

Concentrations of *Campylobacter* spp., *Escherichia coli*, Enterococci, and *Yersinia* spp. in the Feces of Farmed Red Deer in New Zealand

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Abstract

Intensive deer farming can cause environmental issues, mainly by its impact on soils and water quality. In particular, there is a risk to the microbial quality of water, as high quantities of suspended sediment and fecal bacteria can enter into water systems. The feces of farmed red deer (*Cervus elaphus*, $n = 206$) from Canterbury and Southland, New Zealand, were analyzed with regard to the presence of *Campylobacter* spp., *Escherichia coli*, enterococci, and *Yersinia* spp.. Enterococci and *E. coli* were isolated from all samples, with mean concentrations of 4.5×10^5 (95% CI 3.5×10^3 , 5.6×10^7) and 1.3×10^8 (95% CI 1.1×10^6 , 1.5×10^{10}) per gram of dry feces, respectively. *Campylobacter* spp. were isolated from 27 fecal samples, giving an overall prevalence of 13.1%. *Campylobacter* isolation rates were variable within and between regions (Canterbury 7.95% [95% CI 2–14%], Southland 16.95% [95% CI 10–24%]). Five out of 42 composite samples were positive for *Yersinia enterocolitica*, and one sample for *Y. pseudotuberculosis*. The overall prevalence ranges on a per-animal basis were therefore 2.43 to 11.17% and 0.49 to 2.91%, respectively. This study is the first to quantify the concentration of *Campylobacter* spp. present in healthy deer farmed in New Zealand. Deer feces are a potential source of human campylobacteriosis, with all genotypes isolated also previously observed among human cases. The fecal outputs from deer should be regarded as potentially pathogenic to humans and therefore be appropriately managed.

Core Ideas

- Deer feces are a potential source of human campylobacteriosis.
- All *Campylobacter* genotypes were found among human clinical isolates in New Zealand.
- *Campylobacter* spp. prevalence in deer feces is low compared with other livestock.
- The average daily excretion of *Campylobacter*-positive deer and sheep is comparable.
- Access of deer to waterways should be restricted.

NEW ZEALAND is the largest supplier of farm-raised venison worldwide with 1.1 million deer farmed on ~2800 farms in 2012 (Bascand, 2013). Although the New Zealand deer industry has experienced a significant drop since the peak of 1.75 million deer in 2004 (due to higher slaughter rates and competition), it is expected to grow again, and sustainable management practices for deer farming are increasingly important (Deer-NZ, 2016).

Intensive deer farming can cause environmental issues, mainly due to its effects on soils through compaction and erosion, and on water quality and aquatic habitats through eutrophication, sediment loss, and fecal contamination (Klein, et al., 2002; McDowell, 2006). Some behavioral characteristics of deer, such as fence-line pacing and wallowing, can worsen environmental issues such as erosion (Evans et al., 1998; Pollard and Wilson, 2002; McDowell and Paton, 2004). Wallows that are connected with waterways (either permanent or temporary) are a high risk to the microbial water quality, as they cause high quantities of suspended sediment and coliforms to enter the water directly. These “connected wallows” are classified as critical source areas for microbial contaminants (McDowell, 2009). Adopting a practice of “safe wallows” and removing high risk wallow areas can significantly reduce the levels of suspended sediments (soil loss) and bacterial loads (*Escherichia coli*) to waterways (McDowell, 2009). Fecal contamination of waterways by deer can be caused by direct deposition of feces into farm waterways and runoff from overland flow or drains.

Few studies have investigated the microbial quality of waterways around deer farms. Concentrations of *Campylobacter* and *E. coli* downstream of deer farms were found to be high, and water sampling upstream and downstream of two deer farms in the Piakonui catchment, Waikato, revealed that *E. coli* concentrations were 2 to 10 times higher downstream of the deer farms than in upstream reaches, which were already affected by two dairy farms (Eyles et al., 2002; Davies-Colley et al., 2004). These results were confirmed by another study, showing that deer wallows connected to catchment waterways have a significant effect on water quality, including fecal contamination (McDowell, 2009). *Campylobacter* spp. are one of the leading causes of zoonotic enteric infection worldwide, and New Zealand presents with one of the highest

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Abbreviations: cfu, colony forming unit; MBIT, multiplex ligation-dependent probe amplification-binary typing; MPN, most probable number; PCR, polymerase chain reaction; PSBB, peptone sorbitol bile broth.

infection rates worldwide, with 135 infections per 100,000 people in 2015 (ESR, 2016a). The presence of *Campylobacter* spp. in deer herds in New Zealand is not known, but previous studies suggest that the prevalence of thermotolerant species in the streams associated with deer farms is second only to the levels found in dairy catchments and therefore poses a significant potential risk to human health (Eyles et al., 2002). In addition to *Campylobacter* spp., *Yersinia* spp. were chosen as the second pathogen of interest rather than *Salmonella* spp. The number of yersiniosis notifications in New Zealand is increasing (whereas the number of salmonellosis notifications is decreasing) (ESR, 2016b). New Zealand studies on feces of other animals (at slaughter and collected at pasture) showed low incidence of *Salmonella* spp. (1.9% in lambs and 0% in sheep; Moriarty et al., 2011b). Another study investigated the microbial content of bovine feces, and no *Salmonella* spp. was detected (Moriarty et al., 2008). Likewise, an earlier survey of 185 newborn dairy calves in New Zealand failed to isolate any *Salmonella* spp. (Grinberg et al., 2005).

