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Running head: Microbial contamination on campground drinking water

Title: Microbial contamination in drinking water at public outdoor recreation facilities in New Zealand

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Abstract

Aim

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The aim of our study was to assess the presence and risk of waterborne pathogens in the drinking water of outdoor facilities in New Zealand and track potential sources of microbial contamination in water sources.

Methods and results

A serial cross-sectional study with a risk-based sample collection strategy was conducted at 15 public campgrounds over two summer seasons (2011/12 and 2012/13). Drinking water supplied to these campgrounds was not compliant with national standards, based on *Escherichia coli* as an indicator organism, in more than half of the sampling occasions. *Campylobacter* contamination of drinking water at the campgrounds was likely to be of wild bird origin. Faecal samples from rails (pukeko and weka) were 35 times more likely to return a *Campylobacter*-positive result compared to passerines. Water treatment using ultra-violet (UV) irradiation or a combination of filtration and UV irradiation or chemicals was more likely to result in water that was compliant with the national standards than water from a tap without any treatment. The use of filters alone was not associated with the likelihood of compliance.

Conclusions

Providing microbiologically safe drinking water at outdoor recreational facilities is imperative to avoid gastroenteritis outbreaks. This requires an in-depth understanding of potential sources of contamination in drinking water sources and the installation of adequate water treatment facilities.

Significance and impact of study

Our study provides evidence that drinking water without treatment or filter-only treatment in public campgrounds is unlikely to comply with national standards for human consumption and extra water treatment measures such as UV irradiation or chemical treatment are needed.

Keywords: campground, *Campylobacter*, drinking water, *E. coli*, indicators, water quality

Introduction

Waterborne diseases are a significant cause of morbidity and mortality due to the consumption of untreated or inadequately treated water. A large proportion of waterborne diseases are associated with organisms from faecal contamination (Gallay et al. 2006; Cabral

2010). For instance, *Campylobacter* spp. and *Escherichia coli* are frequently related to waterborne outbreaks originate from human, poultry, cattle, or swine faeces (Ashbolt 2004). The occurrence and concentration of bacterial pathogens are used to assess the quality and safety of water for human consumption. However, the understanding of local factors leading to the presence of pathogenic bacteria and sources of contamination can help to develop strategies for preventing waterborne gastrointestinal illness (Krolik et al. 2016).

In New Zealand, the microbial quality of drinking water is monitored by the Ministry of Health (MoH) using the Drinking Water Standards for New Zealand (DWSNZ). Maximum acceptable values (MAV)s in the DWSNZ are not given for all main waterborne pathogens of public health significance (Ministry of Health 2017); instead, *E. coli* is used as a faecal indicator bacteria. Suitable drinking water consumption for humans meets the standards when *E. coli* is not detectable i.e., the most probable number per 100 millilitres (MPN/100 mL) is < 1. The limit for the bacteriological transgression of *E. coli* is recommended by the World Health Organisation (WHO 2018) to ensure safe drinking water.

Another waterborne pathogen causing significant gastroenteritis cases in New Zealand is *Campylobacter* spp. Although there are no MAVs for *Campylobacter* spp. in the DWSNZ, this bacteria is the most common cause of gastrointestinal illness in New Zealand and it was identified as the causative agent for a major waterborne disease outbreak in 2016. The presence of *Campylobacter* spp. and potential source causing the contamination can be identified using molecular markers. Genotyping methods, such as multilocus sequence typing (MLST), are important for the identification of the source of contamination and informing control measures to limit further infection. The finding of common pathogenic subtypes in both water and animal faeces would provide evidence for a specific animal source of contamination (Jokinen et al. 2011; Whiley et al. 2013; Mughini-Gras et al. 2016). Two waterborne disease outbreaks have been reported at recreational facilities in New Zealand (Bohmer 1997; Hewitt et al. 2007). One of these outbreaks affected 67 people and it was related to contamination of *Campylobacter* spp. from an unknown source during a summer school holiday camp (Bohmer 1997).

