*E. coli* Plate	Enterococci Plate	*E. coli* qPCR	HuBac qPCR	Arcobacter qPCR
Enterococci qPCR (ent23) (log copies/100 mL)	1.000					
E. coli Plate (CFU/100 mL)	-0.095	1.000				
Enterococci Plate (CFU/100 mL)	-0.039	***0.646***	1.000			
E. coli qPCR (uidA) (log copies/100 mL)	0.136	***-0.279***	***-0.292***	1.000		
HuBac qPCR (log copies/100 mL)	***0.293***	-0.101	-0.116	***0.237***	1.000	
Arcobacter qPCR (log copies/100 mL)	***0.193***	-0.065	-0.091	0.174	***0.577***	1.000

Table 5. Measures of the statistical significance of the Pearson correlation coefficients evaluated in this study.

Fecal Indicator	Enterococci qPCR	*E. coli* Plate	Enterococci Plate	*E. coli* qPCR	HuBac qPCR
E. coli Plate (CFU/100 mL)	0.371	----	----	----	----
Enterococci Plate (CFU/100 mL)	0.739	***0.000***	----	----	----
E. coli qPCR (log copies/100 mL)	0.144	***0.013***	***0.018***	----	----
HuBac qPCR (log copies/100 mL)	***0.015***	0.557	0.385	***0.071***	----
Arcobacter qPCR (log copies/100 mL)	***0.066***	0.615	0.478	0.120	***0.000***

With respect to the other parameters, all of which are non-microbiological measures, we observe two consistent statistically significant factors influencing both the *E. coli* and enterococci plate count results (number of seagulls and water temperature) as demonstrated on Table 6. In both types of plates (*E. coli* and enterococci), we see a positive association between the number of seagulls and the number of colony forming units observed. This

association suggests that as the daily estimate of the number of seagulls increases at the beach, the number of colony forming units per plate holds also increases for both E. coli (r = 0.325, *p* = 0.046) and enterococci (r = 0.327, *p* = 0.009).

Likewise, we observe a similar pattern, as expected, with respect to water temperature. In both types of plates (*E. coli* and enterococci), we see an increased number of colony forming units with increasing water temperature. The association for E. coli (r = 0.372, *p* = 0.044) and enterococci (r = 0.375, p = 0.017*)* are again statistically significant for both plates where the *p*-values are both less than 0.05.

Table 6. Pearson correlation coefficients (r) evaluating associations among the non-microbiological parameters quantified in this study, whereby statistically significant correlations ($p < 0.05$) are bolded and italicized and marginal associations are only italicized ($p < 0.10$).

Parameter	*E. coli* Plate (Correlation r)	*E. coli* Plate (Correlation *p*)	Enterococci Plate (Correlation r)	Enterococci Plate (Correlation *p*)
pH	0.015	0.850	-0.008	0.580
Sp. Conductivity	0.074	0.197	-0.041	0.348
Dissolved Oxygen	-0.188	0.415	-0.276	0.277
Wave Height	0.302	0.114	0.152	0.241
Avg. Wind Speed	0.085	0.352	-0.055	0.902
Daily Precip.	-0.318	0.373	-0.060	0.776
Previous Precip.	0.097	0.986	-0.096	0.211
Total Geese	0.051	0.896	0.206	0.310
Total Gulls	***0.325***	***0.046***	***0.327***	***0.009***
Total People	0.002	0.431	-0.108	0.357
Chlorophyll a	0.261	0.116	*0.154*	*0.097*
Turbidity	*0.347*	*0.068*	0.201	0.208
Water Temp.	***0.372***	***0.044***	***0.375***	***0.017***
Phycocyanin	0.095	0.727	-0.011	0.857

Among these other parameters evaluated as potential factors influencing colony forming unit observations, turbidity and chlorophyll *a* results are also statistically relevant with marginally significant *p*-values ($p < 0.10$). With increasing turbidity, we observed an increased number of *E. coli* (r = 0.347, $p < 0.068$). A similar finding was observed with enterococci, but this positive association failed to achieve our level for determining any significance (r = 0.201, *p* = 0.208). Chlorophyll *a*, similarly appears to be associated with one fecal indicator (enterococci), but failed to demonstrate any significant association with *E. coli* densities.