Although the contamination of surface water and groundwater from diffuse sources of fecal pollution, particularly grazing livestock, is well recognized in New Zealand and internationally (Davies-Colley et al., 2004; Till et al., 2008), there is limited information on the microbial burden specifically arising from deer feces in New Zealand. Whereas several studies have measured the presence and concentration of fecal indicators and pathogens in the feces of different species of livestock, the current study is the first to enumerate *Campylobacter*, *E. coli*, and enterococci in the feces of farmed red deer (*Cervus elaphus*) in New Zealand. The microbial contamination of waterways with fecal bacteria poses a significant health risk for both humans and livestock. However, there are only limited data available on the levels of microbial pathogens and indicators in cervine feces.

The aim of this study was to examine fresh cervine feces, collected from red deer at slaughter, from a variety of deer farming locations in New Zealand. The concentrations of fecal indicators (*E. coli* and enterococci) and selected pathogens (*Yersinia* spp. and *Campylobacter* spp.) were determined to assess the potential contribution of deer feces to the microbial contamination of environmental waters and the associated public health risk.

Materials and Methods

Sample Selection and Collection

A total of 206 fecal samples from red deer were collected monthly between February and December 2015, with 118 samples from Southland and 88 samples from Canterbury (Table 1). All Canterbury samples were collected on five sampling occasions from a single abattoir, whereas Southland samples came from two abattoirs, collected at six different sampling events. On one sampling day each month, up to 20 samples were collected by the staff at the abattoir. The rectal anal junction of deer after slaughter was collected during evisceration and placed into individual sterile plastic bags (Whirl-Pak, Nasco). The plastic bag was inverted over the hand of the sampler, ensuring that the inside of the bag was not contaminated, and after sample collection, the bag was returned to its original shape containing the rectal anal junction. The samples were returned to the laboratory (Institute of Environmental Science and Research, Christchurch) in a cooled, dark container and processed within 24 h of sampling. The rectal anal junctions

Table 1. Description of sampling schedule in the regions of Canterbury and Southland, New Zealand, to obtain fecal samples from farmed deer for microbial analysis.†

Sampling region	Month of sampling	No. of samples	No. of samples positive for <i>Campylobacter</i> spp.
Southland	Feb. 2015	19	4
Canterbury	Mar. 2015	20	3
Southland	Apr. 2015	20	2
Canterbury	May 2015	20	2
Southland	June 2015	20	13
Canterbury	July 2015	14	2
Southland	Aug. 2015	20	0
Canterbury	Sept. 2015	14	0
Southland	Oct. 2015	19	0
Canterbury	Nov. 2015	20	0
Southland	Dec. 2015	20	1

† Note that samples cannot be tracked back to the single farm, as confidentiality was assured before sampling; however, samples from one sampling day are likely to represent samples from one herd, and different sampling days represent samples from different herds.

were opened under sterile conditions, and the material from each junction constituted an individual sample. Sampling of the rectal anal junction ensured that the integrity of each sample was maintained, without the risk of cross-contamination.

Sample Preparation

The moisture content of each fecal sample was determined by drying a 2-g subsample at 103 to 105°C for 18 h (Clesceri et al., 1989). All microbial concentrations were then expressed per gram of dry weight.

For the enumeration of microorganisms, 25 g of each sample was weighed into sterile filter stomacher bags (Interscience), buffered peptone water (Fort Richards) was added to result in a 10-fold dilution, and the sample was homogenized using a BagMixer (Interscience) for 1 min. A series of 10-fold dilutions in 0.1% peptone water (Fort Richards) was prepared for each sample using 5 mL of the original homogenate.

E. coli and Enterococci

Samples (1 mL) from appropriate dilutions (typically 10⁻⁵, 10⁻⁶, and 10⁻⁷) were filtered in triplicate through 47-mm, 0.45-µm cellulose ester membrane filters (Millipore). After filtration, membranes were placed on Chromocult Coliform agar (Merck), and the plates were incubated at 35°C for 18 to 24 h. All blue-violet *E. coli* colonies and red fecal coliform colonies were counted, and any suspect colonies were restreaked for confirmation. To enumerate enterococci, membranes were incubated on Chromocult Enterococci agar (Merck) at 41°C for 24 h. The red colonies were enumerated as presumptive enterococci, and the membrane was removed, placed onto Bile Esculin Azide agar (Fort Richard) and incubated at 44°C for 4 h. Colonies with a dark halo in the surrounding medium were counted as confirmed enterococci, and any suspect colonies were restreaked for confirmation. Reference strains of *E. coli* (NZRM 916), *Enterococcus faecalis* (NZRM 798), *Shigella sonnei* (NZRM 3227), and *Klebsiella pneumoniae* (NZRM 482) were included in experiments as positive and negative controls to confirm suspect colonies via colony morphology and color comparison.

Campylobacter spp.

Campylobacter spp. were enumerated using a three × five most probable number (MPN) procedure (Moriarty et al., 2008) with enrichment in 30-mL volumes of m-Exeter broth (Fort Richard). After inoculation, tubes were incubated at 42°C for 48 h in microaerophilic conditions, as previously described (Fraser et al., 1992; Moriarty et al., 2015). A 10-μL sample of each MPN tube was streaked onto m-Exeter agar (Fort Richard), incubated at 37°C for a minimum of 4 h, and then transferred to a 42°C incubator for the remainder of a 48-h total incubation period. This method has been proven suitable for detection of *Campylobacter* at low concentrations (Moriarty et al., 2015). Suspect *Campylobacter* spp. colonies were confirmed using biochemical tests (oxidase, catalase), colony morphology, Gram stains, and a multiplex polymerase chain reaction (PCR), as described by Wong et al. (2004). This PCR procedure allows for isolates to be classified as *C. jejuni*, *C. coli*, or thermotolerant *Campylobacter* spp.

