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Microbial water quality at contrasting recreational areas in a mixed-use watershed in eastern Canada

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ABSTRACT

Recreational water use is an important source of human enteric illness. Enhanced (episodic) surveillance of natural recreational waters as a supplement to beach monitoring can enrich our understanding of human health risks. From 2011 to 2013, water sampling was undertaken at recreational sites on a watershed in eastern Canada. This study compared the prevalence and associations of human enteric pathogens and fecal indicator organisms. Beach water samples had lower pathogen presence than those along the main river, due to different pollution sources and the hydrological disposition. Pathogen profiles identified from the beach sites suggested a more narrow range of sources, including birds, indicating that wild bird management could help reduce public health risks at these sites. The presence and concentration of indicator organisms did not differ significantly between beaches and the river. However, higher concentrations of generic *Escherichia coli* were observed when *Salmonella* and *Cryptosporidium* were present at beach sites, when *Salmonella* was present at the river recreational site, and when verotoxigenic *E. coli* were present among all sites sampled. In this watershed, generic *E. coli* concentrations were good indicators of potential contamination, pathogen load, and elevated human health risk, supporting their use for routine monitoring where enhanced pathogen testing is not possible.

Key words: *E. coli*, enteric disease, indicator organisms, public health risk, recreational water, waterborne pathogens

HIGHLIGHTS

- Enhanced surveillance of recreational waters can supplement water quality monitoring and inform our understanding of what contributes to the risk of human illness.
- Water collected at reservoir beaches was less contaminated than sites along the main river.
- Subtyping of pathogens at the beach sites suggested a narrow range of sources.
- The nature of the sampling site influenced the prevalence and types of pathogens.

INTRODUCTION

Canadians regularly visit beaches, rivers, lakes, and ponds to swim and take part in other recreational water activities. Nearly two-thirds of surveyed Canadians report swimming in lakes or rivers at least once per year (Royal Bank of Canada 2016). From epidemiological data and water monitoring studies, we know many Canadians will be exposed to waterborne pathogens from these activities and, consequently, become ill (Butler *et al.* 2016; Napier *et al.* 2017; Janicki *et al.* 2018). Recreational water illnesses can be mild, including nausea or mild diarrhea, to severe, resulting in hospitalization or death (Fewtrell & Kay 2015).

In Canada, recreational water exposure, which includes both pools and natural waters, is responsible for an estimated 24% of all enteric illnesses (Butler *et al.* 2016), posing a significant cost to the Canadian economy, with over 600,000 illnesses and a cost of over $840 million estimated in 2012 (Vinson 2012). Recent research estimates 4 billion surface water recreation visits

each year in the USA, resulting in approximately 90 million illnesses nationwide at a cost of $2.2–$3.7 billion USD per year (DeFlorio-Barker *et al.* 2018).

Surface water monitoring studies are an important component of a robust enteric disease surveillance system, as they help inform risk assessments and public health intervention efforts (Boehm *et al.* 2009; Health Canada 2012; US EPA Office of Water 2012; Marion *et al.* 2014; Korajkic *et al.* 2018; Ontario Ministry of Health and Long-Term Care 2018). In addition to informing source attribution as part of a national surveillance system, water surveillance studies can inform local watershed managers and public health managers on the local water system and be used to develop watershed-specific education and risk mitigation measures.

However, approaches to water monitoring at the local level vary by jurisdiction, often limited to periodic sampling of indicator organisms in targeted waters due to cost-effectiveness and ease of testing. Fecal indicator organisms, including *Escherichia coli* and enterococci, are important for understanding public health risks from the use of surface waters and guiding intervention efforts because pathogen testing of surface water is currently more expensive. Fecal indicator organisms identify potential fecal contamination and are used as indicators for the presence (and sometimes densities) of human pathogens, although the relationship between fecal indicators, pathogen occurrence/densities, and human illness can be variable (Boehm *et al.* 2009; Korajkic *et al.* 2018). Fecal indicator test results that exceed water quality guidelines can result in public health prevention actions such as 'beach closures' or 'beach postings' (Health Canada 2012; Ontario Ministry of Health and Long-Term Care 2018).

Natural recreational waters can be vastly different in their physiography, hydrology, biology, and anthropogenic activities (US EPA Office of Water 2012). Lakes, reservoirs, and rivers make up the vast majority of recreational freshwater venues in Canada. These classes of freshwater recreational waters can have different infectious disease exposure pressures depending on: (i) wildlife habitats (Kruse *et al.* 2004), (ii) pathogen reservoirs in sediments and near-shore environments (Frey *et al.* 2015), (iii) sedimentation and flushing potential (Frey *et al.* 2015), (iv) factors that augment growth and survival of pathogens such as temperature and suspended solids (Blaustein *et al.* 2013), (v) upstream contamination sources including agriculture, wastewater effluents (Canadian Council of Ministers of the Environment 2004), and septic system leaks (Spoelstra *et al.* 2017), and (vi) human use of the water body (Fewtrell & Kay 2015).

This study builds on previous surveillance and research efforts to better understand the drivers of pathogen loading in heavily used/impacted watersheds in eastern Canada (Lee *et al.* 2010, 2014; Edge *et al.* 2012; Johnson *et al.* 2014; Lapen *et al.* 2016; Thomas *et al.* 2016; Swirski *et al.* 2018) as a part of the national, integrated enteric disease surveillance program, FoodNet Canada (Public Health Agency of Canada 2013). This study assesses the prevalence of enteric pathogens and fecal indicator organisms in reservoir beaches and running-river recreational sites that contrast considerably from the perspective of hydrology and both natural and anthropogenic contamination pressures. Associations among waterborne pathogens and fecal indicator bacteria were examined in the context of trends and sources of waterborne pathogen presence. The ultimate purpose of this study was to help guide public health measures to reduce the disease risk from recreational water use and to help inform where and when water quality monitoring resources need to be amplified.