Fecal Indicator qPCR Results

In reviewing the correlations presented earlier in Table 4, several qPCR measurements are significantly correlated with other qPCR measurements. With respect to the enterococci qPCR, we see weak, but still significant, associations with the human-specific molecular marker (HuBac, r =

0.293) and the bacteria pathogen indicator, *Arcobacter* (r = 0.193). These correlations, although both weakly correlated, are statistically significant positive associations. The HuBac marker is also significantly and positively correlated with the *E. coli* qPCR marker (r = 0.237) as well as the bacteria pathogen, *Arcobacter* (r = 0.577).

Among the environmental parameters, many are associated with the qPCR results (Table 7). The most apparent is water temperature, which is negatively associated with the results from all four qPCR markers. This suggests that as water temperature decreased in the study, we observed an increasing amount of the various genetic markers in the water as determined by qPCR. A potential hypothesis for this negative correlation to be observed for all the genetic markers is that the water temperature parameter was related to some other factor that could be influencing the qPCR results. For example, water temperature decreased consistently throughout the study period (July – September). This apparent association is demonstrated in figure 2, which presents the statistically significant associations between decreasing water temperature and the week number of the study. Cooler temperatures, seasonal bias, or a build-up of genetic material in the water are all hypothesized as potential qPCR influences.

Table 7. Pearson correlation coefficients (r) evaluating associations among the qPCR results for fecal indicators with the non-microbiological parameters quantified in this study, whereby statistically significant correlations ($p < 0.05$) are bolded and italicized and marginal associations are only italicized ($p < 0.10$).

Parameter	Ent (ent23) qPCR	*E. coli (uidA)* qPCR	HuBac qPCR	Arcobacter qPCR
pH	-0.272	-0.330	***-0.392***	-0.076
Sp. Conductivity	-0.037	-0.133	0.128	0.118
Dissolved Oxygen	-0.090	0.012	-0.023	***-0.257***
Wave Height	0.207	***0.372***	***0.338***	*0.264*
Avg. Wind Speed	0.112	0.128	***0.498***	0.474
Daily Precip.	0.020	***-0.171***	-0.085	0.123
Previous Precip.	-0.141	-0.087	***-0.331***	-0.163
Total Geese	-0.221	-0.212	***0.267***	-0.123
Total Gulls	***-0.183***	***-0.221***	0.183	-0.029
Total People	-0.024	-0.012	0.222	*-0.242*
Chlorophyll a	0.015	-0.174	0.081	0.017
Turbidity	*0.194*	***0.187***	***0.349***	0.190
Water Temp.	***-0.316***	***-0.325***	***-0.620***	***-0.312***
Phycocyanin	0.003	-0.022	0.112	0.047