Colonies identified as *Campylobacter jejuni* and *Campylobacter coli* were subtyped using a multiplex ligation-dependent probe amplification-binary typing (MBiT) assay (Cornelius et al., 2014). In brief, isolates for MBiT analysis were purified to obtain single colonies, and one colony was then resuspended in 250 μL of 2% Chelex in H₂O. The tube was heated for 5 min at 98°C to denature the DNA, cooled, and centrifuged at 13,000g for 5 min. The supernatant was transferred to a fresh tube, and the multiplex ligation-dependent probe amplification reaction was performed as described in Cornelius et al. (2014). The amplification products were diluted 1:10 in sterile water and separated using the ABI genetic analyzer 3130XL using POP-7 polymer and GeneScan 600 LIZ size standard (Life Technologies). Analysis of electropherograms, subsequent band assignment, cluster analysis, and minimum spanning network analysis were performed using BioNumerics v7.5 (Applied Maths). Thresholds of 5% of the optical density range and 5% of the curve range with correction for peak intensity profile were used. Filtering by relative peak height was also performed using a minimum relative height of 15% and a maximum distance of 30%. Bands were assigned to 18 band classes, using a position tolerance of 0.75%. Manual adjustment of bands was made as necessary. For cluster analysis, a categorical value similarity matrix with unweighted pair group method cluster analysis was applied. Minimum spanning network analysis was undertaken using binary coefficient, with permutation resampling of 40%. The size of each circle in the network diagram represents the number of isolates with that MBiT profile.

Yersinia spp.

Deer feces (5 g) were taken from each of five samples (25 g total) and mixed with 225 mL peptone sorbitol bile broth (PSBB) (Wehr and Frank, 2004) in a single bag (Whirl-pak). The bags were homogenized for 30 s and incubated at 10°C for 10 d. Negative controls included uninoculated PSBB and PSBB inoculated with *E. coli* (NZRM916). Samples of PSBB inoculated with *Yersinia enterocolitica* (NZRM3596) and *Yersinia pseudotuberculosis* (NZRM110) served as positive controls. Ten milliliters of the enrichment was used to extract DNA (ISOLATE II Genomic DNA Kit, Bioline), which served as template in *Yersinia*-specific PCR (undiluted and 10-fold diluted in sterile water).

The PCR primers used were designed to amplify the following genes: 749 bp *Yersinia* subgenus ribosomal RNA (Trebesius et al., 1998), 356 bp *ail* (Hudson et al., 2008), 183 bp *inv* (Kot et al., 2007), and 231 bp *virF* (Harnett et al., 1996). The PCR products indicate the presence of the *Yersinia* subgenus, *Y. enterocolitica*, *Y. pseudotuberculosis*, and of the virulence plasmid (*pYV*), respectively. Each PCR reaction mixture contained (per 50-μL reaction): 19.65 μL water, 3 μL template, 4.5 μL 25 mM MgCl₂, 5 μL 10× PCR buffer, 5 μL 2 mg mL⁻¹ bovine serum albumen, 0.4 μL 200 μM of each deoxynucleotide, 0.25 μL 5 units μL⁻¹ Amplitaq Gold (Applied Biosciences), 2 μL 0.4 μM *ail-a* forward and *ail-b* reverse primers, 2 μL 0.4 μM *Y virF* forward and *virF* reverse primers, 1 μL 0.2 μM *16S-86* forward and *16S-86* reverse primers, and 1 μL 0.2 μM *invA-Kot* forward and *invA-Kot* reverse primers. The PCR conditions were as follows: 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with a final extension of 7 min at 72°C. All PCR reactions including the control DNA amplified from *Y. enterocolitica* NZRM3596 and NZRM110 were run on a MCE-202 MultiNA (Shimadzu) using the DNA-1000 kit. The limit of detection was determined to be 10 colony forming units (cfu) g⁻¹ wet weight composite sample.

Statistical Analysis

The data were analyzed using SigmaPlot version 12.5 (Systat Software, 2011). The concentrations of *E. coli* and enterococci were found to have a non-normal distribution; thus, a Kruskal–Wallis one-way ANOVA on ranks was performed, followed by Dunn's tests. These tests were used to determine if bacterial concentrations varied both within regions and between regions. When bacterial cell counts were less than the detection limit, cfu data were recorded as one [$\log_{10}(1) = 0$]. A simple *t*-test was performed on the *Campylobacter* data.

Results

The percentage of total solids in all red deer fecal samples varied from 14.7 to 45.2% with an arithmetic mean of 30.3% (SD). Canterbury samples presented with a mean of 28.2% (CI 1.025, range 16.4–40.9%), whereas Southland samples showed a mean of 31.8% (CI 1.308, range 14.7–45.2%).

E. coli and Enterococci

Enterococci and *E. coli* were isolated from all samples. Figures 1a and 1b show the variability in concentrations of *E. coli* and enterococci between sampling time points, respectively. Geometric mean *E. coli* concentrations were an order of magnitude higher in Southland samples (2.43×10^8 cfu g⁻¹ dry feces) than in Canterbury samples (5.9×10^7 cfu g⁻¹ dry feces, Table 2). However, enterococci presented with slightly higher mean values in Canterbury deer feces (7.32×10^5 cfu g⁻¹ dry feces) than in Southland samples (3.09×10^5 cfu g⁻¹ dry feces). The mean concentrations of both *E. coli* and enterococci were significantly different between the regions of Canterbury and Southland ($p < 0.001$ and $p = 0.006$, respectively).

Campylobacter spp.