METHODS

As part of a broader enhanced water sampling program by FoodNet Canada and the Ontario Ministry of the Environment, Conservation and Parks exploring sources and influences of contamination on river and recreational water, this analysis investigated water samples collected from five sites along the Grand River Watershed (6,800 km^2) in the Region of Waterloo, Ontario, Canada from 2011 to 2013. The Grand River Watershed is comprised of agricultural row cropping (43%), pastures and range-grasses (27%), forests (12%), urban areas (9%), and wetlands (2%) (Kaur *et al.* 2019). Wastewater treatment plant (WWTP) effluents serve as critical, but regulated, point sources of water contamination and direct river water inputs in this watershed (Hwang *et al.* 2019).

River water samples were collected twice per month throughout the year at two reference sites that form part of the ongoing surveillance program in the region: directly upstream from the drinking water intake (DWI; GR1) and directly downstream of a municipal WWTP (GR2) outflow in the main river (Figure 1; Table 1). These sites served as a means to contrast the recreational water site data.

Recreational water samples were collected twice per month during the swimming season (June–September) at three sites (see Figure 1 and Table 1). The two sandy beach-dominated water recreational sites, Shade's Mills (GR6) and Laurel Creek

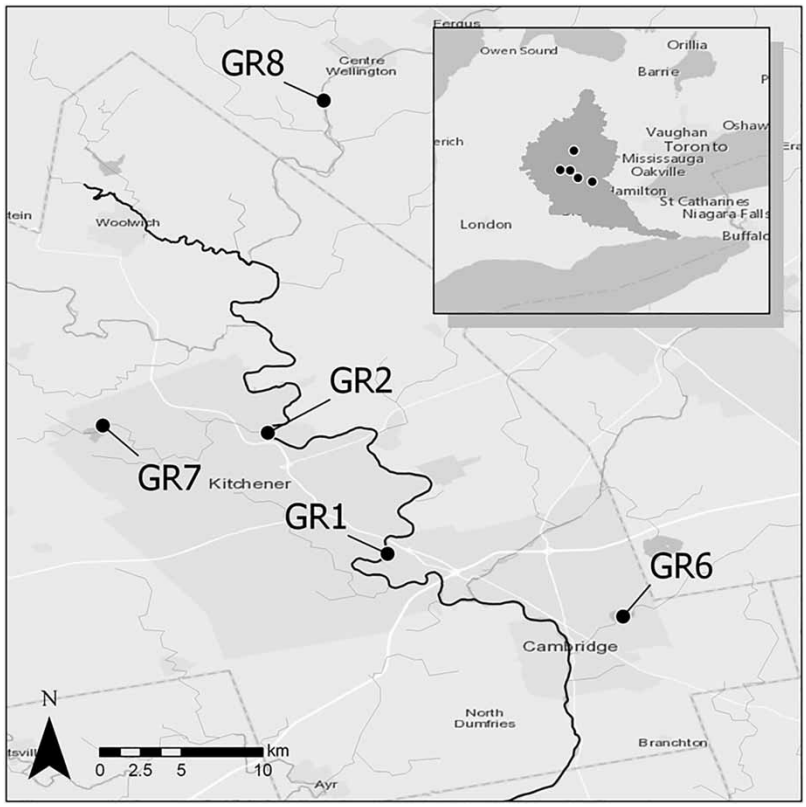

Figure 1 | Location of the water sample sites in the Grand River, Ontario, Canada. Beach sites: Shade's Mills (GR6) and Laurel Creek (GR7), river recreational site at Elora Gorge (GR8), and reference sites upstream of DWI (GR1) and downstream of WWTP effluent site (GR2).

Table 1 | Site designation and testing periods for each microbiological parameter at each Grand River Watershed sampling site, 2011–2013

		Microbiological testing time periods	
Name	**Site ID[a]**	***Campylobacter* spp., *Salmonella* spp., *E. coli*, VTEC[b], enterococci, *Bacteroides***	***Cryptosporidium* spp., *Giardia* spp.**
Rec. Site: beach water at Shade's Mills reservoir	GR6	June–September	June–September
Rec. Site: beach water at Laurel Creek reservoir	GR7	June–September	June–September
Rec. Site: river water at Elora Gorge on river stem	GR8	June–September	June–September
Ref. Site: upstream of DWI on river stem	GR1	Year Round	October–April
Ref. site: WWTP effluent impacted water on river stem	GR2	Year Round	October–May

DWI, drinking water intake; WWTP, wastewater treatment plant.
[a]Site designation per other research studies on this watershed (Lapen *et al.* 2016; Chen *et al.* 2018).
[b]VTEC refers the isolation of verotoxin-producing *E. coli*.

(GR7), are located on dammed reservoirs on tributaries of the main river stem. These sites are used for swimming and other activities such as windsurfing. The Elora Gorge recreational site (GR8) is located on the main river at the point where fast-water enthusiasts exit the river. The DWI site and the WWTP effluent impacted site are both located downstream of the river gorge site on the main river channel. Additional information about sample sites and nearby land use is available elsewhere (Lee *et al.* 2014; Lapen *et al.* 2016; Chen *et al.* 2018).

Microbiological testing

Single sample bacterial and protozoan testing and enumeration were completed as part of the Public Health Agency of Canada's FoodNet Canada water quality surveillance program (Public Health Agency of Canada 2006). Water samples were

collected using 1 L sterile sampling bottles and sent to Laboratory Services Division, University of Guelph for bacteriology testing. Duplicate 25 L samples were sent to Hyperion Ltd for parasitology testing once per month. *Salmonella* and VTEC isolates were submitted to the National Microbiology Laboratory in Guelph, Ontario for further subtyping. Microbial testing methods are further described elsewhere (Lee *et al.* 2010, 2014; Johnson *et al.* 2014; Lapen *et al.* 2016; Swirski *et al.* 2018).