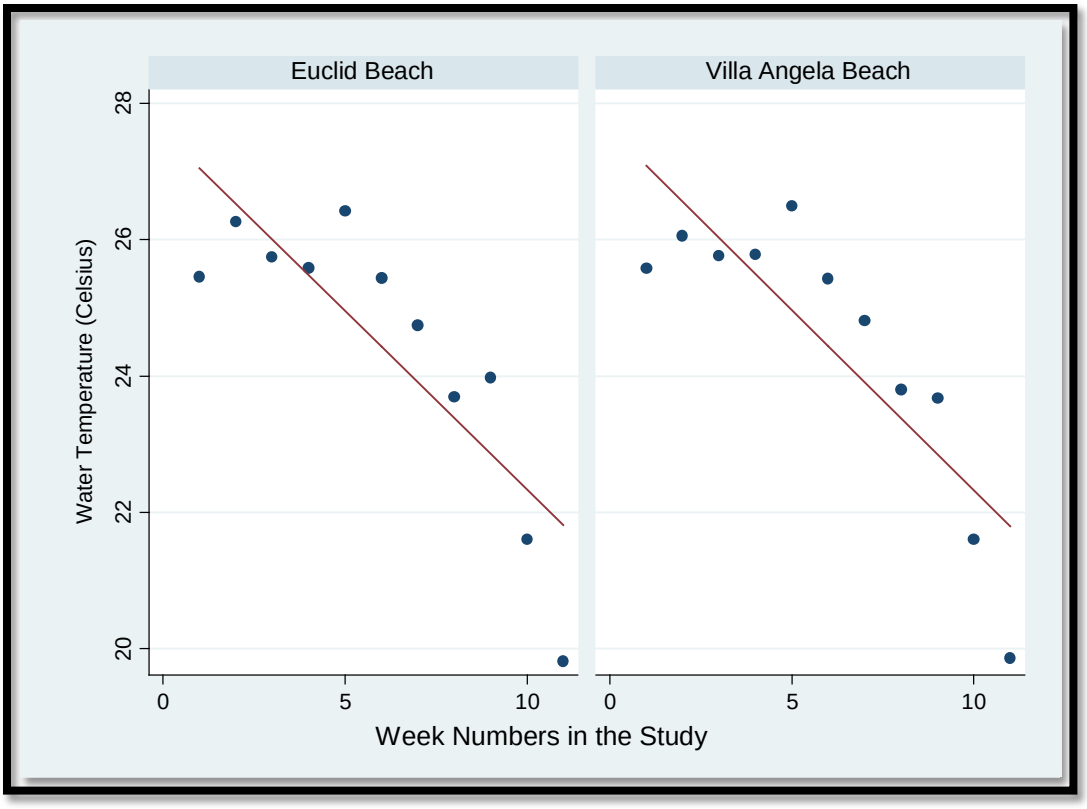

Figure 2. Scatterplots with lines-of-best-fit depicting the association between decreasing water temperature at each study beach with the week number in the study. (Euclid Beach R^2 = 70.8%, *p = 0.0012*; Villa Angela R^2 = 72.2% *p* = 0.0009)

Upon making a quick view at Figure 3, it is apparent that the highest copy numbers for HuBac observed during the study period were during the last weeks in the study. Colder water temperatures, seasonal influences or a gradual accumulation of HuBac in the water column are potential explanations for this observation, which appears to be also true for the other three genetic markers.

Among the other environmental parameters beyond water temperature and time-related variables, both wave height and turbidity are positively associated with all the parameters and both parameters achieve some level of statistical significance with 3 of the 4 genetic markers. Wave height and turbidity associations suggest that the lake sediments and shoreline sands may be acting as reservoirs that harbor genetic material for the specific fecal markers. During windy weather, choppy turbid water could be picking up microbes (potentially viable) and fecal markers detectable by qPCR. These same conditions may also be conditions in which infectious disease risk is also elevated. This is a noteworthy finding warranting further investigation.

