Campylobacter in recreational lake water in southern Quebec, Canada: presence, concentration, and association with precipitation and ruminant farm proximity

Rebecca A. Guy, Julie Arsenault, Serge Olivier Kotchi, Maxime Gosselin-Théberge, Marie-Josée Champagne and Philippe Berthiaume

ABSTRACT

Campylobacter is an important cause of gastrointestinal illness and exposure to recreational water is one potential source of infection. The objective of this study was to investigate the presence and concentrations of *Campylobacter*, and determine the influence of agricultural activities and precipitation on their presence, at lake beaches used for water recreation in southern Quebec, Canada. A total of 413 water samples were collected from June to August, from 22 beaches, between 2011 and 2013. The overall proportion of positive water samples was estimated to be 33.9% (95% CI: 27.7, 40.1) for *C. jejuni* and 49.7% (95% CI: 41.8, 57.6) for *Campylobacter* spp. The concentrations of both thermotolerant *Campylobacter* spp. and *C. jejuni* ranged from 20 to 900 bacteria/L of water. Logistic regressions showed that the presence of *C. jejuni* and *Campylobacter* spp. was significantly associated with the year and season. Other significant predictors of *C. jejuni,* but not *Campylobacter* spp., included the presence of precipitation the day before sampling and the presence of ruminant farms within a 5 km radius of the beach. The present study provides insights into the risk of *Campylobacter* presence in recreational lake water for better understanding public health risks.

Key words | agriculture, *Campylobacter*, real-time polymerase chain reaction, recreational lake water, remote sensing, ruminant

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INTRODUCTION

Campylobacter is the leading cause of bacterial gastroenteritis in industrialized countries and is the leading notifiable enteric disease in Canada. Poultry is recognized as the main reservoir for *Campylobacter*, and numerous epidemiological studies have highlighted that consumption of undercooked poultry meat, or food cross-contaminated by this product, is an important source of human infection. However, other animal species can also be healthy carriers of *Campylobacter*, including cattle, pets, wild birds and wild mammals (Huang *et al.* 2015; Kaakoush *et al.* 2015). Contact with these animals, directly or indirectly through a contaminated environment, represents another potential source of

human exposure to the bacteria. In Quebec, this is supported by a study conducted in the Eastern Townships, reporting that exposures to poultry account for less than half the episodes of campylobacteriosis in the region (Michaud *et al.* 2004).

Among the various environmental sources of *Campylobacter*, water has been identified as an effective and probably underestimated vehicle of transmission (Kaakoush *et al.* 2015). The report of *Campylobacter* as one of the top six causes of drinking water outbreaks in Canada (Schuster *et al.* 2005) supports this notion. Moreover, drinking undisinfected water and swimming in recreational water have been

recognized as risk factors for infection with *Campylobacter* (Kapperud *et al.* 2003; Schonberg-Norio *et al.* 2004; Denno *et al.* 2009; Pintar *et al.* 2017). The latter could be one of the contributing factors for the striking peaks observed in the incidence of campylobacteriosis during summer in Quebec (Michaud *et al.* 2004; Arsenault *et al.* 2012; Lévesque *et al.* 2013) and elsewhere (Kovats *et al.* 2005).

Numerous potential non-point and point sources exist for contamination of environmental water with *Campylobacter*, including sewage effluent (Rechenburg & Kistemann 2009; Hokajarvi *et al.* 2013), agricultural runoff from livestock sources (Vereen *et al.* 2007; Jokinen *et al.* 2010), and direct fecal contamination from bathers, wildlife and pets (Van Dyke *et al.* 2010; Hellein *et al.* 2011). *Campylobacter* is present in freshwater recreational water globally such as in lakes in New Zealand (Till *et al.* 2008), Finland (Hokajarvi *et al.* 2013) and the USA (Hellein *et al.* 2011; Oster *et al.* 2014). While there have been frequent reports of *Campylobacter* in environmental water, little is known about the concentrations and determinants of *Campylobacter* in freshwater recreational lakes in agricultural regions. In the past 15 years, numerous studies have been published on the water quality of Great Lake recreational beaches in the northern USA (Oster *et al.* 2014). However, there are large gaps in our knowledge of the impact of agriculture on small freshwater lakes in agricultural areas that are frequently used for recreational swimming in Canada and other parts of the world. As such, the impact of animal production on the contamination of water in those lakes is unknown.

The goals of this study were: (1) to determine the presence and concentration of *Campylobacter* in recreational waters in Southern Quebec; and (2) to investigate the impact of agricultural activities surrounding the beaches and precipitation on the presence of *Campylobacter* in recreational water samples. Our approach consisted of using molecular methods of direct quantification using real-time polymerase chain reaction (qPCR), combined with classical microbiology.

METHODS

Sampling site description

Beaches were selected in two regions of Southern Quebec, Canada, the Montérégie (12 beaches) and Estrie (10

beaches), stratified by agricultural activities surrounding the beaches. Surface areas occupied by agricultural activities were 65 and 20% for Montérégie and Estrie, respectively.

The list of all beaches participating in the provincial public beach surveillance program was obtained from the Ministry of Sustainable Development, Environment and the Fight Against Climate Change (MDDELCC; Government of Quebec). All beaches were classified according to the presence of farm animals in the zone of influence of the beach (ZIB, defined below under proportion of agricultural areas (PAA)) using the database of registered farms of the Ministry of Agriculture, Fisheries and Food of Québec (MAPAQ) (Québec Gd. 2006). The owners/managers of beaches were contacted individually (using a randomly ordered list) and asked to participate in the study to fulfill the target sample size of 10 beaches in 2011 and 20 beaches in 2012 and 2013, with approximate equal representation of beaches with and without farm animals within the ZIB. The beaches were grouped into optimum route scenarios to minimize the number of kilometers traveled per sampling day.