Water samples were analyzed for the presence of *Campylobacter* spp., verotoxigenic *E. coli* (VTEC), *Salmonella* spp., *Cryptosporidium* spp., and *Giardia* spp., as well as general fecal indicator bacteria, *E. coli* (referred to as 'generic' *E. coli* in the text) and enterococci, and host-specific human and bovine *Bacteroides* as indicators of the source of fecal pollution (Lee *et al.* 2010) (Table 1). *Campylobacter* spp. concentrations were calculated based on the most probable number (MPN) method (Schmidt *et al.* 2013). *Campylobacter* spp. were considered present when the MPN value was 1 or greater. Enumeration was not done for *Salmonella* spp. or *E. coli* VTEC. Concentrations of *Cryptosporidium* spp. and *Giardia* spp. were reported as the number of oocysts or cysts per 100 L, respectively.

Sequencing

As Whole Genome Sequencing (WGS) has proved to be a high-resolution subtyping method, *Salmonella* spp. isolates were sequenced and analyzed to further assess potential upstream contamination sources from agricultural inputs, as possible pathogen reservoirs might originate from animal sources.

All but four *Salmonella* isolates from water samples included in this study were sequenced and compared with 507 *Salmonella* isolates from Foodnet Canada retail sample sources and 259 *Salmonella* isolates from Foodnet Canada farm manure sample sources collected in the same region between 2011 and 2013.

DNA from *Salmonella* isolates was extracted using the EZ1 DNA tissue kit (Qiagen, Valencia, CA). Libraries were prepared using the Nextera XT DNA library prep kit (Illumina, San Diego, CA). Sequencing was performed on the Illumina MiSeq platform or the Illumina NextSeq platform with the MiSeq Reagent Kit V3 (Illumina) or the NextSeq 500/550 Reagent kit V2.5 (Illumina) to obtain an average genome coverage of greater than or equal to 40 $\times$ for all isolates. Raw sequence data were deposited in the Integrated Rapid Infectious Disease Analysis (IRIDA) platform. Sequence analysis was performed with Bionumerics version 7.6.3 (AppliedMaths), using Whole Genome Multilocus Sequence Typing (wgMLST) schemes. A dendrogram was constructed using the categorical (values) similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA for hierarchical clustering). Dendrograms or Minimum Spanning Trees were generated for each serotype to assess the genetic diversity of strains isolated from water sampling and to compare water strains to *Salmonella* spp. isolates from FoodNet Canada retail and farm manure sequences. Isolates were considered possibly related by WGS if they were within 0–10 wgMLST allele differences.

Statistical testing

All statistical analyses were conducted using Stata SE 15.1 (StataCorp, College Station, TX, USA). Two-sided Fisher's exact tests were performed to detect differences in the presence of fecal indicators or pathogens by sample site, both during and outside of the swimming season, and by month. Kruskal–Wallis (K–W) tests were performed using the concentration of fecal indicators or pathogens by sample site and over time, supported by Dunn's test of multiple comparisons to interpret the results of significant K–W tests.

A reference threshold of >400 CFU per 100 mL for generic *E. coli* was used as a relative comparative value across months and sample sites, derived from Canada and Ontario's Recreational Water Quality Standards single sample criteria for beach closures/postings (Health Canada 2012; Ontario Ministry of Health and Long-Term Care 2018). Mann–Whitney U (M–W) tests were performed to determine if the concentrations of pathogens and indicators were different between samples that did or did not exceed the 400 CFU per 100 mL threshold for generic *E. coli*. Spearman's rank correlation was performed to evaluate correlations in the concentration of pathogens or indicators across sample sites and precipitation variables. Only non-parametric tests were performed, as Shapiro–Wilk normality tests showed that none of the distributions for pathogen and indicator concentration were normally distributed ($p < 0.0001$).

Significance was determined at $p \leq 0.05$ across all statistical tests.

RESULTS

A total of 206 water samples for all sites were collected during the 3-year study period.

Table 2 provides summary information on the presence and concentrations of fecal indicators and pathogens over this time. For pathogen detection and isolation of specific subtypes of pathogens, the denominator (N) is the number of samples tested for that pathogen or the number of samples for which subtyping was performed at each site, either during the swimming season or outside of it.

Water quality results

Verotoxigenic *E. coli*

The proportion of samples that tested positive for VTEC was significantly different across sample sites (Fisher's exact $p < 0.001$) both during and outside of the swimming season (Table 2). VTEC was more commonly found at the reference WWTP effluent site (GR2) (83% of samples overall, 88% during the swimming season) compared to the other sites (Table 2). VTEC was detected in a significantly higher proportion of samples at the river gorge (Elora Gorge) site (39%) than at the beach sites (17%; Fisher's exact $p = 0.03$). Differences in the proportion of samples with VTEC isolation were not significant across the months of the swimming season for any sample site (Fisher's exact $p > 0.05$).

E. coli O157 was not detected at any recreational water site and was only reported during the swimming season at the WWTP effluent site (GR2) (Table 2). Overall, *E. coli* O157 was detected in 6/86 samples. The most commonly identified serotype was *E. coli* O103 ($n = 12$), which is in the top seven serogroups of human health significance (Federal VTEC Working Group 2011). *E. coli* O103 was not identified at any of the recreational water sites. *E. coli* O45 was the only serogroup within the top seven priority subtypes identified from recreational water sites, among two samples from the river gorge site.

Campylobacter spp.

Campylobacter spp. were detected in 32% (51/158) of samples where testing was performed, including 61% of recreational water samples (Table 2). The prevalence of *Campylobacter* spp. differed significantly by sample site during the swimming season (Fisher's exact $p = 0.05$). The difference was only marginally insignificant between the river gorge and the beach sites ($p = 0.058$).

The proportion of all samples that were positive for *Campylobacter* spp. increased over the course of the swimming season. This is significant overall (Fisher's exact $p < 0.001$).