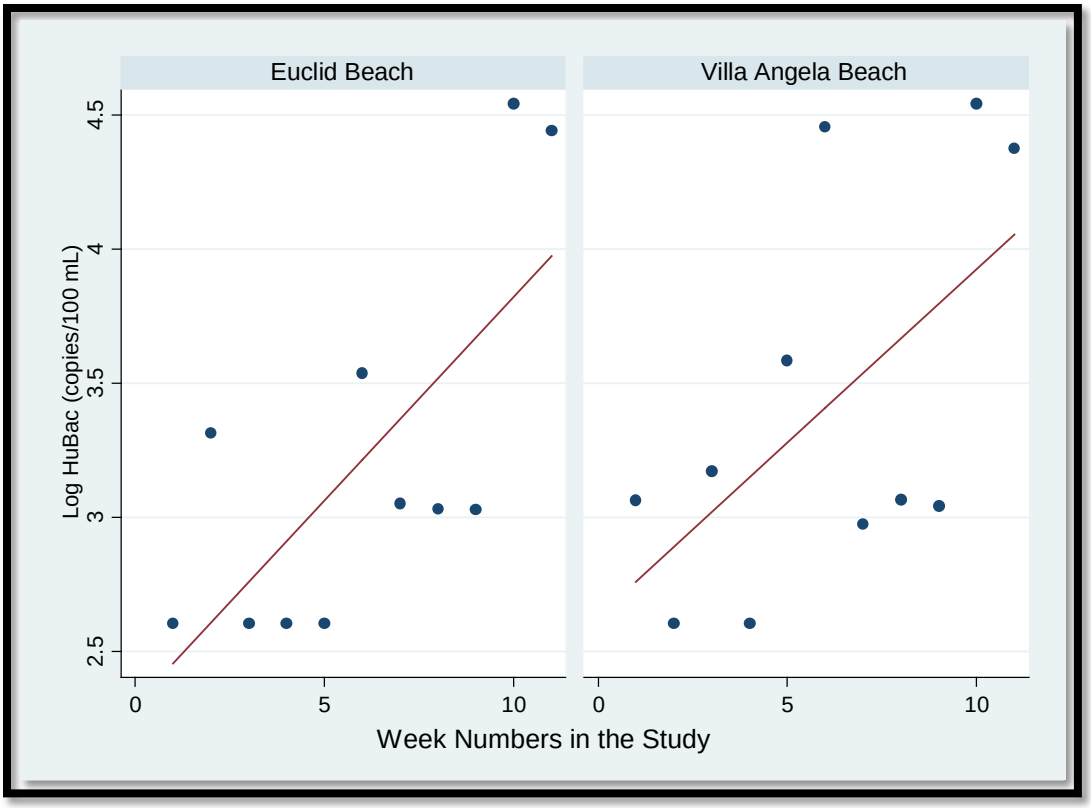

Figure 3. Scatterplots with lines-of-best-fit depicting the association between increasing copies of the HuBac genetic marker across the various weeks in the study at each study beach. (Euclid Beach R^2 = 51.0%, *p = 0.014*; Villa Angela R^2 = 35.1% *p* = 0.055)

Predicting Bacteria Pathogen Densities

Among the genetic markers evaluated in this study, the human pathogen *Arcobacter* was investigated. Two genetic markers were significantly and positively associated with *Arcobacter* densities as demonstrated in Table 4 and 5. Enterococci and HuBac achieved statistical significance ($p < 0.05$) in their association with *Arcobacter* with correlation coefficients (r) of 0.193 and 0.577, respectively. These correlation coefficients can be evaluated as coefficients of determination or R^2 values. In this case, an R^2 of 3.7% is generated from our enterococci density measurements, which means that 3.7% of the Arcobacter density is explained by an enterococci density measurement. This R^2 value is quite low and does not generate much certainty and calls into question the utility of enterococci qPCR measurements for predicting *Arcobacte*r densities. With respect to HuBac and *Arcobacter*, the correlation coefficient (r) of 0.577 translates into an R^2 of 33.3% thereby suggesting that 33.3% of our *Arcobacter* density measurement can be explained by our HuBac density measurement. In our study, with our limited data set, this is a much more reassuring value than enterococci and would lead one to the preliminary conclusion that HuBac is the best predictor of *Arcobacter* when *Arcobacter* is not measured. This is not a surprising finding as *Arcobacter* is associated with human fecal contamination events and the HuBac marker was developed for quantifying human-associated fecal indicator bacteria, such as *Bacteroides* and *Prevotella*. Enterococci and *E. coli* markers are

more general markers, designed for assessing the extent of both animal and human fecal contamination. The strength of the association between densities of *Arcobacter* and HuBac can be observed in Figure 4. The lack of association with the other markers and plate count methods can also be observed in Figure 4.