Campylobacter spp. were isolated from 27 out of 206 fecal samples, giving a prevalence of 13.11% for all sampling sites

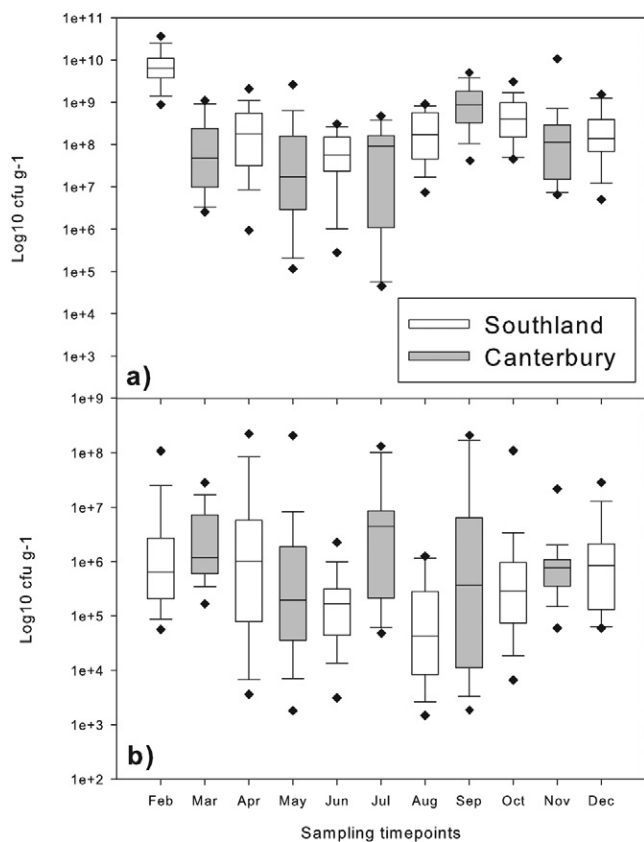


Fig. 1. Variability in (a) *E. coli* and (b) enterococci of deer feces at each sampling time point (per gram dry weight). The horizontal line represents the median, boxes are the 25th to 75th percentile, whiskers represent the 10th and 90th percentile, and diamonds are fifth and 95th percentile values.

combined. The prevalence of *Campylobacter* spp. ranged from 7.95 to 16.95% across both regions, with a prevalence of 7.95% (CI 2–14%) for Canterbury and 16.95% (CI 10–24%) for Southland (Table 3). One sampling event (June) in Southland presented with a high prevalence of 65% (CI 44–86%). A significant difference was detected in the prevalence of *Campylobacter* spp. in fecal samples between Canterbury and Southland samples ($p = 0.036$). *Campylobacter* spp. were enumerated by MPN and suspect isolates verified by PCR, with 25 samples confirmed as containing *C. jejuni* and/or *C. coli* (Table 3). The number of *Campylobacter*-positive samples was found to be highly variable between sampling events (Table 1). The detection limit of

Table 3. Prevalence and concentration of *Campylobacter* spp. in deer feces obtained from abattoirs in Canterbury and Southland, New Zealand.

Parameter†	Region		
	Canterbury	Southland	Combined
<i>N</i>	88	118	206
<i>n</i>	7	20	27
Prevalence (CI, %)	7.95 (2–14)	16.95 (10–24)	13.1 (9–18)
Mean (\pm SD)	6.88×10^3 ($\pm 1.75 \times 10^4$)	2.02×10^3 ($\pm 4.05 \times 10^3$)	3.28×10^3 ($\pm 9.35 \times 10^3$)
Median	1.09×10^2	8.1×10^1	8.86×10^1
Classification <i>Campylobacter</i> spp.			
<i>C. jejuni</i>	5	7	12
<i>C. coli</i>	1	7	8
<i>C. jejuni</i> + <i>C. coli</i>	0	5	5
Not determined	1	1	2

† *N*, total number of samples; *n*, samples positive for *Campylobacter*; CI, confidence interval.

Campylobacter spp. for the method used has been determined to be 1.5 cfu g⁻¹ of fresh feces (Moriarty et al., 2015).

Nineteen genotypes of *Campylobacter* were observed, with 14 genotypes from *C. jejuni* isolates and five from *C. coli* isolates (Fig. 2). Five of the genotypes were observed in both regions, with 12 found in Southland only, and two in Canterbury only. For 16 animals, only one genotype was identified (average of five isolates tested, range 1–12), whereas four animals had two different genotypes of *Campylobacter*, and four animals three different genotypes. All genotypes had been previously found among human clinical isolates in New Zealand. Fourteen of the genotypes have previously been seen in either ovine or bovine samples, with eight of these genotypes also seen among chicken isolates, and six among porcine samples. Of the 19 identified *Campylobacter* genotypes, 16 genotypes have been previously identified in environmental waters in Southland, and five genotypes in Canterbury water samples.

Yersinia spp.

A total of 206 samples of deer feces were combined into 42 composite samples and were tested for the presence or absence of *Y. enterocolitica* and *Y. pseudotuberculosis* using species-specific PCR assays (data not shown). Five out of the 42 composite samples were found to be positive for *Y. enterocolitica*, and one of the 42 composite samples was positive for *Y. pseudotuberculosis*. The

Table 2. Concentration of *Escherichia coli* and enterococci in deer feces obtained from abattoirs in Canterbury and in Southland, New Zealand.