Beaches were sampled during the recreational bathing season. In 2011, sample collection started in early June and lasted for a period of 10 weeks. In 2012 and 2013, sampling started in mid-June, resulting in 8 weeks of weekly sampling. Not all the beaches could be sampled each year due to either denied access to the beach in the subsequent year, or exclusion of the beach because chlorine treatment for disinfection was applied to the water. Thus, over the three-year summer sampling period there was a total of 22 different beaches sampled from 18 lakes. Two different beaches on the same lake were sampled for four of the 18 lakes (i.e. beaches 1 and 2, 5 and 15, 10 and 14, and 9 and 11). The minimum distance between the beaches on the same lake was 3.5 km for all four lakes.

Proportion of agricultural areas

A supervised maximum likelihood classification of satellite images was used to assess the occurrence and PAA in the ZIB. Geomatica 10.3 software (PCI Geomatics, Ill Richmond, ON, Canada) was used to classify 2010 and 2011 images from Landsat-5, Spot-5, GeoEye-1 and

WorldView-2 sensors. The ZIB was defined as the intersection between the watershed of the lake and the circle of 2 km radius centered at the beach (Turgeon *et al.* 2011). Previous work showed that land use and land cover in the 2 km ZIB had a greater influence on the microbial contamination of the beach (Turgeon *et al.* 2011). The PAA was calculated by dividing the area associated with these surfaces by the total area of the ZIB.

Number of farms

Farms located in an area defined by a 5 km radius around the beaches were identified with high resolution satellite images (GeoEye images from Google Earth). A greater radius was used compared to PAA because none of the beaches had poultry farms and only four beaches had ruminant farms in the 2 km radius selected for PAA. In addition, an on-site investigation was used to determine the number of ruminant farms (dairy cattle, beef cattle, veal, sheep, goat, or alpaca) and poultry farms (chicken or turkey).

Total precipitation

Total daily precipitation (TDP) data were obtained from a total of 98 meteorological stations between 2011 and 2013. The meteorological stations used were part of the networks of Environment Canada ($n = 77$) and MDDELCC ($n = 21$). Spatial interpolations were performed to calculate TDP surfaces covering the entire study area based on weighted inverse distance, ordinary kriging and empirical Bayesian kriging methods (Tobin *et al.* 2011). Descriptive statistics, directional trends and semivariograms allowed for the selection of the interpolation method and the data transformations (standardization, removing the directional trend) if needed. The accuracy of the interpolated surfaces was assessed by cross-validation (Luo *et al.* 2008). The most accurate model for each day was used for calculation of interpolated precipitation. Analyses were performed in ArcGIS 10.1 software (ESRI, Redlands, CA, USA).

Water sampling and filtration

Samples were collected on a weekly basis, in 4 L disposable carboys (Pall Corp., Vernon Hills, IL, USA) by holding the carboys at about 15 cm beneath the surface in water that was 70 cm deep. Samples were placed in coolers containing ice packs and shipped to the laboratory within 24 hr. Samples were stored at 4 °C upon arrival and processed within 48 hr of collection.

One liter of each water sample was filtered through 0.45 μm mixed cellulose esters filters (S-Pak, Millipore, Molsheim, France) for isolation of *Campylobacter*. Additional 1 L water samples were filtered through 0.45 μm polyethersulphone filters (Supor-450, Pall, Ann Arbor, MI, USA) for use in qPCR quantification. More turbid waters required more than one filter for processing 1 L. For qPCR quantification, a different filtration system with similar recoveries was used in 2012–2013 than in 2011, to enable faster throughput and processing of the larger number of samples on a three sample vacuum manifold (Millipore, Molsheim, France) attached to an EZ Stream vacuum pump (Millipore, Molsheim, France). In 2011, the filters were placed on individual Nalgene reusable filter holders with receivers (Nalgene, Rochester, NY, USA) that were washed and autoclaved between use.

DNA extraction

DNA was extracted directly from the Supor-450 filters using the Power Water Kit (Mo Bio, Carlsbad, CA, USA) immediately following filtration. Using sterile forceps, the filters were removed from the vacuum manifold, carefully curled into a tube shape with the bacterial side facing inwards and placed in the 5 mL PowerWater bead tube containing beads. One mL of tissue lysis buffer, PW1 (Powerwater Kit) preheated to 55 °C, was added to each tube and tubes were placed in a vortex genie set at maximum speed and vortexed for 10 min to disrupt the bacterial cell walls. Following bead-beating the PW1 solution was carefully removed from the tube and placed in a 1.5 mL microtube to continue with the DNA extraction according to the manufacturer's instructions. When multiple filters were used to filter a sample, two filters were processed through one bead tube, sequentially. In this case the filter was carefully removed from the bead-beating tube so as to remove as little of the PW1 solution as possible, and a second filter was added to the tubes and subjected to bead-beating. All the PW1 used in processing the filter(s) from one water sample was passed through

one silicon column (Power Water kit). The manufacturer's instructions were followed and DNA was eluted from the column in 100 μL of PW6 elution buffer. DNA was stored at −20 °C until use.

DNA for use as qPCR standards was extracted from 48 hr cultures of *C. jejuni* reference strain ATCC 294728, using the QiaAmp DNA extraction kit (Qiagen, Mississauga, ON, Canada) and quantified as previously described (Gosselin-Théberge *et al.* 2016).