The Drinking Water Standards for New Zealand do not apply to the sources from which drinking water is abstracted. However, monitoring water for microbial contamination caused by faeces from wildlife or domestic animals in the catchment can provide a wider picture of

the sources of contamination. Identification and isolation of *Campylobacter* spp. from the source water and their association with a host near the catchment contributes to the development of effective strategies to reduce the risk of gastrointestinal diseases from drinking water (Devane et al. 2018).

Recreational facilities in New Zealand are important for public outdoor activities and nature-based recreation, which are prominent in the local culture and lifestyle, and significant for the tourism industry (a substantial contributor to the national economy). The peak of the camping season in New Zealand occurs during the southern hemisphere summer (December-February). Outdoor and nature-based recreation activities are often associated with high water consumption, especially during dry and warm weather, and therefore it is essential to ensure that recreational facilities provide safe and secure drinking water supplies. Here, we assessed the microbial quality and safety of drinking water from outdoor recreational facilities in New Zealand using two bacteria, both of which can cause waterborne illness. We examined the risk of waterborne disease and tracked the sources of faecal contamination in these waterways linking water and faecal bio-signatures from potential sources. We asked two questions: are outdoor facilities in New Zealand meeting the national drinking water quality standards? And what is the likely source of microbial contamination in drinking water supplied to these facilities?

Materials and methods

Study campground selection

A stratified random sampling procedure was applied to select 15 study campgrounds (eight from the North Island and seven from the South Island) from a sampling frame containing a list of all Department of Conservation (DOC) operated campgrounds in New Zealand (Figure 1). Eligible campgrounds were those classified as standard (11) or serviced (4) and had a surface or roof (only Kohaihai campground) water source (Table 1). Nine campgrounds did not have water treatment facilities, two used filters only, two had ultraviolet (UV) light treatment, one combined filtration with UV light irradiation, and another one used a combination of filter and chemical treatment (Table 1). Campgrounds that had a surface water source through a town or city water supply network were not eligible. Whatamango Bay campground had a spring water source but was erroneously labeled as having a roof water source at the time of campground recruitment and the error was only noticed after the random selection procedure had been completed, so it was retained in this study.

Sample collection

We conducted a serial cross-sectional study with a risk-based sampling strategy in which sample collection coincided with the southern hemisphere summer (December-February). Collection of faecal and water samples from the study campgrounds was carried out in four sampling rounds, two in each of the 2011/12 and 2012/13 summer seasons.

Grab and filter water samples were collected from a tap within the camping area and the source at each campground. Only tap samples were collected at Kohaihai and Whatamango campgrounds because they had roof and spring sources, respectively. Aseptically collected grab samples included 100mL for *Campylobacter* isolation and 100mL for *E. coli* enumeration. Faecal samples from wild birds ($n = 508$), wild mammals ($n = 105$) and domestic mammals ($n = 53$) for *Campylobacter* isolation were collected near the campground catchments using Copan Transystem swabs with transport medium containing Amies agar gel and charcoal (Copan Italia Spa, Italy). All water and faecal samples were stored and transported to the laboratory on ice within 36 hours of collection.

Laboratory sample processing

A modified Colilert (IDEXX Laboratories Inc., USA) enzyme substrate method was used for estimating the most probable number of *E. coli* organisms in water samples (MPN per 100mL). Water samples for *Campylobacter* isolation were filtered through 0.45µm-pore, 47mm-diameter disks (Millipore Corporation; Massachusetts, USA). *Campylobacter* enrichment for both water and faecal samples was carried out on arrival at the laboratory by incubating the filter disks and faecal swabs in Bolton's broth under microaerophilic conditions (85% N₂, 5% O₂, 0% H₂ and 10% CO₂) at 42°C for 48 hours in a Macs-VA500 microaerophilic workstation (Don Whitley Scientific Limited, UK). After enrichment, the broths were cultured onto a selective medium, modified charcoal cefoperazone deoxycholate agar (mCCDA), for 48 hours. From each mCCDA plate with *Campylobacter*-like growth, two colonies were subcultured onto separate horse blood agar plates and incubated in the microaerophilic workstation for 48 hours.