Campylobacter jejuni ($n = 23$), *C. lari* ($n = 5$), and *C. coli* ($n = 2$) were detected in the 29 samples, which had speciation completed, including two samples where multiple types were identified. The most commonly identified serovar at recreational water sites was *C. jejuni*. *C. coli* was only detected at the DWI site (outside of swimming season), while *C. jejuni* was mostly detected at the reservoir beach site, and *C. lari* at the river gorge site.

Salmonella spp.

Salmonella spp. were detected less frequently at recreational sites (8% of samples at the beach sites, 21% of samples at the river gorge site), compared to the reference river sites GR1 (38%) or GR2 (63%) (Table 2).

There was a significant difference in the proportion of *Salmonella* spp. positive samples by month at the beach (Shade's Mills) and the DWI sites (Fisher's exact $p = 0.04$, both). At the reservoir beach site (Shade's Mills), *Salmonella* spp. were detected in 50% of samples in September, and 0% of samples from June to August. The most common serotypes identified at recreational water sites were *S.* Typhimurium (two samples from Elora Gorge) and *S.* Thompson (one sample each from Elora Gorge and Shade's Mills; Table 3).

Comparison across water, retail, and on-farm manure sampling at this FoodNet Canada sentinel site shows a broad diversity of *Salmonella* spp. serotypes (Table 3). Further analysis of *Salmonella* spp. diversity using wgMLST identified one *Salmonella* Typhimurium isolate from the Shade's Mills beach sample site that clustered within five alleles to three retail chicken isolates. All four samples were collected within a time span of 3 months. While there were similarities among strains at the two river reference sites, the remainder of *Salmonella* isolates from recreational water sites varied from each other and from retail and farm samples (Supplementary Material, Table S1). Of the additional 12 clusters containing water isolates from non-recreational water sites (GR1 and GR2) that clustered with another sample source, five (42%) were related to retail chicken sources, three (25%) were related to farm sources, and eight (67%) were related to other surface water samples. For all clusters of water isolates within the 0–10 allele range, the date of sample collection ranged from the same day up to 27 months apart (Supplementary Material, Table S1).

Table 2 | Summary of fecal indicator and pathogen presence and concentration in surface water samples

	Swimming season						Out of season	
	Rec. beach sites			Rec. river site	Reference sites		Reference sites	
	Shade's Mills	Laurel Creek	Combined beach sites[a]	Elora Gorge	Upstream of drinking water intake	Wastewater treatment plant effluent	Upstream of drinking water intake	Wastewater treatment plant effluent
Pathogens								
Pathogenic *E. coli*								
VTEC detection (*n*/*N*, %)	2/23 (8.7)	6/23 (26.1)	8/46 (17.4)	9/23 (39.1)	9/23 (39.1)	20/23 (87.0)	17/47 (36.2)	30/37 (81.1)
E. coli O157 detection (*n*/*N*, %)	0/2 (0)	0/6 (0)	0/8 (0)	0/8 (0)	0/8 (0)	3/17 (15)	1/16 (6)	2/29 (7)
Campylobacter spp.								
Detected *n*/*N* (%)	11/17 (64.7)	4/13 (30.8)	15/30 (50)	13/16 (81.3)	12/16 (75.0)	6/12 (50.0)	5/48 (10.4)	0/36 (0)
Median MPN per 100 mL	2.2	2.0	2.2	0.6	1.7	0.8	161.0	–
Salmonella spp.								
Detected *n*/*N* (%)	3/24 (12.5)	1/24 (4.2)	4/48 (8.3)	5/24 (20.8)	9/24 (37.5)	15/9 (62.5)	14/48 (29.2)	31/38 (81.6)
Cryptosporidium spp.								
Detected *n*/*N* (%)	10/12 (83.3)	8/12 (66.7)	18/24 (75)	10/12 (83.3)	–	–	11/11 (100)	9/12 (75.0)
Median oocysts per 100 L	3.1	4.0	4.0	4.0	–	–	13.3	4.6
Giardia spp.								
Detected *n*/*N* (%)	10/12 (83.3)	5/12 (41.7)	15/24 (62.5)	11/12 (91.7)	–	–	11/11 (100)	12/12 (100)
Median cysts per 100 L	5.5	4.1	5.0	11.7	–	–	37.5	257.2
Fecal indicators								
Generic *E. coli*								
Median CFU per 100 mL (min–max)	56 (4–680)	72 (4–900)	62 (4–900)	108 (44–920)	320 (20–2,100)	66,500 (3,700–1.2×10^6)	280 (4–6,000)	34,500 (400–430,000)
% Samples exceeding threshold[b]	4.2	16.7	10.4	12.5	45.8	100	36.2	97.4
Enterococci								
Median CFU per 100 mL (min–max)	100 (4–460)	103 (4–940)	100 (4–940)	115 (40–1,400)	140 (56–2,200)	21,000 (12–320,000)	310 (20–7,200)	11,500 (470–1.3×10^7)
Bacteroides								
Total *Bacteroides* median cells per 100 mL	50,000	39,000	43,500	28,500	160,000	2.9×10^7	300,000	9.9×10^6
Bovine-specific *Bacteroides* detected *n*/*N* (%)	6/15 (40)	9/15 (60)	15/30 (50)	12/16 (75)	13/15 (75)	15/15 (100)	20/22 (91)	24/24 (100)
Human-specific *Bacteroides* detected *n*/*N* (%)	9/15 (60)	5/15 (33)	14/30 (47)	11/16 (69)	15/15 (100)	13/15 (87)	19/22 (86)	23/24 (96)

VTEC, verotoxigenic *E. coli*; MPN, most probable number (of *Campylobacter* spp.) in positive samples; CFU, colony forming units.

Note: – refers not tested or not applicable.

[a]Beach sites=both sites.

[b]Reference *E. coli* threshold of >400 *E. coli* CFU per 100 mL.