qPCR quantification

Real-time PCR targets were the 16S rRNA for quantification of thermotolerant *Campylobacter* spp. (Josefsen *et al.* 2004) and the *mapA* gene for quantification of *C. jejuni* (Best *et al.* 2003). Our rationale for selection of these assays and the primers/probe sequences we used were previously published (Gosselin-Théberge *et al.* 2016 (see supplemental Table 3)). The probes were purchased from IDT Technologies (Coralville, Idaho, USA) as ZEN probes with FAM reporters and BHQ_2 quenchers. The qPCR assays were performed using either the Stratagene Brilliant core kit or the Brilliant III mastermix (Stratagene, La Jolla, CA, USA). Each 25 μL reaction included 5 μL of DNA template, 5 mM $MgCl_2$, 0.8 mM dNTPs, and 1.25 U SureStart *Taq* DNA polymerase. The concentration of primers and probes were 800 nM primers/120 nM probe for *Campylobacter* spp. quantification, and 240 nM primers/200 nM probe for *C. jejuni* quantification. Glycerol (molecular biology grade, Sigma St. Louis, MO, USA) was added at a final concentration of 8%, when using Stratagene Brilliant core kits. Bovine serum albumin (Fraction V, Sigma, St. Louis, Missouri, USA) was added to the mastermixes, at a concentration of 300 ng/μL BSA, to reduce the impact of qPCR inhibition. The cycling conditions for the *Campylobacter* spp. 16S assay consisted of a 3 or 10 min incubation (depending on qPCR kit used) at 95 °C followed by 40 cycles of alternating temperatures of 95 °C for 10 sec and 58 °C for 60 sec. The cycling conditions for *C. jejuni mapA* consisted of a 3 or 10 min incubation at 95 °C followed by 40 cycles of alternating temperatures of 95 °C for 15 sec, and 60 °C for 60 sec. The PCR reactions were run in an HRM 6500 Rotor-gene Q instruments (Qiagen). Fluorescence data (three data points) were collected at the end of each cycle and each sample was run in triplicate wells.

qPCR inhibition determination

qPCR inhibition was measured using the SPUD qPCR assay targeting the *Solanum tuberosum* phyB gene (Nolan *et al.* 2006), with modifications as previously described (Guy *et al.* 2014). No inhibition was observed in SPUD amplification in samples from each beach that were collected at weeks 1, 5 and 8 for each year of the study.

PMAqPCR

A subset of 131 beach samples (32%), selected based on previous results showing high frequencies of qPCR detection, was tested using a propidium monoazide qPCR (PMAqPCR) assay in order to estimate the number of live *Campylobacter*. PMA treatment was conducted on the filters following filtration of the water sample. The filter was removed from the unit, placed in a Petri dish and 300 μL of PMA (Biotium, Hayward, CA, USA), at a final concentration of 25 μM (in 2012) or 12.5 μM (in 2013), was applied directly onto the filters. The filters were placed in the dark for 10 min to allow the PMA to enter damaged cells, and then exposed for 5 min to a 500 watt light source at a distance of 20 cm to photoactivate the PMA. During photoactivation the Petri dishes containing the filters were placed on ice to counter the high temperature effects during light exposure. The remaining PMA solution was removed by vacuum filtration prior to extracting DNA from the filters, as described above.

Campylobacter cultivation

Campylobacter were enriched from filters containing water concentrates, in 100 mL of Bolton broth containing Bolton Selective Supplement (Oxoid, Basingstoke, Hampshire, UK) for 4 hr at 35 ± 1 °C, followed by an incubation of 44 hr at 42 ± 1 °C, under microaerobic conditions. Isolation was performed on modified Charcoal-Cefoperazone-Deoxycholate Agar (mCCDA) (Oxoid) following 24–72 hr incubation at 42 ± 1 °C (Jokinen *et al.* 2010; ISO 17995:2005, Water quality – Detection and enumeration of thermotolerant *Campylobacter* species).

Typical colonies were isolated and identified at the genus level using gram coloration, catalase and oxidase tests. Multiplex PCR (mPCR) was used to determine the presence of *Campylobacter* spp. and *C. jejuni/C. coli* (Denis *et al.* 1999). Isolates that were *Campylobacter* spp. positive but *C. jejuni* and *C. coli* negative in the mPCR were tested using a cpn60 PCR, targeting a gene coding region of the 60 kDa chaperonin protein subunits, and sequenced (Hill *et al.* 2006). A maximum of five colonies were analyzed per each water sample collected and tested using both biochemical and molecular methods. When there were ≤5 colonies per sample then all colonies were tested.

Data analysis

Raw data obtained from the qPCR runs were analyzed using the Rotor-Gene Q Series Software, version 2.3.1. Optimization of the quantification analysis of the qPCR data, including use of a digital PCR-calibrated standard curve, was performed in another study (Gosselin-Théberge *et al.* 2016). A water sample was considered positive for *C. jejuni* when the bacteria were detected by either qPCR or culture, and positive for thermotolerant *Campylobacter* spp. when any *Campylobacter* species was detected either by one of the two qPCR assays or by culture.

The proportion of positive water samples with a 95% confidence level was estimated for *C. jejuni* and *Campylobacter* spp. This estimate was adjusted for sampling weight and stratification by region using the 'Surveyfreq' procedure of SAS software version 9.4 to take into account our sampling design (i.e. the probability of a beach to be selected depended on the presence of animal farms within the ZIB, and thus they were not all equally represented in our sample). A multi-level (sample, beach) logistic regression was used to model the presence or absence of *C. jejuni* according to sample-level (i.e. year, season, week, precipitation) and beach-level (i.e. ruminant farms, poultry farms, PAA) variables as fixed effects. The same model was used to model the presence or absence of *Campylobacter* spp. Only the presence/absence status was modeled, as preliminary analyses showed a poor fit or lack of convergence when using either a Poisson or a negative-binomial regression modeling the counts of *C. jejuni* or *Campylobacter* spp. All

explanatory variables were categorized. For the PAA, the 66th percentile of the distribution was used as a cut-off, whereas the rounded 90th percentiles were used to discriminate low and high volumes of rain for the total precipitation (mm) the day before sampling and the total precipitation (mm) over the 3 days before sampling. All variables were first tested in univariable analyses, and were then selected for inclusion in a full model if their *P*-value was less than 0.20. Prior to inclusion, associations between each pair of selected explanatory variable was explored in contingency tables and tested using exact chi-square test. In the presence of two strongly associated variables, only one variable was kept based on comparison of the Akaike Information Criteria (AIC) and Bayes Information Criterion (BIC) of alternative full models including one of the two correlated variables. A backward procedure was used to select the final model, with *P* > 0.05 as the rejection criteria. Two-way interactions between meteorological and agricultural variables were tested in the final model. Odds ratios were used to quantify the association between the presence of *C. jejuni* or *Campylobacter* spp. and each selected risk factor. For variables with more than two categories, pairwise comparisons were used to present the results with Bonferroni adjustment for the *P*-value. The glimmix procedure of SAS software version 9.4 was used for modeling the data using the Laplace estimation method.