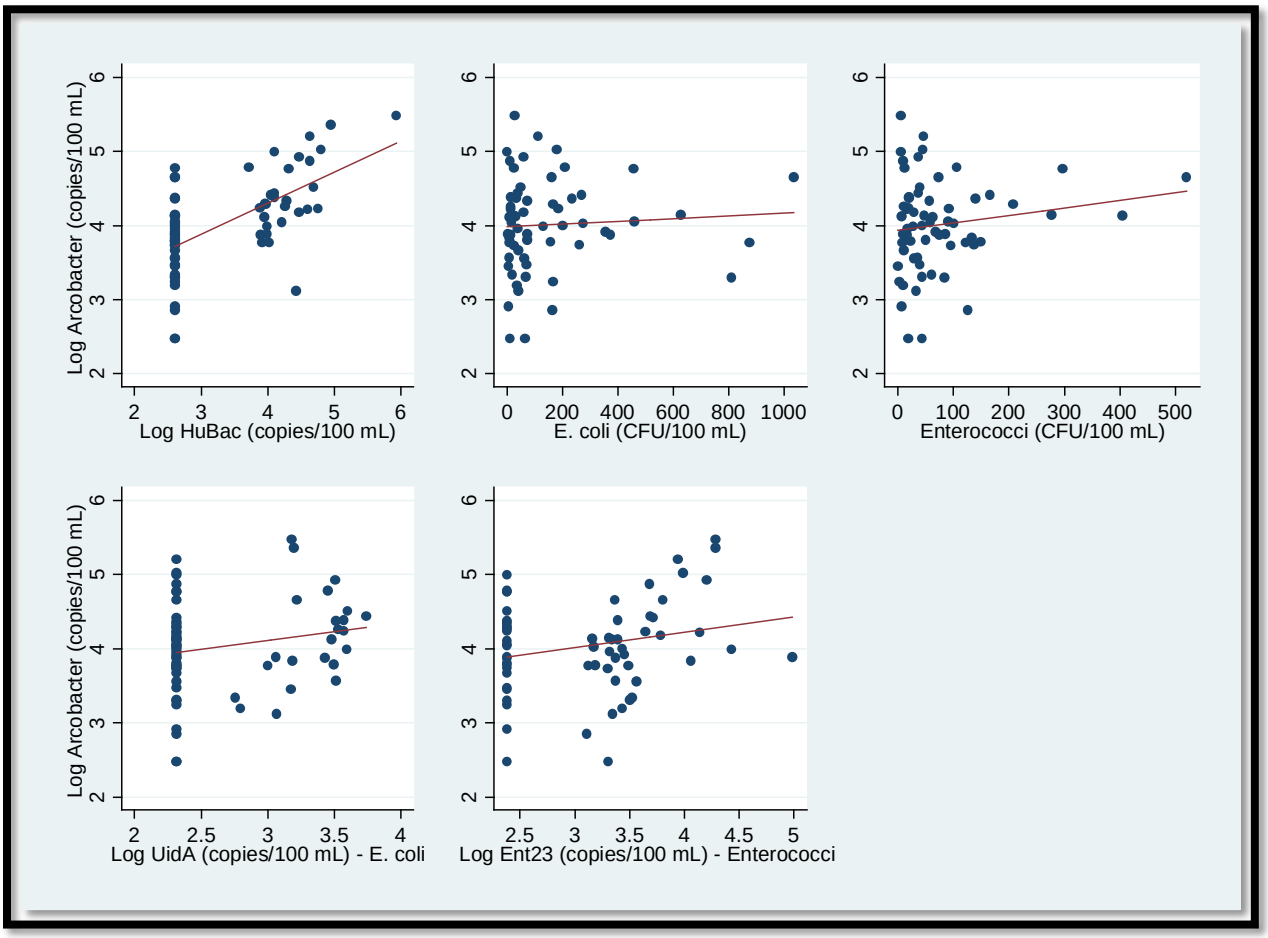

Figure 4. Scatterplots with lines-of-best-fit depicting the association between increasing copies of the HuBac genetic marker across the various weeks in the study at each study beach. (Euclid Beach R^2 = 51.0%, *p = 0.014*; Villa Angela R^2 = 35.1% *p* = 0.055)

In an attempt to better understand factors influencing *Arcobacter* densities at these two Lake Erie Beaches, we employed multivariable linear regression with a reverse selection procedure. In doing so, we identified four statistically significant environmental parameters and one genetic marker that partially explain *Arcobacter* densities at these two beaches (Table 8). Collectively, this five-variable model achieves an overall R^2 of 59.2% and is statistically significant whereby $p < 0.0001$. Nearly 60% of the measured *Arcobacter* densities observed in this study can be explained using HuBac, pH, dissolved oxygen, wave height and the observed precipitation (Figure 5).

Table 8. Summary of multivariable regression variables (factors) used for the five-term multivariable model for predicting *Arcobacter* densities in this study.