	<i>E. coli</i>			Enterococci		
	Canterbury (<i>N</i> † = 88)	Southland (<i>N</i> = 118)	Combined (<i>N</i> = 206)	Canterbury (<i>N</i> = 88)	Southland (<i>N</i> = 118)	Combined (<i>N</i> = 206)
	colony forming units g ⁻¹ dry wt. feces					
Mean‡	5.90×10^7	2.43×10^8	1.33×10^8	7.32×10^5	3.09×10^5	4.46×10^5
Median	1.11×10^8	2.67×10^8	1.52×10^8	7.85×10^5	2.85×10^5	4.07×10^5
95% CI§	4.17×10^5 , 8.32×10^9	3.39×10^6 , 1.74×10^{10}	1.12×10^6 , 1.51×10^{10}	5.01×10^3 , 1.10×10^8	2.14×10^3 , 3.98×10^7	3.55×10^3 , 5.62×10^7
Min.	4.10×10^4	2.71×10^5	4.10×10^4	1.58×10^3	1.42×10^3	1.42×10^3
Max.	1.12×10^{10}	3.65×10^{10}	3.65×10^{10}	2.16×10^8	2.28×10^8	2.28×10^8
<i>p</i> value	<0.001			0.006		

† *N* = number of samples.

‡ Mean values are geometric means.

§ Confidence intervals (CI) were calculated after log transformation of data.

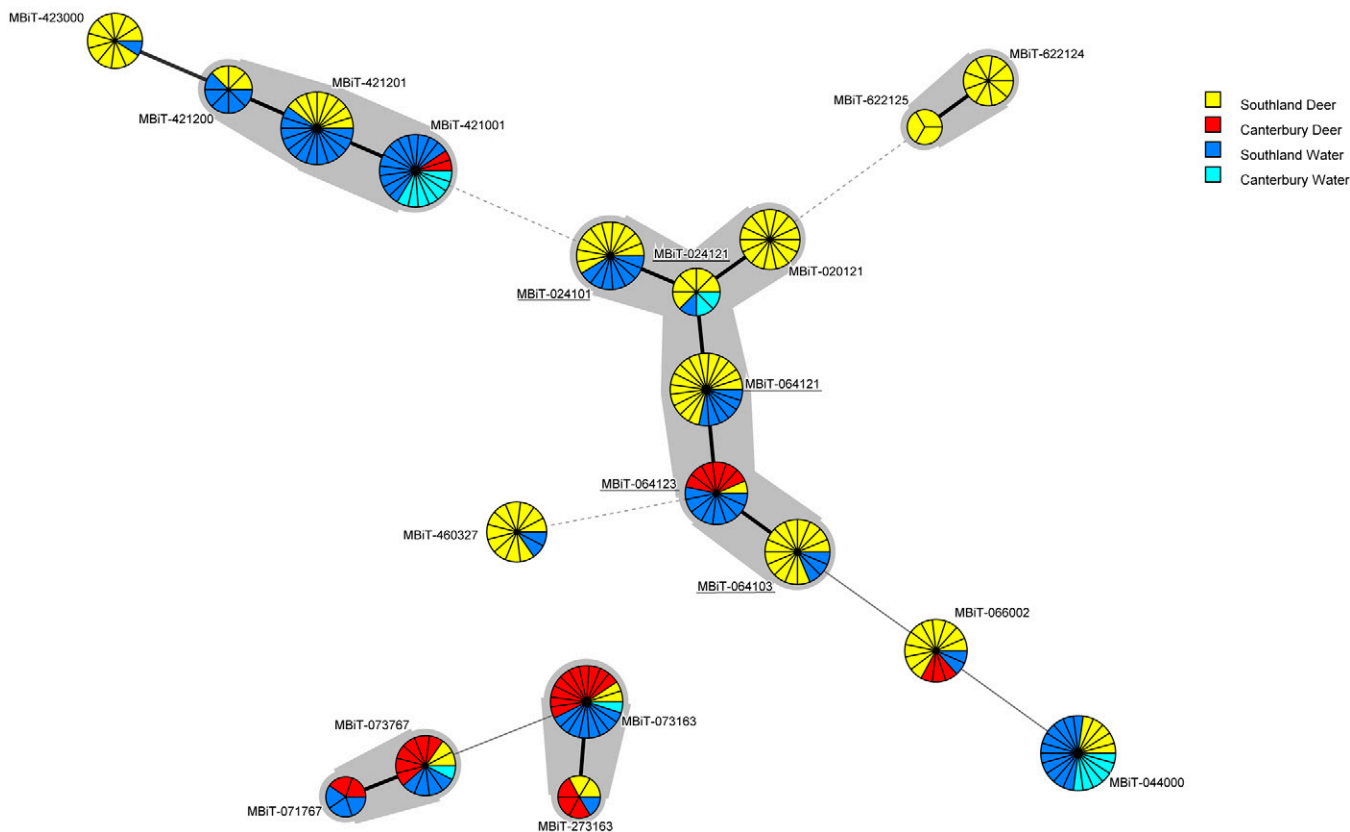


Fig. 2. Minimum spanning network of *Campylobacter* genotypes with colors representing isolates as follows: Southland deer feces (yellow), Canterbury deer feces (red), Southland water samples (dark blue), and Canterbury water samples (light blue). Underlined multiplex ligation-dependent probe amplification-binary typing (MBiT) numbers represent *C. coli*, whereas *C. jejuni* numbers are not underlined. Circle size represents the number of isolates with that MBiT profile. Partition shading indicates one locus difference. Subsequent branches are bold if there were differences in two loci, a thinner solid line represents differences in three loci, a dashed line indicates there are differences four loci, and no line indicates five or more differences.

overall prevalence of PCR-positive samples on a noncomposite (per animal) basis was therefore 2.43 to 11.17% for *Y. enterocolitica* and 0.49 to 2.91% for *Y. pseudotuberculosis*. The prevalence range for *Y. enterocolitica* was 1.14 to 5.68% in the sampling region of Canterbury and 3.39 to 15.25% for Southland. The sample positive for *Y. pseudotuberculosis* was taken at the February sampling event in Southland.