RESULTS

Description of beaches and land use

Of the 22 beaches that were sampled between 2011 and 2013, two were part of a summer camp, four were within provincial parks, eight were within municipal beaches (one of which was adjacent to a quay), and eight were within trailer camping parks. The lakes were either natural ($n = 12$) or artificial ($n = 6$) and ranged in size from 71.6 to 0.01 km^2 (Table 1). One artificial lake (beach 4) was part of a reservoir. The PAA as well as the number of farms for each beach is presented in Table 1. While most ruminant farms were dairy ($n = 39$) or beef cattle ($n = 27$) farms, veal calf ($n = 1$), sheep ($n = 4$), goat ($n = 2$) and alpaca ($n = 2$) farms were also present.

Table 1 | Beach, lake and land use characteristics and sampling frequency

Beach ID	Region[a]	Year(s) sampled[b]	Total samples collected	Lake area (km²)	PAA[c] (%)	Number of farms[d]		
						Ruminant	Poultry	Total
1	E	11/12/13	26	1–5	1.7	0	0	0
2	E	12/13	16	1–5	2.8	1	0	1
3	E	11/12/13	26	1–5	3.9	0	0	0
4	M	11/12/13	26	1–5	5.1	5	0	5
5	E	12/13	16	>10	5.5	2	0	2
6	M	11/12/13	26	1–5	7.8	4	0	4
7	M	12/13	16	<1	8.0	3	7	10
8	E	12/13	16	>10	8.5	0	0	0
9	E	12/13	16	>10	9.9	8	0	8
10	M	11/12/13	26	>10	10.3	0	0	0
11	E	12/13	16	>10	11.3	0	0	0
12	E	11/12/13	25	>10	13.1	0	0	0
13	M	12/13	16	1–5	13.3	5	0	5
14	M	12/13	16	>10	13.6	0	1	1
15	E	11/12/13	26	>10	16.5	2	0	2
16	M	12/13	16	1–5	18.7	4	0	4
17	E	11/12/13	26	<1	23.3	9	0	9
18	M	12	8	<1	43.7	5	0	5
19	M	11/12/13	26	<1	48.2	14	4	18
20	M	11	10	<1	68.9	3	0	3
21	M	12/13	15	<1	72.7	0	1	1
22	M	13	8	<1	77.9	10	0	10

[a]E, Estrie; M, Montérégie.
[b]Water samples were collected during the summer season from 2011 to 2013.
[c]PAA, proportion of agricultural area in the 2 km zone of influence (ZIB).
[d]Number of farms in a 5 km radius surrounding beaches.

Frequency of detection of *Campylobacter* in recreational water

A total of 413 water samples were analyzed using the two approaches: molecular-based quantification and culture isolation. The percentage of positive samples according to the specific detection method is presented in Table 2. Combining qPCR and culture positives lead to an increased proportion of positive samples in all years with the exception of *C. jejuni* in 2012 (Table 2). The overall proportion of positive beach water, adjusted for sampling weight and stratification, was estimated to be 33.9% (95% CI: 27.7, 40.1) for *C. jejuni* and 49.7% (95% CI: 41.8, 57.6) for *Campylobacter* spp.

At the beach level, both thermotolerant *Campylobacter* spp. and *C. jejuni* were frequently present in the 22 different beaches sampled and their frequency of detection was consistently higher using qPCR compared with culture (Figure 1). All beaches had qPCR positive samples at least once during the different sampling years, whereas only 50% of the beaches had culture positive samples. The most frequently isolated species was *C. jejuni* (30/33). *C. lari* was isolated from three separate beaches in July 2012.

Concentrations of *Campylobacter* in recreational water

All 22 beaches were qPCR positive for thermotolerant *Campylobacter* spp. at least once in the three years of

Table 2 | Percent of *Campylobacter jejuni* and thermotolerant *Campylobacter* spp. in recreational water in the summers of 2011–2013 as determined using two detection methods, direct qPCR and culture

		Percent of positive samples					
		C. jejuni			**Thermotolerant Campylobacter spp.**		
Year	No. samples	qPCR	Culture	Culture ± [a]qPCR	qPCR C. spp.	qPCR (C.j. + C. spp.)	Culture ± [a]qPCR (C.j. + C.spp.)
2011	96[b]	39.6	9.5	42.7	37.5	39.6	58.3
2012	158	15.2	4.4	15.2	25.3	26.6	31.0
2013	159	42.8	10.7	46.5	37.1	39.6	62.9
All years	413	31.5	8.0	33.9	32.7	34.6	49.7

[a]Culture-positive and/or qPCR-positive samples.
[b]One sample was missing for the culture method; thus the percent of positive results for culture ± qPCR are based on 95 samples in 2011.

study, whereas 21/22 beaches were qPCR positive for *Campylobacter jejuni*. The concentration of thermotolerant *Campylobacter* spp. and *C. jejuni* fluctuated weekly with the majority of positive samples (86% for *Campylobacter* spp.; 62% for *C. jejuni*) displaying low concentrations of genomic DNA, to the equivalent of 20 bacteria/L. This concentration was the lower limit of quantification for both qPCR assays. qPCR concentrations of thermotolerant *Campylobacter* spp. never exceeded 20 bacterial equivalents/L in 50% of the beaches, whereas *C. jejuni* concentrations never exceed 20 bacterial equivalents/L in 32% of the beaches (Figure 2). Only thermotolerant spp. was detected by qPCR in beach 18 (Figure 2). Sporadic spikes in the concentration of both *Campylobacter* spp. and *C. jejuni* for individual beaches were observed, and in 2013 more beaches (40/159) exceeded 20 bacterial/L *C. jejuni* than in 2011 (6/96) or 2012 (3/158). Concentrations exceeding 100 *C. jejuni*/L were observed at six beaches throughout the study period. Because the concentration of thermotolerant *Campylobacter* spp. and *C. jejuni* was determined using genomic DNA, both live and dead cell DNA were quantified, corresponding to both live and dead cells. Based on the difference between qPCR and PMAqPCR detection frequencies on the same samples, 86 and 95% of samples were estimated to contain viable *Campylobacter* spp. and *C. jejuni*, respectively, from the subset of tested water samples (Figure 3).