Parameter (Factor)	Coefficient	Standard Error	(*p*)
Log HuBac (copies/100 mL)	0.446	0.069	0.000
pH	0.795	0.317	0.015
Dissolved Oxygen (mg/L)	-0.053	0.016	0.001
Wave Height (cm)	0.007	0.002	0.004
Observed Precipitation (cm)	1.069	0.361	0.005
Constant Term	-3.809	2.772	0.175

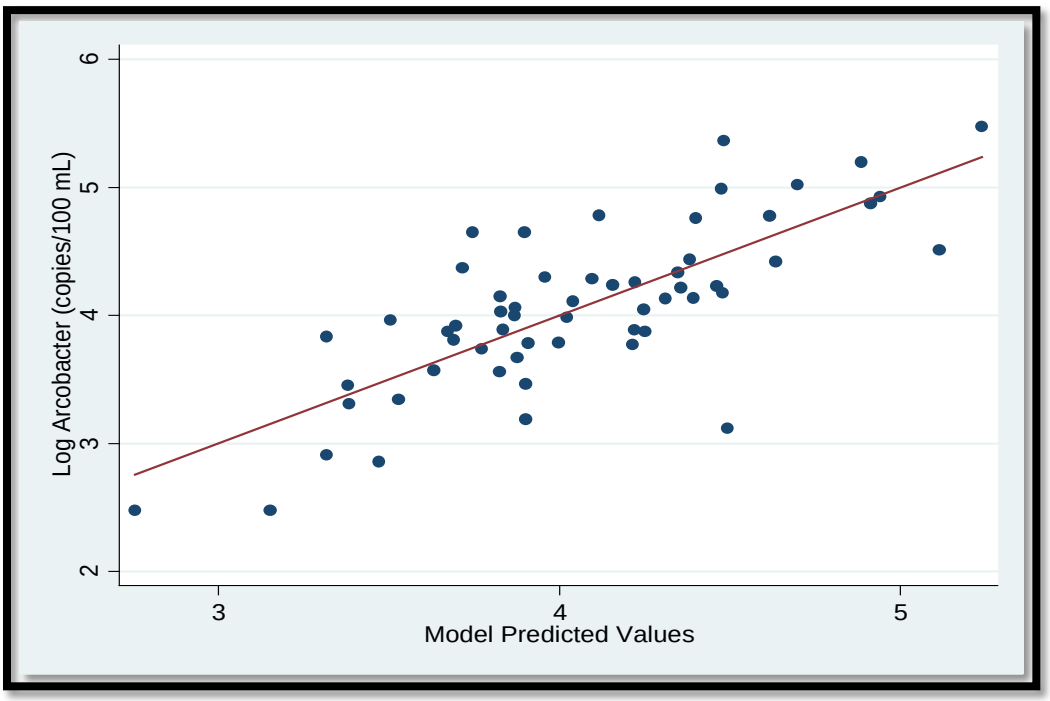

Figure 5. Scatterplot with line-of-best-fit demonstrating positive association between measured *Arcobacter* densities and model-predicted densities (R^2 = 59.2%, $p < 0.001$).
Predicting Viral Pathogen Densities

Predicting Viral Pathogen Densities

Among the 66 samples dedicated for virus quantification, only two samples generated positive detections for enterovirus (entV). All adenovirus and norovirus samples generated results below the limit of detection. This is a good finding indicating that viral densities at these two beaches appear to be low; however, virus infections can occur at very low levels.

Overall, in evaluating the characteristics of the EntV(+) samples versus the EntV(-) samples, only specific conductivity and *E. coli* qPCR generated statistically significant differences between EntV(+) versus EntV (-) results. The mean qPCR *E. coli* result using the uidA gene was 3.187 log copies/100 mL for when EntV was detected. When EntV was undetected, the mean density for

the uidA gene was only 2.613 log copies/100 mL, which was significantly lower (p = 0.035) as observed in Table 9. Given the low number of EntV (+) results, even this interpretation should be done cautiously.

Table 9. A comparison of the differences in the means (as determined by the t-test) for various microbiological and non-microbiological parameters when human enterovirus was detected or not detected with marginal differences ($p < 0.10$) indicated in italics only and statistically significant differences ($p < 0.05$) illustrated in bold + italics.