Discussion

This study helps to define the relative impact of different animal feces with respect to environmental contamination. *Campylobacter* was detected in 27 of 206 red deer fecal samples (13.1%). A limited number of studies worldwide have been conducted regarding the microbial burden of fresh red deer feces, with most studies concentrating on wild animals. Fresh fecal samples were collected after hunting events, and the prevalence in these studies ranged between 0 and 2.8% positive samples (Carbonero et al., 2014; Diaz-Sanchez et al., 2013), hence *Campylobacter* prevalence in New Zealand farmed red deer appears to be significantly higher. Reasons for this finding may include the different food sources and the proximity of deer to one another in a herd facilitating intraherd transmission of pathogens in an intensive farming environment. A number of studies investigating the prevalence of *Campylobacter* spp. in feces of lambs, sheep, and cattle at slaughter found prevalence rates of 80.9 and 91.7% for lambs, 43.8% for sheep, and 54.6% for cattle (Stanley et al.,

1998; Milnes et al., 2008; Moriarty et al., 2011b). Source analysis using MBiT revealed that most of our *Campylobacter* isolates had types that had previously been observed among human clinical cases and other farmed animals, including sheep, cows, pigs, and chickens. These animals have been shown to be potential sources for transmission of *Campylobacter* spp. (French et al., 2010; Moriarty et al., 2011a, 2011b; Anderson et al., 2012). Farmed red deer will more likely acquire pathogens from other animal species living in close proximity on the farm than wild red deer. Higher-resolution genotyping may allow further distinction among these animals, but it is feasible that strains can move freely among these different types of farm animals.

The prevalence of *Y. enterocolitica* found in this study was lower than in a New Zealand study on red deer (2.43–11.17% vs. 10.5–36.8%) undertaken by Henderson (1984), whereas the *Y. pseudotuberculosis* prevalence was in a similar range (0.49–2.91% vs. 0.8–7.0%). The lower results in our study may have methodological origin, since we used a PCR-based method, whereas Henderson (1984) used an isolation-based method, including an enrichment step. Sampling regions in both studies were similar: Canterbury and Southland locations, and additional Otago samplings in the Henderson study. Henderson postulated that the low rates of *Y. pseudotuberculosis* found in the feces of red deer were unexpected due to the widespread diagnosis of yersiniosis in deer. In contrast, *Y. enterocolitica* rates exceeded recently described isolation rates for wild red deer in Eastern Europe (0.7%) (Gnat et al., 2015). Another European study found

isolation rates of *Y. enterocolitica* in pig feces of 8 to 12.5% and higher isolation rates from pig tonsils (56–67.2%), raising the question whether the carrier state for *Yersinia* spp. may not be adequately characterized by fecal testing (Bucher et al., 2008). Furthermore, Henderson (1984) reported frequent changes in the fecal flora of deer with respect to *Yersinia* spp., and that the excretion of *Yersinia* sp. seems to be intermittent; thus, a sporadic shedding of *Yersinia* spp. can be expected. The author used this scenario to explain the differences in the prevalence of *Y. enterocolitica* between sampling locations (deer farms in the regions of South Canterbury, Otago, and Southland), ranging from 10.5 to 36.8% across regions, and to explain variations in repeated sampling results from one location.

Several New Zealand studies have enumerated the concentrations of *E. coli*, enterococci, and *Campylobacter* in the feces of other animals, as summarized in Table 4. Adding data from this study helps to determine the relative impact of different animal feces with respect to potential environmental contamination. Studies investigating the prevalence of microbial indicators and pathogens in feces of lambs and sheep at slaughter and at pasture concluded that lambs (and to a probably lesser extent, sheep)

potentially play a significant role in the microbial pollution of streams and rivers in the farm environment via surface runoff (Moriarty et al., 2011b). The role of deer, however, still needs to be determined. In deer, the mean concentration of *E. coli* was higher than enterococci, which was the case for most animal species surveyed except for horses and ducks. Although the concentration of *E. coli* in wet deer feces was comparable with the concentration in lamb feces, the mean daily excretion of *E. coli* per animal was found to be the highest in deer compared with all other animals investigated. Of course, this can be attributed to the higher body weight of deer and thus a higher daily excretion of feces (Table 4).

In contrast, deer feces were found to contain a concentration of enterococci (cfu g⁻¹ wet weight) that compared with other animal species as ducks > lambs > horses > gulls > deer = black swans > sheep > Canada geese > dairy cows. The mean daily excretion of enterococci per animal was similar for deer, lambs, and ducks. Only horses excreted more enterococci per animal, whereas all other livestock and wildfowl were found to excrete less enterococci as deer on a per-animal basis.

Table 4. Estimated mean daily excretion of *Escherichia coli*, enterococci and *Campylobacter* spp. in the feces of various livestock. All concentrations are expressed as cfu per gram of wet feces, except for *Campylobacter* spp. where the units are MPN per gram of wet feces (WW)^a