Risk factors for the presence of *Campylobacter*

The distribution of the various risk factors is presented in Table 3. From the results of the univariable analyses for

C. jejuni, the following variables were selected for multivariable analyses: year, season, week, total precipitation the day before sampling, total precipitation 3 days before sampling, and presence of ruminant farms. However, the two precipitation-related variables showed a strong correlation with each other and so did the season and week variables. Consequently, only the total precipitation for the day before sampling and the season were kept, the decision being supported by those variables producing smaller AIC and BIC values for the model. All these selected variables were retained in the final model. The interaction between total precipitation the day before sampling and the presence of ruminant farms was tested in the final model, but was not retained as it was not significant ($P = 0.49$). The estimated odds ratios for the final model are presented in Table 4. Overall, the odds of *C. jejuni* being present were higher in 2011 and 2013 compared to 2012, and were also higher at the end of summer compared to the end of spring or early summer. The odds of *C. jejuni* being present were also higher when it was raining the day before sampling, but did not vary significantly according to the amount of rain. Finally, the odds were significantly higher in the presence of ruminant farms surrounding the beach.

For *Campylobacter* spp., only the year, week, season and total precipitation the day before sampling were considered for multivariable modeling. As was the case for *C. jejuni*, the week was not considered for multivariable analyses of *Campylobacter* spp. due to the strong correlation with the season. After adjustment for
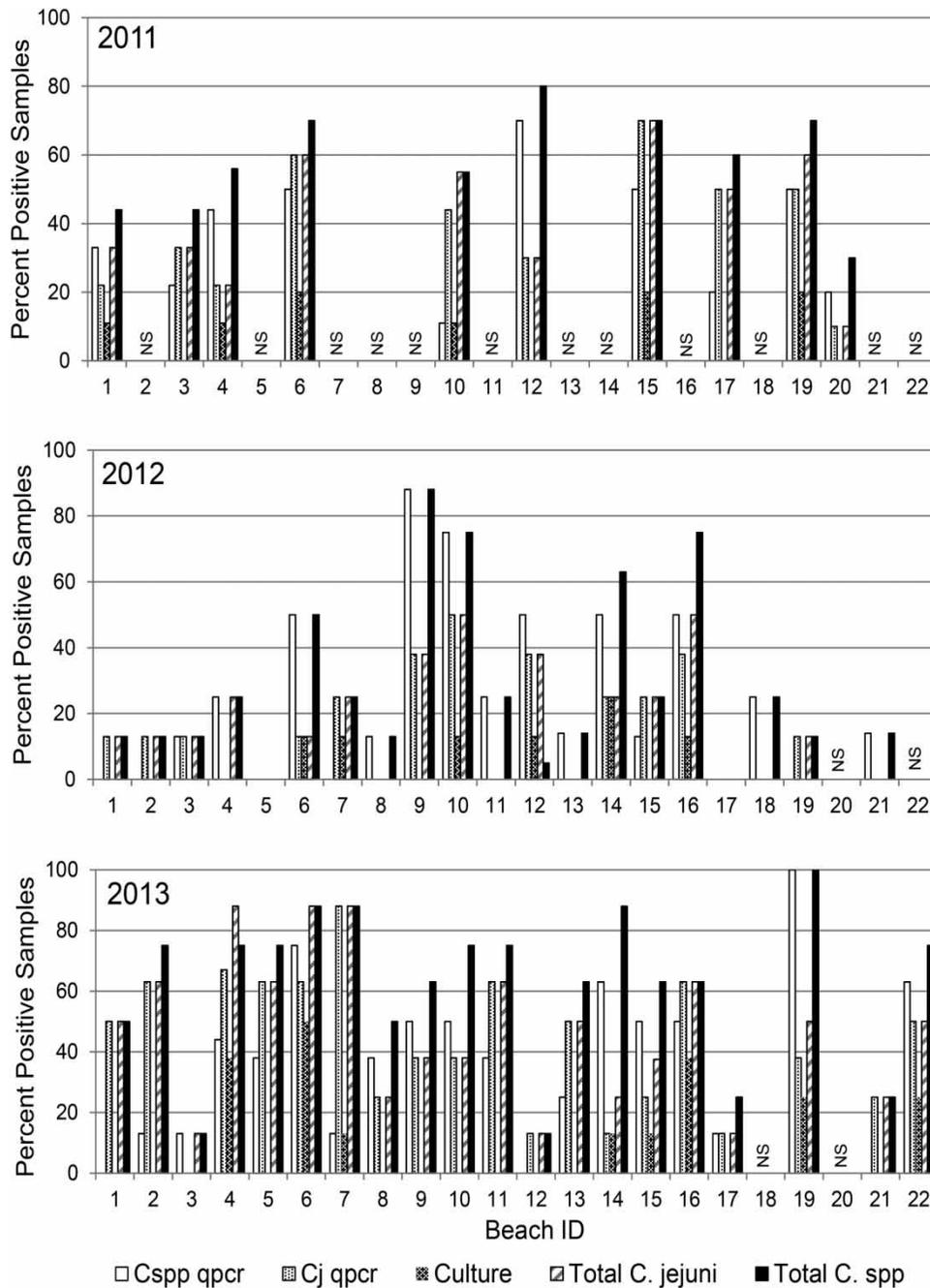
Figure 1 | Occurrence of *Campylobacter* in individual recreational water beaches over a three year summer bathing period. The percent of positive water samples is shown for individual detection methods (*C. jejuni* qPCR, *Campylobacter* spp. qPCR, and culture) and for combined values to give the total positive *C. jejuni* (Cj qPCR ± Cj culture) and *Campylobacter* spp. (Cj qPCR ± *Campylobacter* spp. qPCR ± culture). NS refers to beaches that were not sampled in a specific year.