Parameter	EntV(+) Mean	EntV (-) Mean	Non-Equal (p)
Ent qPCR, ent23 (log copies/100 mL)	3.353	3.089	0.420
E. coli Plate (CFU/100 mL)	42.778	170.525	0.241
Enterococci Plate (CFU/100 mL)	44.833	92.004	0.442
E. coli qPCR , uidA (log copies/100 mL)	***3.187***	***2.613***	***0.035***
HuBac qPCR (log copies/100 mL)	3.054	3.336	0.601
Arcobacter qPCR (log copies/100 mL)	3.668	4.070	0.229
pH	*8.255*	*8.519*	*0.060*
Sp. Conductivity (uS)	***249.667***	***296.000***	***0.030***
Dissolved Oxygen (ppm)	13.226	10.997	0.304
Wave Height (cm)	18.667	24.229	0.667
Avg. Wind Speed (mph)	6.217	7.237	0.487
Daily Precip. (in.)	0.000	0.103	0.253
Previous Precip. (in.)	0.000	0.094	0.311
Total Geese (individuals)	1.667	8.100	0.432
Total Gulls (individuals)	10.000	46.500	0.126
Total People (individuals)	1.833	1.233	0.504
Chlorophyll a (ppb)	4.420	6.954	0.352
Turbidity (NTU)	4.617	8.619	0.368
Water Temp. (°C)	23.742	24.507	0.620
Phycocyanin (ppb)	11.102	12.651	0.501

CONCLUSIONS

In summary, our results support the following major findings:

1) Enterococci qPCR should be used with caution for estimating infectious disease risk at these two Lake Erie beaches as the qPCR results lack association with traditional fecal indicators.

2) Future epidemiology studies should be considered at these beaches or similar Lake Erie beaches to understand whether or not enterococci qPCR is predicitive or effective in estimating illness risk among water users when qPCR results do not correlate with culture-based fecal indicators.

3) A human-specific marker (HuBac) appears to be an effective indicator for predicting densities of the pathogen *Arcobacter* in Lake Erie waters, which also suggests that *Arcobacter* at these beaches may be linked to human-associated fecal pollution.

IMPLICATIONS FOR BEACH WATER MANAGEMENT

In this study, enterococci qPCR was evaluated against culture-based methods and qPCR with other targets that are related to fecal contamination (HuBac, etc.) It is apparent that enterococci qPCR and the traditional measures of fecal contamination were not associated or correlated with one another in this study, which is a cause for concern. The traditional measures of fecal contamination, culture-based *E. coli* and culture-based enterococci were positively and strongly correlated between them ($r = 0.646$, $p = 0.000$). If qPCR is to be employed as a new rapid measure for determining the extent of fecal contamination at a beach or the extent of infectious disease risk, many different outcomes pertaining to beach closure can be expected for these two beaches.

Overall, this study was limited to two adjancent beaches with significant fecal contamination. Furthermore, this study evaluated water quality for only one swimming season. Additionally, this study relied on single daily samples as opposed to many throughout the day. In summary, this study was limited to relatively small number of samples and may not capture or refelct the true associations observed at other Lake Erie beaches or during other swimming seasons. However, this study does demonstrate a significant need for concern regarding the impacts of new recreational water quality criteria relying on qPCR and for continued beach monitoring using the traditional surveillance system that utilizes culture-based methods for *E. coli*.

It is plausible that the traditional culture-based fecal indicators (*E. coli* or enterococci) are still capable of being used effectively for estimating disease risk as they were designated by USEPA in 1984 (Dufour 1984), although there are not received in a timely manner (18-24 hours later). Furthermore, it is also plausible that qPCR for enterococci measured in our study outperforms the culture-based methods for estimating disease risk as demonstrated by EPA by Wade et al. (2008) and can present results within six hours. However, given the lack of association between the qPCR enterococci results with the plate-based methods, there is a cause for concern. Among the qPCR markers, HuBac was well-associated with the pathogen *Arcobacter*; whereas, qPCR enterococci was only weakly associated. The use of HuBac or other human-specific marker as a qPCR-based method may hold more promise than qPCR-enterococci for estimating or predicting illness risk at these two Lake Erie beaches.

DISSEMINATION OF INFORMATION

1) C. Lee, S. Agidi, J.W. Marion, and J. Lee. 2012. *Arcobacter* in Lake Erie beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. *In press*. *Applied and Environmental Microbiology.*

2) J. Lee, S. Agidi, C. Lee, and J. Marion. An emerging gastrointestinal pathogen associated with human-originated contamination sources in Lake Erie beach waters. 4th International EcoSummit, Columbus, September 2012.

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