Table 3 | Top 10 *Salmonella* serotypes identified and sequenced from water sites, and corresponding number of isolates sequenced from the retail and farm components of the FoodNet Canada Ontario sentinel site, 2011–2013

	Water					Retail				Farm manure			
Serotype	GR6	GR7	GR8	GR1	GR2	Chicken Breast	Frozen Breaded Chicken	Ground Chicken	Ground Turkey	Broiler Chicken	Beef Cattle	Dairy Cattle	Swine
Thompson	1		1	1	7		1	2					
Newport			1	4	1			3					
Paratyphi B				1	5								
Heidelberg				2	3	27	39	53	8	73	5	2	
Infantis		1		2	2		4	3		1		1	1
Typhimurium			2	1	2	8	6	7	1			4	5
Hadar				1	3	5	7	5	1		1		
Hartford				2	2								
Agona					3	1	3			1	1		4
Give				2								4	

Cryptosporidium spp.

Cryptosporidium spp. were identified in 81% (48/59) of samples tested, including 75% of beach samples, 83% of samples from Elora Gorge, and 87% (20 / 23) of samples from reference river sites (Table 2). Samples from river reference sites were only tested for *Cryptosporidium* spp. outside of the swimming season. Differences in the presence of *Cryptosporidium* spp. between recreational sample sites were not significant during the swimming season.

Overall, the proportion of samples where *Cryptosporidium* spp. were detected was the lowest in June (44%) increasing to 100% in September across all recreational water sites (Fisher's exact $p = 0.04$).

Concentrations of *Cryptosporidium* spp. did not differ significantly across recreational water sites (K–W $p = 0.94$), nor did the concentration at any of the recreational water sites differ significantly by month during the swimming season ($p > 0.05$).

Thirteen distinct *Cryptosporidium* genotypes were detected in the samples where genotyping was performed, including 12 samples identifying multiple genotypes (Table 4). The most commonly identified genotypes were Andersoni ($n = 12$), Ubiquitum ($n = 10$), Parvum ($n = 7$), and Baileyi ($n = 7$), with Baileyi being the dominant genotype at the beach sites, most commonly associated with bird sources.

Table 4 | *Cryptosporidium* genotypes identified, by site type

Cryptosporidium genotypes identified	Human health risk level	Common sources[a]	Beach reservoir sites[b]	River sites[c]
Andersoni	Low	Cattle	0	12
Ubiquitum	Moderate	Deer, sheep	1	3
Baileyi	Low	Birds	6	1
Parvum	High	Human, ruminants, others	2	3
Hominis	High	Human	0	2
Muskrat	None	Muskrat, vole	1	1
Skunk genotype	Low	Skunk, raccoon	0	2
Deer Mouse	None	Deer Mouse	0	1
Felis	Low	Cats	0	1
Vole	None	Vole	0	1
W25	None	Wildlife	1	0

[a]References: Van Dyke *et al.* (2012), Wilkes *et al.* (2013), Guo *et al.* (2015), and Thomas *et al.* (2016).
[b]Beach Reservoir sites at Shade's Mills and Laurel Creek.
[c]River Sites at Elora Gorge and upstream of DWI.

Giardia spp.

Overall, *Giardia* cysts were detected in 83% ($n = 49/59$) of samples, including 100% of samples tested from reference river sites (Table 2). The differences in the presence of *Giardia* spp. across recreational sites during the swimming season was significant (Fisher's exact $p = 0.04$). *Giardia* spp. were present in a higher proportion of samples from Elora Gorge (92%) than from beach sites (63%).

Among recreational water sites, Elora Gorge had significantly higher concentrations of *Giardia* spp. (11.7 cysts per 100 L), compared to the beach sites (Shade's Mills (5.5) and Laurel Creek (4.1)) (Dunn's $p < 0.01$ and $p = 0.02$, respectively).

Generic *E. coli* and enterococci

Generic *E. coli* and enterococci were detected in all samples during the study period. Differences in *E. coli* and enterococci concentrations were not significant between the recreational sites (beaches or Gorge, $p > 0.05$). While samples from GR1 (intake site) had greater variability and higher median concentrations of *E. coli* and enterococci than the three recreational sites (Table 2), concentration differences were only significant for *E. coli* at one beach location (Shade's Mills, $p < 0.05$). *E. coli* and enterococci concentrations at the WWTP site (GR2) were orders of magnitude higher (Table 2; $p < 0.05$) than all other sites. The concentration of *E. coli* increased across the swimming season at each sample site, but this was only significant at GR7 with concentrations in June significantly lower than July and September ($p < 0.05$).

E. coli concentrations at the three recreational sites rarely exceeded the 400 CFU per 100 mL threshold limit over the 3 years of study, with one sample from Shade's Mills beach site, four samples from Laurel Creek beach site, and three samples from Elora Gorge exceeding the threshold. None of the recreational sites exceeded a secondary contact threshold (1,000 *E. coli* CFU per 100 mL) during the study period (Health Canada 2012). The reference DWI intake site (GR1) exceeded the 400 per 100 mL threshold in 16% (11/24) of samples, but only three samples exceeded the 1,000 *E. coli* CFU per 100 mL threshold. In all instances, the samples taken at the site downstream of the WWTP effluent exceeded both thresholds.

Bacteroides

Total *Bacteroides* were detected from all samples, with concentrations lowest at the recreational sites (Table 2). Differences in the concentration among the three recreational sites were not observed ($p > 0.05$); however, differences ($p < 0.05$) were observed between all recreational sites and the intake and WWTP effluent sites.

The detection of human and bovine-specific *Bacteroides* markers varied among sites, with the recreational sites generally showing lower occurrences (Table 2). Human-specific *Bacteroides* occurrence was significantly different between sites ($p < 0.05$), with the lowest occurrence (33%) at one of the beach sites (Laurel Creek). The Laurel Creek samples were positive for the bovine marker 60% of the time. The Shade's Mills beach site showed a higher proportion of samples positive for human marker (60% of samples), compared to the bovine marker (40%). A similar occurrence of bovine and human markers was observed at the river gorge site at 75 and 69%, respectively (Table 2). Differences were not observed in the levels of human or bovine markers among the recreational sites (data not shown, $p > 0.05$).