the year, the precipitation variable was no longer significant ($P = 0.18$) and thus was removed from the multivariable model. The estimated odds ratios from the final model are presented in Table 4. The effect of year was similar to that described for *C. jejuni*. The odds of observing *Campylobacter* spp. were higher in summer (both early and late summer) compared to spring.
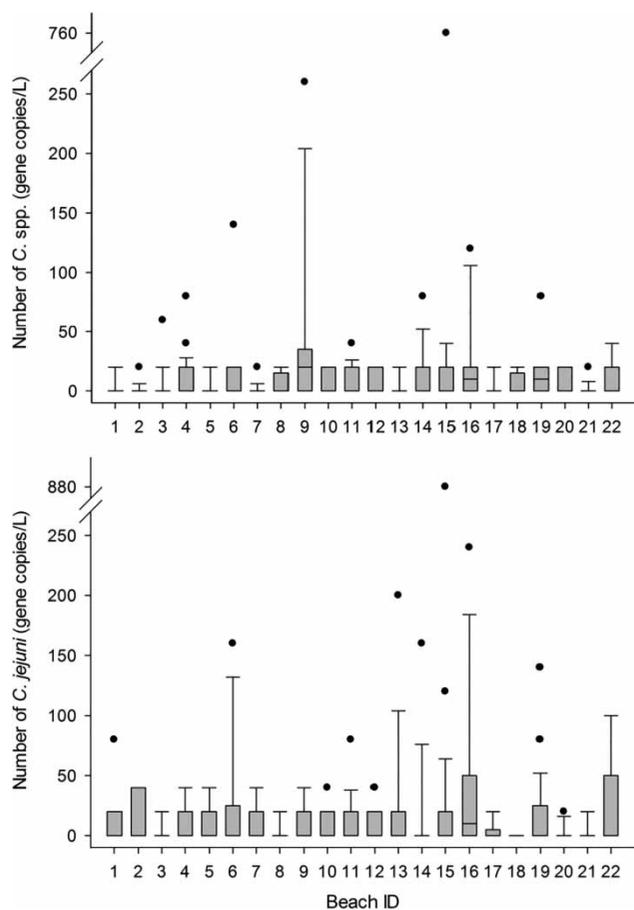
Figure 2 | Distribution of qPCR concentrations of *Campylobacter* in water samples from 22 individual beaches over the summers of 2011–2013. There were 8–26 samples per beach boxplot depending on the number of years the beach was sampled. The boxes lower and upper boundaries indicate the 25th and 75th percentile, a horizontal line within a box indicates the median, the whiskers indicate the 10th and 90th percentiles, and the outliers are represented individually as black dots.

DISCUSSION

Campylobacter was often present in water samples during the three summer bathing seasons in this study and at similar detection frequencies as reported in Finland (Hokajärvi *et al.* 2013) and New Zealand (Till *et al.* 2008). Higher frequencies have been reported for some of the Great Lake beach studies in North America, whereby up to 70% of samples from seven beaches contained *C. jejuni*; however, those beaches had water quality impairments (Oster *et al.* 2014).

Low concentrations of *Campylobacter* in recreational water as seen in this study have been previously reported.

Using the most probable number (MPN) method, Khan *et al.* (2013) observed concentrations of thermotolerant *Campylobacter*, ranging from 3 to 30 mpn/L in water samples from five freshwater beaches in Lake Simcoe, Ontario, Canada. A handful of studies have applied qPCR for estimating concentrations of *Campylobacter* in freshwater beaches, such as reported concentrations ranging from 10^2 to 10^3 gene copies/L in freshwater beaches in Florida, USA (Hellein *et al.* 2011). High levels, of up to 10^6 gene copies/L, of *C. jejuni* were reported from the highly impacted beaches in the Great Lakes, USA (Oster *et al.* 2014).

Recently, Banting *et al.* (2016) demonstrated that the primers of certain *Campylobacter* PCR-based assays cross-amplify *Arcobacter*, potentially leading to an overestimation of *Campylobacter* in the environment. We had previously shown that the assays we used in this study were specific to *Campylobacter* (Gosselin-Théberge *et al.* 2016). However, there have been numerous *Arcobacter* gene sequences submitted to GenBank since our *in silico* analyses performed in 2013. We re-validated the Josefsen *et al.* (2004) and Best *et al.* (2003) primers in 2017 and did not observe any potential cross-amplification with *Arcobacter* (data not shown); indicating it is unlikely to have affected our results.

While there are over 20 species of *Campylobacter* identified from different animal hosts, over 90% of human infections are caused by *C. jejuni* with less than 10% infections due to *C. coli* and *C. lari* (Lévesque *et al.* 2013). We observed a high proportion of *C. jejuni* positive samples in this study suggesting the potential risk for humans if recreational water was ingested. This predominance of *C. jejuni* in recreational lake water samples has been previously reported (Hokajärvi *et al.* 2013; Khan *et al.* 2013). *C. lari* was occasionally isolated in our beach water and has been isolated in other recreational beaches (Khan *et al.* 2013). *C. coli* was neither isolated in our recreational beaches nor in beach water in Ontario (Khan *et al.* 2013); however this species is present at high levels in the Bras d'Henri river basin of Quebec, an intensive hog farming region (Khan *et al.* 2014), and thus may pose a potential risk in those regions.