Multilocus sequence typing of Campylobacter

DNA was extracted from a loopful of colonies on each blood agar plate and stored at -20°C before further processing, while the rest of the colonies were preserved in glycerol at -80°C

for future reference. Polymerase chain reactions (PCR)s specific to *Campylobacter coli* (Denis et al. 2001), *C. jejuni* (Stucki et al. 1995) and *C. sp. nova* I (French et al. 2014) were applied to a pair of isolates per sample. The species of the isolates were determined using the typing scheme of seven house-keeping genes for *Campylobacter* (Dingle et al. 2001). Samples negative by PCR for all three species were subjected to a *Campylobacter* genus PCR (Linton et al. 1996). A sample was declared *Campylobacter*-positive if at least one of its two isolates returned a positive result for any of the four PCRs. A mixed-species result was obtained if the PCR results indicated a different species for each of the two isolates. PCR amplification products were sequenced using the chemical reaction Big-Dye Terminator v. 3.1 reagents in an automated DNA sequencer (ABI 3730XL, Applied Biosystems Inc.). Gene sequences were referenced to the PubMLST database to identify their sequence types and clonal complexes.

Data analysis

Risk of waterborne disease

To determine the risk of waterborne disease associated with campground drinking water, we assessed the non-compliance with the DWSNZ for tap water i.e. (> 1MPN/100mL) at the campgrounds (Ministry of Health 2017).

Microbial source assessment

Source of microbial contamination in campground catchments were quantified by estimating the likelihood of returning a *Campylobacter*-positive (*C. coli*, *C. jejuni*, *C. sp. nova* I and/or *Campylobacter* spp.) PCR-result in faecal samples using a fixed-effects generalized linear regression model belonging to the binomial family. We specified a binary response variable $y_i = 1$ if the i th sample was PCR-positive and zero otherwise. This was parameterized as a function of the faecal source x_1 and the Island (North or South) in which the sample was collected x_2 . With the assumption that samples were mutually independent, the fixed-effects generalized linear regression model takes the form:

$$(1) \quad \log\left(\frac{p_i}{1-p_i}\right) = \alpha_i + \beta_1 x_{1i} + \beta_2 x_{2i} + \varepsilon_i$$

where α is the intercept term and β_1 and β_2 represent the regression coefficients for the effect of faecal source and Island on the probability of a *Campylobacter*-positive result while ε is

the residual term. The faecal source levels are passerines, ducks, rail, ruminant and gull faeces and Island levels are North and South.

For tap water, we extended the model in Eq. (1) to a mixed-effects generalized linear regression model in which a binary response variable y_i was equated to one if the i th tap water sample had an *E. coli* concentration ≥ 1 MPN/100mL and zero otherwise. The probability of a water sample having an *E. coli* concentration ≥ 1 MPN/100mL was parameterized as a function of the type of water treatment at the campground x_1 and the presence of ruminants in the catchment x_2 . Assuming that samples from the same campground were more likely to be similar than those from different campgrounds, the mixed-effects generalized linear regression model takes the form:

$$(2) \quad \log\left(\frac{p_i}{1-p_i}\right) = \alpha_{ij} + \beta_1 x_{1i} + \beta_2 x_{2i} + \varepsilon_i$$

here the intercept term α was allowed to vary according to campground j . The treatment type levels are none, filter only, and other methods and ruminants were either present or absent. Finally, for tap water we used a Poisson regression model to determine the relative risk of increasing MPN/100mL where:

$$(3) \quad y \sim \text{Poisson}(\lambda) \\ \log(\lambda) = \alpha_i + \beta_1 x_{1i} + \beta_2 x_{2i} + \varepsilon_i$$

and y is *E. coli* MPN/100mL per tap sample, α is the intercept term and β_1 and β_2 represent the regression coefficients for the effect of the water treatment method and presence or absence of ruminants in the catchment, while ε is the residual term.