Precipitation

Spearman's rank correlation was performed to explore associations between sample day (D1) and up to 2 days previous (D1, D2, and D3) cumulative precipitation in mm (Wilkes *et al.* 2011), at all sites except the WWTP effluent site. At the DWI site, precipitation was significantly correlated with generic *E. coli* and enterococci concentrations on the sampling day (D1) and on the days prior (Supplementary Material, Figure S1), and with enterococci (but not *E. coli*) and precipitation on the sampling day and the previous day (D1, D2: $R = 0.46$; $p = 0.03$).

At the beach sites combined (Laurel Creek and Shade's Mills), there was a significant correlation between cumulative precipitation on the sampling day and the previous 2 days (D1, D2, and D3) and concentrations of both *E. coli* and enterococci (Supplementary Material, Figure S2). At the river gorge site (Elora Gorge), cumulative precipitation on the sampling day (D1) was significantly correlated with concentrations of *E. coli*, but not enterococci (Supplementary Material, Figure S2).

There were no significant correlations between pathogen enumeration and precipitation for any sites (not shown).

Associations between pathogens and indicators

The concentration of generic *E. coli* at beach sites and Elora Gorge was significantly higher when *Salmonella* spp. were present (M–W test $p < 0.05$; Figure 2), and at beach sites, the concentration of *E. coli* was higher when *Cryptosporidium* spp. were detected (Figure 2). The concentration of generic *E. coli* was not significantly associated with the presence of any

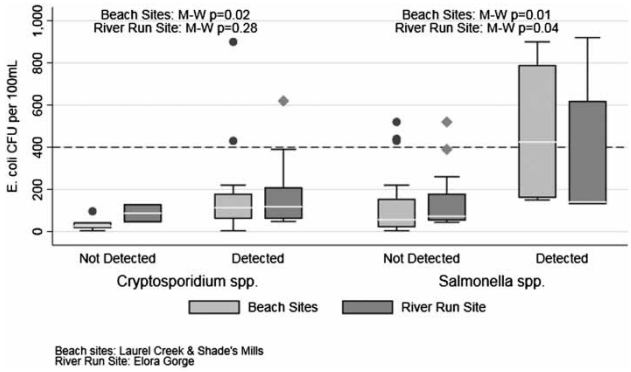

Figure 2 | Boxplots and M–W test *p*-values for *E. coli* concentration by detection of *Cryptosporidium* spp. and Salmonella spp. at beach sites and river run site. Dashed line indicates the 400 *E. coli* CFU/100 mL threshold.

other pathogens at the beach sites or at Elora Gorge (not shown). However, there was a significant association between concentrations of generic *E. coli* and the detection of human *Bacteroides* at beach sites (M–W $p = 0.04$).

For beach sites (Laurel Creek and Shade's Mills combined), there were significant associations between the presence of both *Salmonella* spp. and VTEC and exceedance of the generic *E. coli* threshold of 400 CFU per 100 mL (Fisher's exact $p \leq 0.05$). This was also true at the DWI site ($p < 0.05$). VTEC presence was also significantly associated with *E. coli* >400 CFU per 100 mL ($p = 0.05$) at the Elora Gorge site.

A significant association was observed at the beach sites for the presence of bovine-specific *Bacteroides* and the presence of any pathogen ($p = 0.03$). At the Elora Gorge site, there was a significant association between the presence of protozoa and bovine *Bacteroides* ($p = 0.02$).

Spearman's rank correlation tests were performed on enumeration data during the swimming season, by site. No significant correlations were observed between concentrations of *Campylobacter* spp., *Cryptosporidium* spp. oocysts, and *Giardia* spp. cysts. As expected, significant ($p < 0.01$) correlations were evident at all sites between concentrations of generic *E. coli* and enterococci; correlation was moderate at Elora Gorge ($R = 0.55$), strong at the DWI ($R = 0.70$) and downstream from WWTP effluent ($R = 0.73$) and very strong ($R = 0.85$) at beach sites (Laurel Creek and Shade's Mills). At Elora Gorge, strong correlation ($R = 0.75$, $p = 0.03$) was also observed between concentrations of *Giardia* spp. oocysts and *Bacteroides*.

DISCUSSION

Surface water surveillance initiatives focusing on waterborne human enteric pathogens can provide crucial information on public health risks and the mitigation efforts necessary to help alleviate water pollution problems (Howard 2002; Public Health Agency of Canada 2006; Richardson *et al.* 2009; Parmley *et al.* 2013).

Recreational water sites can vary widely in their biophysical disposition (e.g. lakes and rivers) and sources and relative impact of point-source water pollution (e.g. WWTP and industrial effluent) (Hwang *et al.* 2019) and non-point-source water pollution (e.g. agricultural drainage, septic leaks, and wildlife fecal input) (Baxter-Potter & Gilliland 1988; Somarelli *et al.* 2007; Wilkes *et al.* 2014; Arnold *et al.* 2016). Contrasting differences in bacterial water quality among biogeographically different recreational water sites provide additional insights on how and where to place public health risk management and surveillance efforts (Prüss 1998).

This study focused on two different kinds of water recreation sites: (i) low-relief beaches on reservoirs where water levels and flow are regulated by local dams and (ii) a faster flowing river in a gorge with a larger upstream contributing area and a greater degree of upstream point pollution sources. Due to water flow control structures at the beach/reservoir sites, flow velocities are almost negligible. The DWI and the recreational gorge site (both on the main river) are impacted by upstream wastewater treatment effluents, urban, wildlife, and upstream agricultural land use (>70% of land coverage), while the beach sites are potentially impacted by a combination of upstream land uses, including agriculture (34–48%), forest (24–40%) and urban (7–14%; although no WWTPs) inputs, and wildlife (Chen *et al.* 2018). There are large differences in absolute catchment areas (potential pollution source areas) and pollution source proximity, which need to be contextualized for comparative studies focusing on water quality in natural watershed-scale systems (Wilkes *et al.* 2011; Frey *et al.* 2013,

2015). This study illustrated that the reservoir beach sites generally showed lower prevalence and concentration of pathogens and fecal pollution indicators than the other sites located along the main river. In particular, *Salmonella* occurrence was lower at beaches compared to the river sites, and prevalence increased over the swimming season as might be expected in the context of cumulative seasonal inputs of fecal pollution from wildlife, in particular birds that frequent these sites (Frey *et al.* 2013, 2015).