The presence of ruminant farms within a 5 km radius of the beach was associated with higher odds of finding *C. jejuni* in the recreational beach water. The significant associations with agricultural activities observed in our study are consistent with similar reports in a handful of
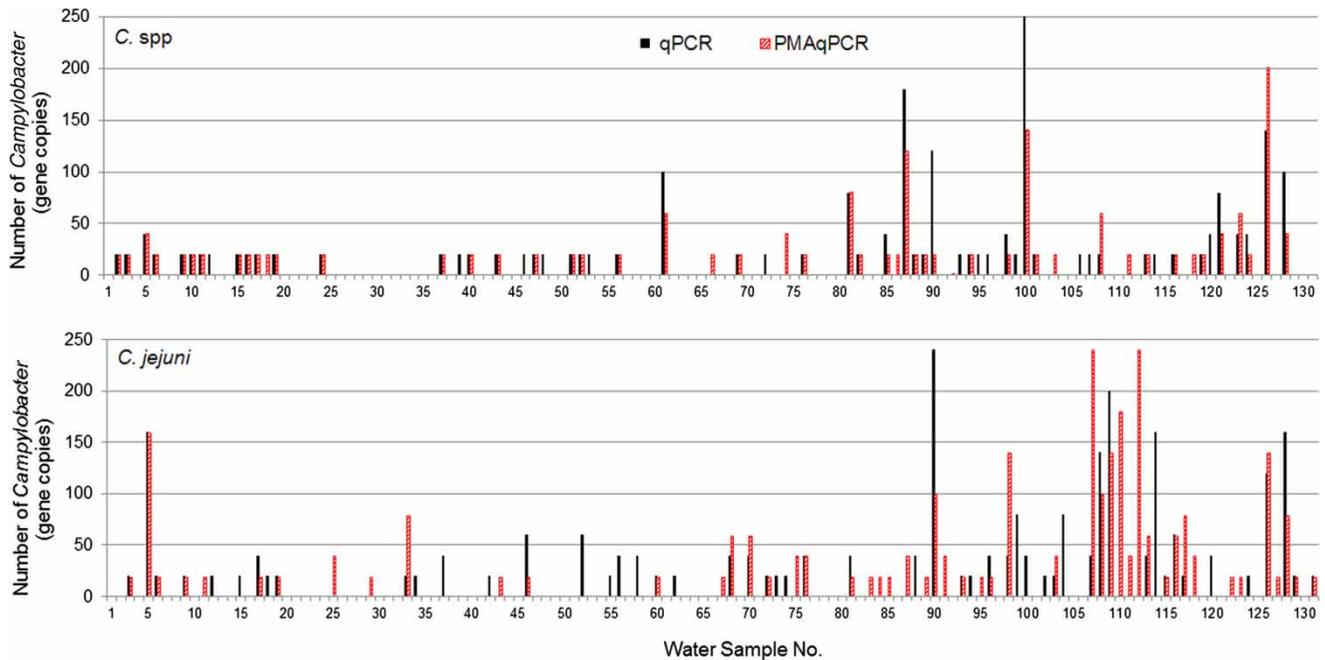
Figure 3 | A subset of water samples (*n* = 131) were tested using both qPCR and PMAqPCR to estimate the number of viable *Campylobacter* spp. and *C. jejuni*. These water samples were collected in 2012 (*n* = 28) and 2013 (*n* = 103).

studies (Vereen *et al.* 2007; Wilkes *et al.* 2011; Khan *et al.* 2014). However, these previous studies were carried out in streams and rivers in intensive agricultural areas and not at more protected lake water sites used for recreational swimming, as in the present study. The biological relevance of cattle as a source of environmental contamination in our study sites is supported by a previous study conducted in the Montérégie region, reporting that 50% of dairy cattle herds were positive for *C. jejuni* (Guévremont *et al.* 2014). We are presently analyzing our comparative genomic fingerprinting data of the *Campylobacter* isolated from the recreational beaches to aid in understanding the potential sources of contamination (manuscript in preparation). In this study PAA was examined as another approach to investigate the impact of agriculture on recreational water. Our results showed an absence of association with PAA, which could be due to an absence of livestock grazing or manure spreading in the agricultural fields within the ZIB; however, this could not be evaluated with our data.

The presence of rain the day before sampling was also associated with higher odds of *C. jejuni* presence in water samples in this study. Heavy rainfall events were previously reported to increase the risk of freshwater beach

contamination by *Campylobacter* spp. (Hellein *et al.* 2011) and a coastal watershed in a highly agricultural region (Vereen *et al.* 2007). The effect of rain could be driven by water movement allowing for resuspension of lake sediments contaminated by *Campylobacter*. Concentrations of *Campylobacter* 2.5 times higher in sediments of bathing water sites compared to water samples were previously reported in the UK (Obiri-Danso & Jones 1999). Another explanation for the rainfall effect could be the transport of bacteria from surrounding contaminated soils by rainwater runoff. It should be noted that no interaction was observed between the intensity of rain and the presence of cattle farming in this study. Although a lack of statistical power cannot be ruled out, this suggests that the potential contamination through runoff water was not limited to agricultural sources, and that the contamination from cattle farms was not influenced by rain. Wild birds, such as gulls or waterfowl, including geese, might be more frequently infected and excreting *Campylobacter* when they have access to agricultural sites, hence contributing to lake water contamination even in the absence of surface water runoff. Contamination by waterfowl has been previously reported (Van Dyke *et al.* 2010; Lévesque *et al.* 2013; Ishii *et al.* 2014) and *C. jejuni* was

Table 3 | Distribution of potential risk factors and results from univariable multi-level logistic regression analyses