The regression models and model diagnostics were implemented using the R packages lme4 (Bates et al., 2014) and sjPlot (Lüdtke 2018), respectively.

Source attribution using MLST

We used the *Campylobacter* sequence typed water isolates for the attribution of water contamination to a specific source. We compared the water isolated with those from different animal faecal sources and augmented the number of sequences types from a curated MLST database that included isolates from human ($n = 5351$), poultry ($n = 3699$), ruminant ($n =$

1574) and wild birds ($n = 815$) hosted by the Molecular Epidemiology Laboratory (^mEpiLab) at the Horkirk Research Institute, Massey University, Palmerston North, New Zealand. The attribution was performed using a Bayesian-based Island genetic model implemented by the R package islandR (<http://github.com/jmarshallnz/islandR>). We further investigated the possible source of contamination by constructing a minimum spanning tree using both water and faecal MLST data generated in the current study using the BioNumerics version 7.0 software (<http://www.applied-maths.com>).

Results

Water sampling

A total of 102 water samples were collected from all campgrounds, of which 48.0% (49) were from sources and 52.0% (53) from taps (Table 1 and Figure 2). Enumeration of *E. coli* showed that 57% (30/53) of the tap samples were non-compliant with the DWSNZ (Table 1). Five campgrounds (Catchpool, Port Jackson, Stony Bay, Waikawau Bay and Whites Bay) were non-compliant with the standards on all sampling occasions. In contrast, three campgrounds (Waikaremoana Motorcamp, Kerr Bay and Totaranui) had tap water that was compliant with the DWSNZ during all sampling occasions.

Overall, 9.8% of the water samples (seven sources and three taps) returned a positive result for one or more of the four *Campylobacter* PCRs (Table 1). Source samples positive for *C. jejuni* were identified in single samples from Catchpool and Otaki Forks and in two samples from Stony Bay. Single source samples were positive for mixed-species of *C. jejuni*/*Campylobacter* spp. from Pelorus Bridge, and *C. jejuni*/*C. sp. nova* I from Stony Bay. *Campylobacter* spp. were identified in one sample from Port Jackson. One positive tap sample collected from Whatamango Bay had mixed-species *C. jejuni*/*Campylobacter* spp. and two samples from Stony Bay were found with *C. sp. nova* I and *C. jejuni*. The presence of *E. coli* showed no significant correlation ($p = 0.96$) with the presence of *Campylobacter* (Figure 2).

Faecal sampling

A total of 668 faecal samples were collected from 508 (76.0%) wild birds (duck, $n = 92$; pukeko, $n = 94$; weka, $n = 5$; gulls, $n = 15$; passerines, $n = 302$), 105 (15.7%) wild mammals (deer, $n = 6$; possum, $n = 73$; rabbit, $n = 24$; rodents, $n = 2$) and 55 (7.9%) domestic animals (cattle, $n = 45$; sheep, $n = 8$; dog, $n = 2$) (Table S1). A total of 142 (21.2%) faecal samples

were PCR-positive comprised of *C. sp. nova* I (9.1%) followed by *C. jejuni* (9.0%), *C. coli* (2.2%) and *C. jejuni/C. sp. nova* I (0.9%). Deer, dog, possum, rabbit, rodent and duck samples were all PCR-negative for *Campylobacter* whilst some ruminant (cattle and sheep), rail (pukeko and weka), passerine and gull samples were positive (13.3% (6/45), 24.2% (23/95), 3.3% (10/302), 26.6% (4/15), respectively).