Salmonella serotype diversity was lower at recreational sites but serotypes of human health significance were detected throughout the swimming season, similar to other Canadian watershed studies (Jokinen *et al.* 2015). Common sources for these pathogens include poultry and swine (Boumart *et al.* 2012; US CDC 2013a, 2013b; Arruda *et al.* 2019). *S*. Typhimurium, detected in two samples from the river gorge water recreational site, is among the top three serovars detected among FoodNet Canada human cases in addition to farm manure samples from broiler or swine sources during the study period (2011–2013). *S*. Thompson was identified in samples from the Shade's Mills beach site, and at Elora Gorge, potentially linked to wild bird contamination. On sampling days, large numbers of gulls and geese were consistently observed at the beach sites (during the swimming season, the local Conservation Authority regularly rakes the beach to remove bird feces). These reservoirs are preferred by migrating waterfowl and the beaches and manicured grassed areas near them are commonly populated by waterfowl. By comparison, the Grand River and its tributaries have minimal shoreline and at the sampling locations are less likely to be influenced by large numbers of migrating waterfowl in this region. The lower occurrence of human and bovine-specific *Bacteroides* at beach sites, compared to the main river channel, might further support impacts from wildlife in these areas.

Of nine recreational water isolates with *Salmonella* spp. typing, only one *Salmonella* Typhimurium isolate from the Shade's Mills beach sample site was considered to be genetically related within 10 alleles to three retail chicken isolates. As these samples were collected within a fairly close time span of 3 months, this suggests the possibility of a common upstream source. However, the low prevalence of *Salmonella* spp. among recreational water samples, especially at beach sites, limits the ability to further identify potential sources of contamination.

The majority of all water wgMLST clusters were related to samples detected on retail chicken in the same region (46%), suggesting a potential link with upstream poultry sources. However, it is important to note that a higher proportion of isolates from retail chicken samples were included in this analysis compared to other farm and retail sample sources. For all clusters of *Salmonella* detected in this study, within the 0–10 allele range, the date of sample collection ranged from the same day up to 27 months apart, suggesting that some strains are persistent in the environment over time, including *S*. Heidelberg and *S*. Infantis strains. *Salmonella* Thompson, Newport, and Paratyphi B isolates were among the most common serotypes isolated from water sources, however, were not genetically related to any farm or retail sources taken during this study period, suggesting the possibility of an alternative source of contamination.

At the beach sites, the impact of the upstream tributary is muted by reduced flow. Lower flow allows suspended particles, including transient pathogens, to sediment out of the water column, potentially reducing exposure among recreational water users despite similar upstream land uses. Sand and exposed sediments can be sources of bacteria during erosion or re-suspension events (Alm *et al.* 2006; Frey *et al.* 2015; Staley *et al.* 2016, 2018). Our results suggest that sources of fecal pollution are much less varied at the reservoir beach sites than at other river sites with greater sources upstream in this watershed. For example, samples from the WWTP effluent point in the river indicate a consistently higher prevalence and concentration of indicators and pathogens than from other sampling sites. It is important to note that since the undertaking of this study, this specific wastewater treatment facility has undergone extensive upgrades. While the WWTP effluent site is upstream of the DWI site, there are other wastewater effluent points along the Grand River, potentially impacting the pathogen presence at downstream sites (not sampled). Research on other watersheds has suggested that wastewater effluent is a strong influence on contamination and the presence of pathogens at recreational water sources (Zhang *et al.* 2016; Sunger *et al.* 2019).

E. coli O157 and *E. coli* O103 were the most common serogroups identified at the reference river sites, but were not identified at recreational water sites. VTEC was detected in 17% of samples from beach sites and 39% of samples from Elora Gorge; however, only one of the seven priority serogroups of human health significance (*E. coli* O45) was identified at the recreational gorge site (Federal VTEC Working Group 2011). Research on the presence and diversity of VTEC at these and other sites along the Grand River watershed (up to 2012) found serogroups of human health significance in 12% of samples, although O45 was not identified from any samples (Johnson *et al.* 2014), and O45 was not identified among human cases reported to the National Enteric Surveillance Program (NESP) in 2013 (Government of Canada 2015).

Cryptosporidium spp. and *Giardia* spp. are usually present at the sample sites, including recreational sites, as has been previously observed in this watershed and others in Canada (Van Dyke *et al.* 2012; Wilkes *et al.* 2013; Lapen *et al.* 2016; Thomas *et al.* 2016). This higher occurrence, compared to bacterial pathogens, may reflect sampling practices, because parasite testing requires the processing of much larger volumes of water, increasing the likelihood of a positive.

Cryptosporidium strains detected at recreational and other sites varied, suggesting a range of sources impacting sites, including birds, wildlife, agriculture, and wastewater. Genotypes with human health risk potential were detected at all sites; however, at the beach reservoir sites, *Cryptosporidium baileyi* ($n = 6$) and *C. parvum* ($n = 2$) were most commonly identified, and at the river run recreational site, *C. ubiquitum*, *C. parvum*, *C. hominis*, and *C. andersoni* were the most common serotypes ($n = 2$ for each). *C. parvum* and *C. hominis* are the genotypes most commonly associated with human illness, although other genotypes present in these samples have also been associated with human illness (Robinson *et al.* 2008). *C. parvum* was detected at all sites, whereas *C. hominis* was only detected at Elora Gorge, which may be due to an upstream sewage treatment facility. The elevated detection of human-specific *Bacteroides* at this site also supports this observation.