Animal (reference)	Microorganisms	Concentration†	Prevalence	Mean daily excretion of feces‡	Mean daily excretion of organisms per animal§	Mean daily excretion by 100 animals¶
			%	kg d ⁻¹	colony forming units	
Deer (current study)	<i>E. coli</i>	3.19 × 10 ⁸	100	5.2	1.66 × 10 ¹²	1.66 × 10 ¹⁴
	Enterococci	2.34 × 10 ⁶	100		1.21 × 10 ¹⁰	1.21 × 10 ¹²
	<i>Campylobacter</i>	9.05 × 10 ²	13.1		4.71 × 10 ⁶	6.17 × 10 ⁷
Horse (Moriarty et al., 2015)	<i>E. coli</i>	4.78 × 10 ⁵	98.3	12.5–21	8.0 × 10 ⁹	7.87 × 10 ¹¹
	Enterococci	1.01 × 10 ⁷	100		1.69 × 10 ¹¹	1.69 × 10 ¹³
	<i>Campylobacter</i>	13	3.4		2.16 × 10 ⁵	7.40 × 10 ⁵
Sheep (Moriarty et al., 2011a)	<i>E. coli</i>	1.67 × 10 ⁷	100	1–2	2.51 × 10 ¹⁰	2.51 × 10 ¹²
	Enterococci	6.80 × 10 ⁵	100		1.02 × 10 ⁹	1.02 × 10 ¹¹
	<i>Campylobacter</i>	2.08 × 10 ³	30.4		3.12 × 10 ⁶	9.48 × 10 ⁷
Lamb (Moriarty et al., 2011a)	<i>E. coli</i>	6.04 × 10 ⁸	100	1–2	9.06 × 10 ¹¹	9.06 × 10 ¹³
	Enterococci	1.44 × 10 ⁷	100		2.16 × 10 ¹⁰	2.16 × 10 ¹²
	<i>Campylobacter</i>	3.33 × 10 ⁵	80.9		4.99 × 10 ⁸	4.04 × 10 ¹⁰
Dairy cattle (Moriarty et al., 2008)	<i>E. coli</i>	8.2 × 10 ⁴	99.05	24.8	2.03 × 10 ⁹	2.01 × 10 ¹¹
	Enterococci	4.5 × 10 ²	93.3		1.12 × 10 ⁷	1.05 × 10 ⁹
	<i>Campylobacter</i>	4.3 × 10 ²	63.9		1.06 × 10 ⁷	6.77 × 10 ⁸
Black swan (Moriarty et al., 2011b)	<i>E. coli</i>	1.91 × 10 ⁶	94	0.418	7.98 × 10 ⁸	7.50 × 10 ¹⁰
	Enterococci	1.10 × 10 ⁶	79		4.59 × 10 ⁸	3.63 × 10 ¹⁰
	<i>Campylobacter</i>	2.04 × 10 ²	45		8.53 × 10 ⁴	3.84 × 10 ⁶
Duck (Moriarty et al., 2011b)	<i>E. coli</i>	9.4 × 10 ⁷	95	0.336	3.18 × 10 ¹⁰	3.02 × 10 ¹²
	Enterococci	1.01 × 10 ⁸	100		3.39 × 10 ¹⁰	3.39 × 10 ¹²
	<i>Campylobacter</i>	5.92 × 10 ¹	29		1.99 × 10 ⁴	5.77 × 10 ⁵
Canada goose (Moriarty et al., 2011b)	<i>E. coli</i>	3.62 × 10 ⁴	95	0.250	9.03 × 10 ⁶	8.57 × 10 ⁸
	Enterococci	2.51 × 10 ⁴	98		6.25 × 10 ⁶	6.13 × 10 ⁸
	<i>Campylobacter</i>	4.84 × 10 ³	40		1.21 × 10 ⁶	4.84 × 10 ⁷
Gull (Moriarty et al., 2011b)	<i>E. coli</i>	1.87 × 10 ⁷	96	0.05	9.35 × 10 ⁸	8.98 × 10 ¹⁰
	Enterococci	8.96 × 10 ⁶	99		4.45 × 10 ⁸	4.41 × 10 ¹⁰
	<i>Campylobacter</i>	7.66 × 10 ²	59		3.83 × 10 ⁴	2.26 × 10 ⁶

† Microbial concentrations are expressed per gram of wet feces to enable comparison with published results of other animal species.

‡ Based on data from: McDowell (2006, deer); Lawrence et al. (2003, horse); Muirhead et al. (2011, dairy cattle); Mitchell and Wass (1995, black swan); Geldreich (1966, duck) Hussong et al. (1979, Canada goose); Wood and Trust (1972, gull).

§ Estimated from daily excretion volume × mean concentration of organism for a positive animal.

¶ Estimated from mean daily output of organisms for a positive animal × prevalence.

The prevalence of *Campylobacter* spp. was relatively low in deer compared with other livestock species, ranging from horses (3.4%) < deer (13.1%) < sheep (30.4%) < cattle (63.9%) < lambs (80.9%) (Moriarty et al., 2015). However, one sampling event presented with a prevalence of 65% of *Campylobacter* spp., which clearly shows that strong fluctuations are possible, which could be due to variations in feed, transport, farming practices, or contact with other animal species. This particular sampling event was performed in southern hemisphere winter (June), a time when the prevalence of infection with *Campylobacter* spp. is usually low in cattle (Moriarty et al., 2008), sheep (Stanley and Jones, 2003), humans (Lal et al., 2012; Nichols et al., 2012), and other mammals (Taema et al., 2008). Possible reasons for the high prevalence might be that deer in this particular herd were exposed to a variety of domesticated and wild animals including sheep, pigs, rabbits, cats, dogs, and birds. Previous studies have shown these animals can be potential sources for transmission of *Campylobacter* spp. (Moriarty et al., 2011a, 2011b; Anderson et al., 2012), and that sharing pastures with other livestock increases the likelihood of deer to acquire pathogens (French et al., 2010). Further investigations are necessary to pinpoint the variability of infection with and shedding of *Campylobacter* spp. in deer, focusing also on factors that lead to changes in the microbial gut flora, and thus the fecal flora as reported by Henderson (1984) for *Yersinia* spp. *Campylobacter* counts per gram of wet feces for the different animals investigated decreased in the following order: lambs >> Canada geese > deer > gulls > dairy cows > black swans > ducks > horses. Lambs present with *Campylobacter* counts in the order of 10^5 cfu g⁻¹ and have the highest prevalence rate (80.9%) of all animals investigated (Moriarty et al., 2011b). Taking the mean daily excretion of feces per animal into account, one deer excretes $\sim 4.71 \times 10^6$ cfu, an amount of *Campylobacter* similar to one sheep (3.12 ± 10^6 cfu) or one Canada goose (1.21×10^6 cfu). The per-animal excretion of *Campylobacter* by lambs and dairy cows is, on average, two and one order of magnitude higher (4.99×10^8 and 1.06×10^7 cfu, respectively) than that of deer. The mean daily excretions of *Campylobacter* for horses, black swans, ducks, and gulls range between 1.99×10^4 and 2.16×10^5 cfu.