Multilocus sequence typing analysis

Forty *Campylobacter* isolates from faecal samples (six from domestic ruminants and 34 from wild birds) were subjected to MLST (Table S2). The isolates analysed using MLST included 13 *C. coli* (one water and 12 faecal) and 34 *C. jejuni* (six water and 28 selected faecal) isolates. Five of the water isolates were from sources and two from taps (Tables 2 and S2). Two source samples presented previously unreported allele sequences and allelic profiles in the PubMLST database. All the isolates from cattle, passerines and gulls showed sequence types (ST) already reported in the PubMLST database, while about half of the pukeko isolates presented STs that have not been reported before. The island genomic model attributed the water isolates to wild bird sources (Figure 3). This finding was supported by a minimum spanning tree that showed closer clustering of water isolates with pukeko and passerine isolates (Figure 4).

Regression analysis

Model estimates show that tap water treated with UV or a combination of filtration and UV or chemicals is approximately 100 (i.e., 1/0.01) times more likely to be compliant with the DWSNZ than water from a tap without any treatment (Table 3). The filter-only water treatment systems were not significantly associated with the DWSNZ compliance status of tap water, compared to no treatment (p -value = 0.99). *E. coli* MPN reduced by 37% (1/0.73) if tap water was filtered compared to no treatment (p -value = 0.89) after accounting for the presence of ruminants in the campground catchment (Table 4). In contrast, MPN reduced 100-fold (1/0.01) if other treatment methods (i.e. UV or filter/chemical) were used. Faecal samples from rails (pukeko and weka) were 35 times more likely to return a *Campylobacter*-positive test result compared to faecal samples from passerines, regardless of the Island from which they were collected, while faecal samples from ducks were nine times less likely to be *Campylobacter*-positive compared to those from passerines, after accounting for Island (Table 5).

Discussion

We examined the presence of faecal indicator bacteria that represent potential risks of gastrointestinal diseases in drinking water supplied at 15 public campgrounds in New Zealand. This assessment of risk was achieved by examining the occurrence of microbes associated with water quality over two summer seasons (2011/12 and 2012/13). Sample collection coincided with the peak of the camping season at the campgrounds, so the risk of disease was estimated when a high number of members of the public engaged in outdoor activities and were most likely to be exposed to drinking water-related diseases. The microbial content analysis of tap water provided a framework for assessing the effectiveness of the existing water treatment methods and the direct risk of infection to the public consuming water from these campgrounds. Detection and analysis of microbes at the point of water abstraction gave insights into the level of contamination in the source water and an indication of which animals in the catchment were contaminating the source water with faeces.

Drinking water provided by the campgrounds was non-compliant with DWSNZ on more than half of the sampling occasions. Campgrounds without water treatment facilities were non-compliant with DWSNZ on all sampling occasions. Furthermore, potential pathogens such as *C. jejuni* and *C. coli* were isolated in tap water from campgrounds without water treatment facilities. In contrast, tap water that was compliant with the DWSNZ on all sampling occasions were from campgrounds fitted with a filter/chemical water treatment system or UV irradiation. These findings were supported by regression analysis showing a strong positive association between water treatment with filtration/chemical or UV irradiation and compliance with DWSNZ. Filtration-only systems were found to be ineffective in eliminating microbial contamination. This could be for a number of reasons, including incorrect fitting or poor maintenance. Detection of *Campylobacter* spp. in tap water from the spring that supplied water to the Whatamango Bay campground implies that the spring was unsecured or there was infiltration into the system, possibly due to aging pipes. Hence remedial measures could include securing the spring and/or replacement of the pipe infrastructure.