The prevalence of *C. parvum* and *C. ubiquitum* at recreational water sites are similar to observations from the South Nation watershed in Ontario (Ruecker *et al.* 2007; Lapen *et al.* 2016). These are associated with a range of hosts including ruminants, wildlife, humans, and rodents (Perz & Le Blancq 2001; Fayer *et al.* 2010; Zahedi *et al.* 2016). The detection of *C. baileyi* at the beach sites but not at other site types suggests that birds may be a source of *Cryptosporidium* spp. at beaches (Zahedi *et al.* 2016). This could be related to the slower flow of water at these sites and the congregation of birds at beach reservoirs, which was commonly observed during the swimming season. Samples with *C. baileyi* detected at the beach sites also had a higher prevalence of *Salmonella* spp. and *Campylobacter* spp. than samples without *C. baileyi*. These results illustrate the importance of birds as a source of fecal contamination at freshwater beaches and potential human exposure to these three pathogens.

Our results confirm that *Giardia* is prevalent in this surface water system, but it is not clear whether detected cysts are of human health significance, as genotyping was not performed on the samples, a method used for microbial source tracking in other Canadian watersheds (Prystajecky *et al.* 2014). Comparing between sites, *Giardia* spp. were more likely to be found at the recreational gorge site than the two reservoir beach sites, which again may be indicative of different inputs and hydrology between these recreational site types.

In this study, we observed the prevalence of *Salmonella* spp., *Campylobacter* spp., and *Cryptosporidium* spp. and the concentration of generic *E. coli* increases over the course of the swimming season, from June to September each year. Previous FoodNet Canada analysis of *Campylobacter* spp. infection incidence by season in the Region of Waterloo showed that human illness is associated with behavior rather than source contamination (David *et al.* 2017) and that, based on comparative genomic fingerprinting, surface water exposure was not a dominant source of human campylobacteriosis in the region (Ravel *et al.* 2017).

The increase in *Cryptosporidium* spp. prevalence across the swimming season at recreational sites is in line with previous research, showing seasonal increases in prevalence in this watershed and others in Canada (Wilkes *et al.* 2013; Lapen *et al.* 2016; Thomas *et al.* 2016). Cryptosporidiosis, the infection caused in humans by *Cryptosporidium* spp., in this region has been shown to be associated with swimming in rivers and lakes and commonly shows seasonal spikes typically in late summer and early fall (Pintar *et al.* 2009).

There can be many reasons for this increase in the prevalence of several pathogens later in the swimming season, including accumulative effects of wildlife and human activities in the area, as well as other environmental conditions such as increasing water and air temperatures and changes in flow conditions that can impact microbial activity. While the exact mechanisms are unknown, this information can help to inform public health communication and decision-making by targeting enhanced risk communication toward the latter half of the swimming season, or by implementing enhanced water monitoring during the latter summer months.

Bacterial indicator data tell an important story. Despite differences in pathogen load between the beach sites and the recreational gorge site, the presence and concentration of indicator organisms did not differ significantly between these recreational sites. However, analysis shows that the concentration of generic *E. coli* and the presence of *E. coli* exceeding the 400 CFU per 100 mL threshold are significantly associated with the presence of several pathogens at these sites. Higher concentrations of generic *E. coli* were observed when *Salmonella* spp. and *Cryptosporidium* spp. were present at beach sites, and when *Salmonella* spp. were present at Elora Gorge, and for all sites sampled, generic *E. coli* concentrations were higher when VTEC were detected. This aligns with previous research showing increased number of pathogens and

increased presence of specific pathogens associated with fecal indicators in this and other Canadian watersheds (Edge *et al.* 2012; Van Dyke *et al.* 2012). This suggests that while generic *E. coli* concentrations and threshold exceedances may not be directly predictive of the presence of specific pathogens, in this watershed they are good indicators of potential contamination, pathogen load, and elevated human health risk.

Human and bovine-specific *Bacteroides* were more frequently detected at non-recreational sites during the swimming season and the highest in samples from the WWTP effluent site followed by the DWI site. As shown in previous studies, high levels of *E. coli*, total *Bacteroides*, and human-specific *Bacteroides* markers have been detected at the WWTP effluent site, compared to other sites in the Grand River. The DWI has also previously shown elevated levels of these indicator bacteria (Lee *et al.* 2014; Kadykalo *et al.* 2020).

In addition to knowledge regarding upstream pollution sources and human and wildlife impacts on recreational water, understanding hydrological factors that impact sites can be an important factor in reducing public health risk. In this study, pathogen and indicator load in recreational water sites are impacted by precipitation. Increased rainfall was associated with increased levels of both generic *E. coli* and enterococci at the DWI site and at the beach reservoir and river gorge recreational water sites. This finding supports other research that demonstrates the impacts of precipitation on water quality (Wilkes *et al.* 2011) and highlights that public health measures and messaging should consider the breadth of potential inputs which affect recreational water quality and safety. The physiography, hydrology, and contamination sources at each site can influence the prevalence and types of pathogens detected. Risk assessment and management decisions should vary based on the recreational site type, and consider point-source and non-point-source water pollution sources as well as the water flow velocities and precipitation events.

CONCLUSION

The nature of the sampling site influences the prevalence and types of pathogens detected. The beach sites generally had lower prevalence and concentration of pathogens and fecal indicators than sites located along the river proper. Pathogen subtypes at the beach sites suggested a more narrow range of sources, including birds. Fewer human pathogenic strains of the target pathogens were identified than might have been expected at beaches, so there is likely low risk in the context of other sources of exposure. By comparison, the river recreational site and the two reference sites demonstrated higher prevalence of pathogen detection and wider range of sources.

Enhanced pathogen-focused sampling of a surface water system is an important component of an integrated surveillance system for identifying sources of waterborne illness and can help to contextualize routine bacterial indicator monitoring efforts by local water managers and public health authorities to gauge public health risk for local residents who frequent these sites during the swimming season.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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