Although a prevalence of 13.1% of *Campylobacter* spp. generally suggests a slightly smaller role of deer feces in campylobacteriosis in New Zealand compared with cattle or sheep, we found that the daily excretion of *Campylobacter* is comparable between *Campylobacter*-positive sheep and *Campylobacter*-positive deer. Therefore, we propose that deer feces are a considerable source of human campylobacteriosis, either through direct contact or from contaminated water. This might be especially the case in areas with a very high prevalence of *Campylobacter* spp. in deer herds. The majority of *Campylobacter* genotypes isolated from deer feces were also found in environmental waters in Southland and Canterbury, and all genotypes have been previously associated with human cases. The species identifiable in this study, *C. jejuni* and *C. coli*, are those most often associated with reported campylobacteriosis in humans, with 90% of disease caused by *C. jejuni* and the majority of the rest attributed to *C. coli* (Gillespie et al., 2002). New Zealand has one of the highest rates of campylobacteriosis in the developed world, with campylobacteriosis also being the most commonly reported gastrointestinal disease in New Zealand. Therefore, understanding

more about *Campylobacter* spp., including its distribution and potential reservoirs, is a priority for New Zealand.

Limited information is available on the microbial burden of deer feces (Guber et al., 2015), its potential impact on the environment, especially in areas with intensive deer farming, and the role of deer in the transmission of zoonotic microorganisms. A number of diseases in New Zealand's deer herds are considered to be important waterborne diseases (Ball and Till, 1998; Gill, 1998). These include diseases that are common to pastoral mammals, such as leptospirosis, "Johne's disease," internal parasites, cryptosporidiosis, and yersiniosis (Klein et al., 2002). However, the routes of transmission and the possible excretion of the pathogens via feces in deer are poorly understood. One study in the United States (Singh et al., 2015) investigated the transmission of pathogens across different livestock species including cattle and deer. The authors proposed that deer feces were significantly less likely to contain pathogens in spring (March) than in summer (June), with frequencies of shiga toxin-producing *E. coli*, enterohemorrhagic *E. coli*, and enteropathogenic *E. coli* of 1, 6, and 22%, respectively, for the June sampling (Singh et al., 2015). French et al. (2010) analyzed feces collected from 30 white-tailed deer farms in Ohio and isolated several pathogens including *E. coli* O157 (3.3%), *Listeria monocytogenes* (3.3%), *Y. enterocolitica* (30%), and *Clostridium difficile* (36.7%).

The fate and transport of fecal microorganisms strongly affects the effective environmental impact of deposited fecal material. The concentrations of *E. coli* in freshwaters such as rivers and enterococci in marine and brackish waters are used as indicators for microbial water quality. Cervine feces deposited close to surface waters may affect these waters via overland flow. A study by Guber et al. (2015) investigating *E. coli* survival in and release from feral deer feces found substantial differences from other source materials, such as feces of livestock and manures. Generally, studies on feces from cattle found about 2-log₁₀ increases in *E. coli* populations within the first few days (Wang et al., 1996, 2004; Muirhead et al., 2005), followed by a decrease in the subsequent weeks, depending on water content, temperature, and pH (Reddy et al., 1981; Weaver et al., 2016). However, in deer feces, the first stage of *E. coli* increase lasted much longer (up to 8 d), and the increase was up to 3 log₁₀ and thus much more pronounced (Guber et al., 2015). This implies that *E. coli* concentrations measured in the fresh feces used in our study are likely to underestimate potential *E. coli* inputs in waterways. When estimating *E. coli* loads in watersheds, the higher potential of *E. coli* growth in deer pellets should not be neglected. Studies on feces of sheep found a pattern similar for *E. coli* and enterococci, but *Campylobacter* spp. were found not to grow and were rapidly inactivated at a rate that tended to be faster at higher temperatures (Moriarty et al., 2011a). Although there are no published data available on the survival and growth of enterococci, *Campylobacter* spp., and *Yersinia* spp. in deer feces, we assume that enterococci will present with a pattern similar to *E. coli* and that *Campylobacter* spp. will reflect the findings for sheep (Moriarty et al., 2011a). To our knowledge, no published data are available on the growth or die-off rates of *Yersinia* spp. in animal feces. However, as the bacterium can survive in soil and cattle manure for 10 d (between -4 and 30°C), and even

longer at low temperatures in water (Guan and Holley, 2003), similar survival times in deer feces can be assumed.

This study focused on the enumeration of indicator organisms (i.e., *E. coli* and enterococci) and the identification of reservoirs of pathogens (i.e., *Campylobacter* and *Yersinia*) in the feces of farmed red deer, as the contamination of waterways with pathogenic bacteria and the associated public health risk is of high priority in New Zealand. Here, intensive farming is the main contributor to nonpoint source fecal water contamination, which occurs via direct deposition, surface runoff, or bypass flow. Future studies should target the identification of *E. coli* and *Enterococcus* spp. Combined with daily fecal output of deer and access to water, the potential contribution of deer to microbial contamination of surface water should be considered when assessing elevated concentrations of microbial indicators in waterways.

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