The isolation of *C. jejuni* and *C. coli* in tap water indicated the existence of faecal contamination and potentially serious disease risk to the public (Mughini-Gras et al. 2016). The strain ST-538 isolated from the Catchpool water source has previously been reported to cause human infection in New Zealand and other countries, including Australia, the

Netherlands, the United Kingdom and the United States of America (<https://pubmlst.org/campylobacter/>). This strain was also isolated from two faecal passerine samples in this study and has been isolated from chicken and cattle in other countries (Kärenlampi et al. 2007). Strains ST-3302 and ST-3640, identified in water samples, have also been isolated from rails in this study whilst ST-3660 and ST-8715 are rail-associated lineages commonly isolated from environmental water in New Zealand (Carter et al. 2009). Our analyses suggested that rail and passerine *Campylobacter* spp. isolates are more likely to be associated with the water isolates, indicating that they are sources of *Campylobacter* spp. contamination in water at the campgrounds. The public health significance of the presence in drinking water of *C. sp. nova* I, a lineage also associated with rails (French et al. 2014; Grange et al. 2016), is currently unknown. *Campylobacter* spp. strains isolated from these birds are not known to be zoonotic (i.e., they have not been reported as causing human illness), but their close relationship with known pathogens presents a potential disease risk to the public.

Logistical constraints associated with gaining access to remote sample sites and the subsequent small number of observations available for both source (49 observations) and tap (53 observations) samples may limit inferences on our regression estimates. This may affect the correlation between pathogens. The presence of *E. coli* in water sources is considered an indicator of the existence of *Campylobacter* (Lund 1996). The occurrence of *E. coli* in our study did not correlate with the detection of *Campylobacter* spp. in the water, which has also been demonstrated elsewhere (Savill et al. 2001; Hörman et al. 2004; St-Pierre et al. 2009). There were 2–4 samples from either source or tap water at each campground with the presence of both *E. coli* and *Campylobacter* spp. A small sample size might limit the use of *E. coli* for assessing the occurrence of *Campylobacter* spp.; nonetheless, it should be noted that all samples with the presence of *E. coli* also showed the presence of *Campylobacter* spp. (Figure 2). Also, the small number of observations and isolation of *Campylobacter* spp. from non-fresh (cultured 24 or more hours after defaecation) faecal samples may have adversely affected our model's ability to adequately estimate the relationship between the microbial quality of water and the presence of animal sources in the campground water catchment (Mughini-Gras et al. 2016). Model diagnostics showed no evidence that our implementation adversely affected statistical inferences about the model parameters.

This study highlights the potential risk of disease associated with drinking water at campgrounds. Remedial measures proposed for these campgrounds include the protection of

drinking water sources and installation of water treatment facilities such as a combination of filtration and UV or chemical treatment. Providing microbiologically safe drinking water at outdoor recreational facilities is imperative to avoid gastroenteritis outbreaks (Boccia et al. 2002; Nygård et al. 2004; ter Waarbeek et al. 2010; Arvelo et al. 2012) and protect a large number of local and overseas visitors using the campground facilities.

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Conflict of interest

No conflict of interest declared.

Author contributions

BJP, NPF and MAS conceived and designed the study. BJP and ADR collected the data. BJP, PJB, JCGR and DTSH processed the data and performed the analyses. PJB and JCGR wrote the manuscript. All the authors read and commented on the manuscript.

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Table captions

Table 1. Campground class, type of water treatment, total number of samples (N), *Campylobacter* PCR-positive samples (Campy +ve), and number of samples with *E. coli* concentrations in drinking water that are non-compliant with DWSNZ (≥ 1 MPN/100mL) at DOC campgrounds in New Zealand included in this study.