		C. jejuni		Campylobacter spp.	
Explanatory variables	**Number of observations**	**% pos.**	***P*-value**	**% pos.**	***P*-value**
Sample level (n = 413)					
Year of sampling					
2011	96	42.7	<0.001	58.3	<0.001
2012	158	15.2		31.0	
2013	159	46.5		62.9	
Season of sampling[a]					
End of spring	64	17.2	<0.001	26.6	<0.001
Early summer	150	28.0		47.3	
End of summer	199	43.2		58.8	
Week of sampling					
June 6–7	10	10.0	<0.01	20.0	0.001
June 13–14	10	10.0		20.0	
June 17–21	44	20.5		29.6	
June 24–28	50	18.0		42.0	
July 1–5	50	32.0		48.0	
July 8–12	50	34.0		52.0	
July 15–19	50	38.0		54.0	
July 22–26	50	52.0		74.0	
July 30–August 2	50	50.0		58.0	
August 5–9	49	32.7		49.0	
Total precipitation (mm) the day before sampling					
0	99	16.2	<0.001	35.4	<0.01
<10	259	39.4		54.4	
≥10	55	38.2		52.7	
Total precipitation (mm) over the three days before sampling					
0	40	17.5	0.07	42.5	0.19
<20	326	35.0		49.1	
≥20	47	38.3		59.6	
Beach level (n = 22)					
Presence of ruminant farms within a 5 km radius					
No	8	25.8	0.05	44.5	0.29
Yes	14	37.8		51.6	
Presence of poultry farms within a 5 km radius					
No	18	31.7	0.77	48.1	0.56
Yes	4	34.2		53.2	
Proportion of area used for agriculture (PAA) within a 2 km ZIB					
<30	17	34.8	0.21	51.0	0.39
≥30	5	23.1		42.3	

[a]Defined as end of spring (from June 6 to June 21), early summer (from June 24 to July 12) and end of summer (from July 15 to August 9).

Table 4 | Odds ratio estimates from a multivariable logistic regression analysis modeling the presence of *Campylobacter* in beach water samples

	Odds ratio		
	Estimate	**95% CI**	***P*-value[a]**
Model A. Presence of Campylobacter jejuni			
Sample level (n = 413)			
Year of sampling			
2011 vs. 2012	5.46	2.69, 11.10	<0.001
2013 vs. 2012	5.31	2.92, 9.62	<0.001
2011 vs. 2013	1.03	0.57, 1.86	1.00
Season of sampling[b]			
End of summer vs. end of spring	5.61	2.54, 12.41	<0.001
End of summer vs. early summer	2.64	1.57, 4.44	<0.001
Early summer vs. end of spring	2.13	0.94. 4.83	0.21
Total precipitation (mm) the day before sampling			
< 10 vs. 0	2.85	1.43, 5.68	<0.01
≥ 10 vs. 0	3.73	1.58, 8.82	<0.01
≥ 10 vs. <10	1.31	0.67, 2.56	1.00
Beach level (n = 22)			
Presence of ruminant farms within a 5 km radius			
Yes vs. No	1.98	1.07, 3.68	0.03
Model B. Presence of Campylobacter spp.			
Sample level (n = 413)			
Year of sampling			
2011 vs. 2012	5.66	2.90, 11.02	<0.001
2013 vs. 2012	4.74	2.81, 8.00	<0.001
2011 vs. 2013	1.19	0.64, 2.24	1.00
Season of sampling[b]			
End of summer vs. end of spring	6.48	3.18, 13.18	<0.001
End of summer vs. early summer	1.78	1.10, 2.88	0.06
Early summer vs. end of spring	3.64	1.74, 7.46	<0.01

[a]Adjusted for multiple comparisons with Bonferroni correction.
[b]Defined as end of spring (from June 6 to June 21), early summer (from June 24 to July 12) and end of summer (from July 15 to August 9).

the predominate species of *Campylobacter* found in these birds. The estimation of wild bird density around the sampled beaches was beyond the scope of our study, limiting the interpretation of our results. Finally, we observed a yearly variation in beach water contamination, suggesting

that unmeasured factors with heterogeneity between years are present, which would warrant further investigations over a larger period of time.

While reports vary as to the infectious dose required to initiate human infections, doses lower than 500 bacteria can infect humans (Hara-Kudo & Takatori 2011). The low concentrations of *Campylobacter* spp. and *C. jejuni* observed in our study, along with the small amount of water generally ingested during bathing activities (Dufour *et al.* 2017), suggest a low risk of acquiring *Campylobacter* infection through recreational water activities at the beaches we studied. However, it is possible that the bacteria were not evenly distributed in our water samples, but rather, aggregated on biofilms (Maal-Bared *et al.* 2012) or organic material which could allow the ingestion of the minimal infective dose. The high viability of *Campylobacter* found in our study also supports a non-negligible risk of infection. Bathing in recreational water has been identified in many studies as a significant risk factor for campylobacteriosis (Schonberg-Norio *et al.* 2004; Unicomb *et al.* 2008; Denno *et al.* 2009; Doorduyn *et al.* 2010). Interestingly, the gradual increase in the proportion of *Campylobacter* positive samples we observed over the bathing season is similar to the reported seasonal increase in campylobacteriosis incidence in Quebec, which has been reported to peak during the first week of August (Arsenault *et al.* 2012). Exposure to recreational waters could contribute to the seasonality in human cases; alternatively, environmental factors driving both the risk of human infection and beach contamination could be involved.

CONCLUSIONS

Campylobacter was found frequently in lake water used for recreational activities, including potentially human infective *C. jejuni*. Although concentrations measured using qPCR and PMAqPCR were frequently low, most *Campylobacter* were deemed viable. The presence of ruminant farms in the vicinity of beaches and rain events the day before sampling both increased the odds of finding *C. jejuni*. Very little is known about the specific burden of campylobacteriosis attributable to recreational activities at beaches located on fresh water lakes. This study provides original and relevant data for further studies aimed at evaluating the risk to public health from exposure to recreational fresh water.

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