Campground	Class	Treatment	Tap			Source		
			N	Campy +ve	Exceed DWSNZ	N	Campy +ve	<i>Campylobacter</i> species
North Island								
Anaura Bay	Standard	None	4	0	3	4	0	<i>C. jejuni</i>
Catchpool	Standard	None	4	0	4	4	1	
Otaki Forks	Standard	Filter	4	0	2	4	1	
Port Jackson	Standard	None	3	0	3	3	1	
Stony Bay	Standard	None	3	2	3	3	3	
Waikaremoana	Serviced	Filter/chemical	4	0	0	4	0	<i>C. jejuni, C. jejuni/C. sp nova I</i>
Waikawau Bay	Standard	Filter	4	0	4	4	0	
Whangaiterenga	Standard	None	4	0	3	4	0	
South Island								
Kerr Bay	Serviced	UV	2	0	0	2	0	<i>C. jejuni/C. spp.</i>
Kohaihai	Standard	None	2	0	1			
Momorangi Bay	Serviced	None	4	0	2	4	0	
Pelorus Bridge	Serviced	Filter/UV	4	0	1	2	1	
Totaranui	Standard	UV	4	0	0	8	0	
Whاتمango Bay	Standard	None	4	1	1			
Whites Bay	Standard	None	3		3	3	0	
Total			53	3	30	49	7	

Table 2. *Campylobacter jejuni* and *C. coli* multilocus sequence types (ST) and allelic profiles isolated from water samples collected in DOC campgrounds during 2011/2012 and 2012/2013 summer seasons. An asterisk (*) indicates a new strain.

Water type	Campground	Species	Strain	aspA	glnA	gltA	glyA	pgm	tkf	uncA
Source	Catchpool	<i>C. jejuni</i>	ST-538	4	7	10	4	42	25	1
Source	Otaki Forks	<i>C. jejuni</i>	ST-3675	237	2	254	340	435	349	256

Source	Pelorus Bridge	<i>C. jejuni</i>	ST-8836*	236	306	254	339	433	695	255
Source	Stony Bay	<i>C. jejuni</i>	ST-8715*	175	638	216	282	359	293	102
Source	Stony Bay	<i>C. jejuni</i>	ST-3640	1	6	5	4	261	7	3
Tap	Stony Bay	<i>C. coli</i>	ST-3302	130	155	69	113	276	257	67
Tap	Whatamango Bay	<i>C. jejuni</i>	ST-3660	192	295	216	282	359	293	102

Table 3: Generalised linear mixed model estimating the likelihood of tap water presenting *E. coli* concentrations ≥ 1 MPN per 100 mL. P values for the overall variables are given, along with individual-level odds ratios with 95% confidence intervals (CI) and p values.

Variable	Odds ratio (CI)	P-value	Observations
Intercept	3.61 (0.73-17.52)	0.11	
Water treatment		0.02	
None	1.00		29
Filter only	1.02 (0.07-13.94)	0.99	8
Other types	0.01 (0.00-0.32)	0.01	14
Ruminants		0.82	
Absent	1.00		35
Present	1.27 (0.14-11.03)	0.82	16

Table 4: Generalised linear mixed model estimating the relative risk of tap water presenting *E. coli* concentrations as MPN per 100 mL. P values for the overall variables are given, along with individual-level odds ratios with 95% confidence intervals (CI) and p values.

Variable	Relative risk (CI)	P-value	Observations
Intercept	30.36 (2.37-389.23)	0.01	
Water treatment		<0.05	
None	1.00		29
Filter only	0.73 (0.01-70.26)	0.89	8
Other types	0.01 (0.00-0.38)	0.01	14
Ruminants		0.92	

Absent	1.00		35
Present	1.08 (0.03-33.66)	0.82	16

Table 5: Generalised linear model estimating the presence of *Campylobacter* in faecal samples collected from the DOC campgrounds during 2011/2012 and 2012/2013 summer seasons. CI = Confidence Intervals.

Variable	Odds ratio (CI)	P-value	Observations
Intercept	0.19 (0.13-0.28)	0.00	
Faecal source		0.00	
Passerine	1.00		302
Duck	0.11 (0.01-0.36)	0.00	92
Rail	34.93 (18.36-72.24)	0.00	99
Ruminant	1.22 (0.53-2.61)	0.62	53
Gull	1.66 (0.44-5.26)	0.41	15
Island		0.61	
North	1.00		300
South	1.15 (0.67-1.95)	0.61	252

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